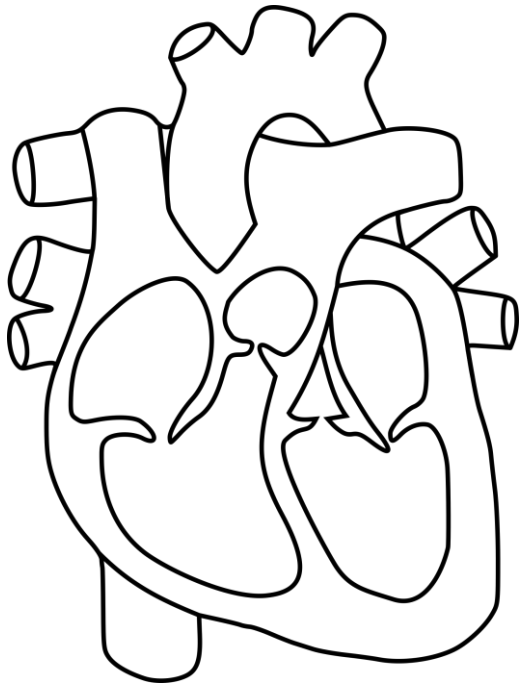
Santa Ana College, Biology Department

Bio249 Human Anatomy & Physiology Laboratory Manual

Written by Jennie Beltran. Contributions by Rosie Santos.



Last updated on:
2/4/2019

Santa Ana College

Bio249 Human Anatomy and Physiology Laboratory Manual

IF FOUND PLEASE CONTACT STUDENT OWNER:

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ACKNOWLEDGEMENTS

I am very excited to contribute to the human physiology course at Santa Ana College. I remember being a student and performing similar labs to help me solidify my understanding of the human body. I am very thankful to be able to contribute my own spin on these core laboratory exercises and I hope to continuously update this manual to increase student success and understanding. My goal is to set a sound foundation for future healthcare professionals, scientists, educators, and other interested individuals. I would like to acknowledge the full-time and adjunct anatomy and physiology professors at SAC for all their hard work in developing our programs. My colleagues and I are here to serve our students to make sure they are prepared for their future careers and to help them grow as people.

Go Dons,

Jennie L. Beltrán, M.S.

Scientists use the metric system to communicate volume, length, mass, and temperature. Below is a quick review.

The Metric System:

Measurement	International System of Units	Basic Unit
Length	Meter	m
Volume	Liter	L
Mass	Gram	g
Temperature	Degrees Celsius Degrees Fahrenheit	ºC ºF

Degree Celsius = (5/9) (degrees Fahrenheit – 32)

Degrees Fahrenheit = (9/5) (degrees Celsius) +32

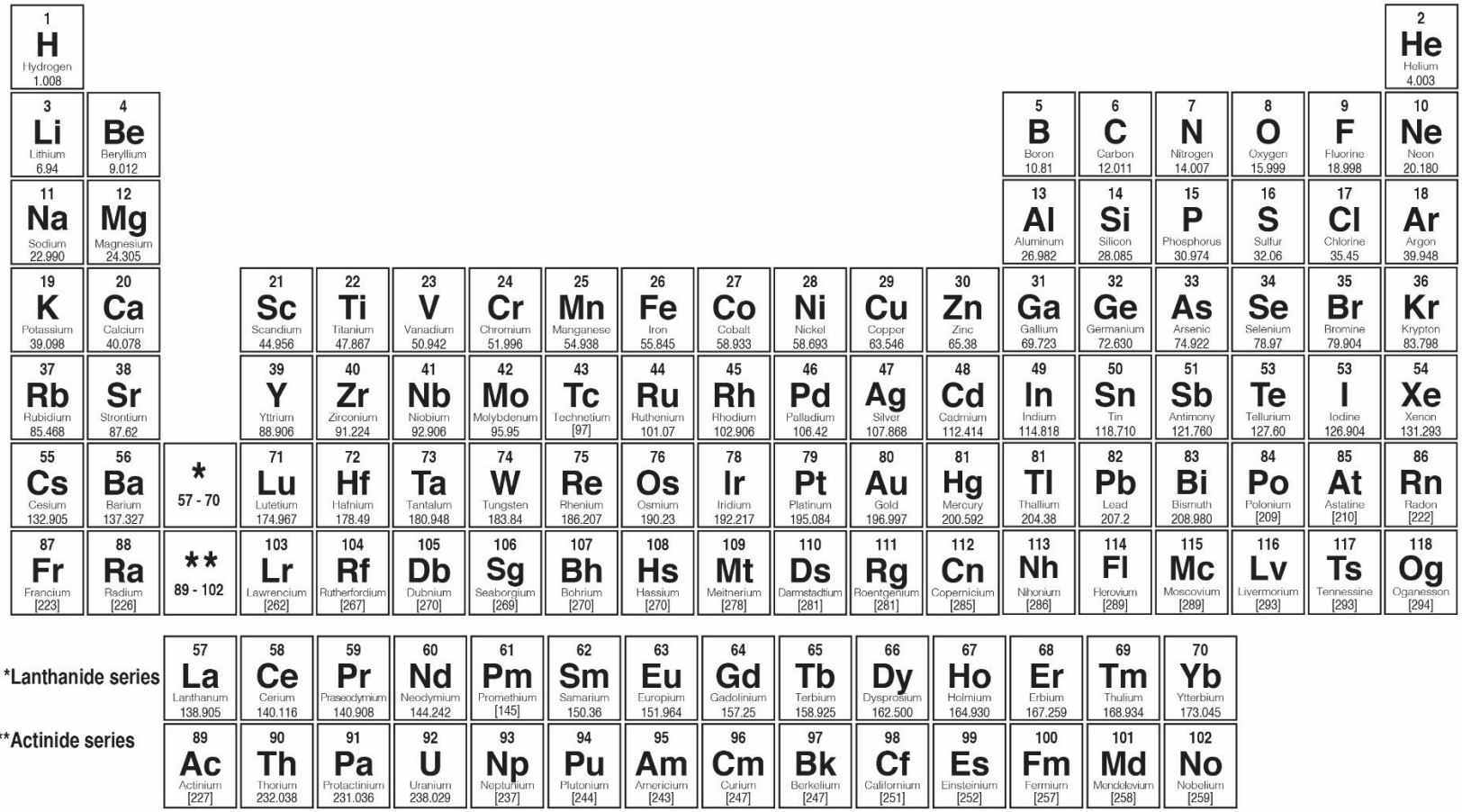
Metric Prefix	Abbreviation	Relative Size to the Basic Unit	Relative Size to the Basic Unit
Kilo-	k	10^3	1,000.0
Basic unit	---	10^0	1.0
Deci-	d	10^{-1}	0.1
Centi-	c	10^{-2}	0.01
Milli-	m	10^{-3}	0.001
Micro-	µ	10^{-6}	0.000001
Nano-	n	10^{-9}	0.000000001

1 milliliter (mL) = 1 cubic centimeter (cc)

1 milliliter of water = 1 gram of water

Order of operations: PEMDAS (parentheses, exponents, multiplication, division, addition, subtraction)

Periodic Table of Elements



LAB EXERCISE #1:

The Process of Science and Experimental Design

Summary

The process of the scientific method (research/observations, question, hypothesis, experiment, analysis, conclusions, scientific communication) is used to help us learn about the world around us. We can make observations about the world around us and through this process we can answer certain types of questions. There are some questions that the scientific method cannot answer, however they may be addressed philosophically, ethically, morally, etc. When scientists ask the right questions, design good experiments, and can reliably repeat their experiments, then we can find answers and add to the body of scientific knowledge.

Identifying various parts of an experiment is a skill that every scientist must acquire. Today we will learn to distinguish between independent variable(s), dependent variable(s), and standardized variables. We will also learn about control experiments and we will talk about the need to understand basic statistics in order to support or reject our hypotheses (replication, sample size, graphing, statistical tests). Additionally, we will learn how to choose the correct type of graph to plot our data and we will use MS Excel to help us graph our class data.

The independent variable is the variable the investigator wishes to test. The investigator may deliberately vary it. However, it is not always possible or necessary for an investigator to directly manipulate an independent variable in order to test its effect. It is typical to test only one independent variable at a time. The dependent variable is what is expected to vary in response to the experimenter's manipulations. It is the variable that will be measured or counted during the investigation. Standardized variables are maintained constant between experiments in order to be certain that any change are due to changes in the independent variables and not to other factors. Control treatments are another necessary part of any well-designed experiment. A control treatment is a treatment in which the independent variable is either eliminated or set at a standard value. The results of the control treatment are compared to the results of the experimental treatments. A good experimental design should be repeated (replicated) many times with similar results. Sample size is another aspect of replication. One way to replicate an experiment is to repeat it over and over. Another way is to perform it on a large sample size simultaneously. The most convincing results come from experiments done with both replication and adequate sample size. Statistics (mean, mode, median, range, t-test, ANOVA, etc.) are performed to help us analyze the results of our experimentation.

Goals

Physiology students will learn about and perform the process of the scientific method. By the end of this lab session, each student should be able to:

- Explain the scientific method.
- Design a good experiment.
- Identify a good question.
- Synthesize a testable and falsifiable hypothesis.
- Determine the experimental variables: dependent variable, independent variable, and standardized variables.
- Determine the need for a control experiment: positive control and negative control.
- Determine the significance of control experiments, replications, and sample size.
- Collect data and analyze data properly by running statistics and graphing using MS Excel.

Graphing Requirements

- Graphs must include the following:
    - Title, Axis labels & units
    - Independent and dependent variables are found on the correct axis
    - Key if appropriate (note: colors and dimensions add another variable)
    - Scatter plot: draw a best fit line and add an equation
    - Line graph: connect the points with a straight line only
    - Bar graph: bars should be separate

Lab Exercises:

A. Experimental Design-The Mystery Container

Equipment

- Mystery Container sealed and filled with varied mystery items
- Technology & Research: Potential items
- Technology & Research: Dial-O-Gram Balance
- Work in groups

Protocol:

1. Your instructor will assign you a mystery container that holds a variety of items. Create a scientific question for this experiment that will lead you to a testable hypothesis of observable phenomena.

2. Protocol #1. When you receive your container, make observations using any method that is available without opening the Mystery Container.
    a. Create a hypothesis of what the Mystery Container may hold. Based on observations and/or previous research you may have a **hypothesis** (“educated guess”) and which is really just a more formal way of restating a scientific question or problem.
    b. Explain which observations led you to make this hypothesis.
    c. Think about various types of information, equipment, and/or methodology that might have been useful in determining the contents of the Mystery Container.
3. Protocol #2: Advances in research and technology
    a. Your instructor will now provide you with a variety of items that may be found in your Mystery Container.
    b. When you receive your new materials make new observations using any method that is available without opening the Mystery Container.
    c. Create a new hypothesis of what the Mystery Container may hold.
    d. Explain which new observations led you to make this hypothesis.
    e. Think about various types of information, equipment, and/or methodology that might have been useful in determining the contents of the Mystery Container.
4. Protocol #3: Even more Advances in research and technology
    a. Your instructor will now provide you with a Dial-O-Gram Balance.
    b. When you receive your new materials make new observations using any method that is available without opening the Mystery Container.
    c. Create a new hypothesis of what the Mystery Container may hold.
    d. Explain which new observations led you to make this hypothesis.
    e. Think about various types of information, equipment, and/or methodology that might have been useful in determining the contents of the Mystery Container.
5. Protocol #4: Ask your instructor for details.
6. Clean Up:
    a. Put the contents of the Mystery Cup back.

b. Seal the Mystery Cup and return it to your instructor.
c. Return the potential items and Dial-O-Gram back to your instructor.

B. MS Excel and Graphing: Heart Rate and Weekly Exercise

Equipment

- Class spreadsheet and practice MS Excel file.
- A laptop (your own or a class laptop)
- Internet access
- Work in pairs or work alone.

Protocol:

1. Your instructor will walk you through a quick tutorial on how to use MS Excel.
2. Download the spreadsheet from the Blackboard website.
3. Create the proper graph using the class data.
4. Save your work (you may want to email the file to yourself or save it on a USB).
5. Try creating many different types of graphs to practice.
6. Shut down the laptop and put the computers away neatly.

Name: _____ **Score:** _____

Bio249 Lab Section: _____

Scientific Pre-Lab Assignment: Due at the beginning of lab.

- Bring your personal laptop. Bring a basic scientific calculator to lab.

- Understand the procedures before coming to lab. Understand waste management before coming to lab.

- Use your textbook and resources to explain the process of the scientific method in your own words.

- You are an investigator who wants to determine the dose of penicillin that is most effective at combating strep throat infections.
    - Identify the independent variable._____
    - Identify the dependent variable._____
    - Identify two standardized variables._____
    - Identify the control experiment(s)._____
- Circle your answer: You are an investigator who is determining if there is a relationship between arm span and height. You collected data from all the SAC students. What type of graph should you use plot your data?
    - Bar graph
    - Line graph
    - Scatter plot
- Circle your answer: You are an investigator who is determining the average running speed of your pet iguana at 80ºF, 100 ºF, and 120 ºF. What type of graph should you use to plot your data?
    - Bar graph
    - Line graph
    - Scatter plot
- Circle your answer: You are an investigator who is determining the monthly growth rate of a dog who has low growth hormone production. What type of graph should you use to plot your data?
    - Bar graph
    - Line graph
    - Scatter plot

THIS PRE-LAB IS DUE AT THE NEXT LAB MEETING

Name: _____ **Score:** _____

Bio249 Lab Section: _____

Scientific Method Post-Lab Assignment: DUE AT THE NEXT LAB MEETING.

A. Experimental Design-The Mystery Container

- Create a good question for the Mystery Container experiment.

- Protocol 1:
  - List the available technology.

  - List your observations below.

  - Hypothesis: What do you think is in the Mystery Container and why? Make sure it is testable and falsifiable.

  - What types of information, equipment, and/or methodology might have been useful in determining the contents of the Mystery Container?

- Protocol 2:
  - List the available technology.

- List your observations below.

- Hypothesis: What do you think is in the Mystery Container and why? Make sure it is testable and falsifiable.

- What types of information, equipment, and/or methodology might have been useful in determining the contents of the Mystery Container?

- Protocol 3:
  - List the available technology.

  - List your observations below.

  - Hypothesis: What do you think is in the Mystery Container and why? Make sure it is testable and falsifiable.

  - What types of information, equipment, and/or methodology might have been useful in determining the contents of the Mystery Container?

- Protocol 4:
  - What did you find?

- Was your hypothesis supported or rejected? Explain why some groups made good hypotheses, however they were incorrect when they opened their Mystery Container.

B. Experimental Design-Design your own experiment

- Design a simple experiment to investigate the following question: Women who practice sprinting the 100-yards daily will decrease the time that they run the mile.
  - Identify the question.
  - Identify the hypothesis.
  - Identify the independent variable.
  - Identify the dependent variable.
  - Identify the proper control treatment.
  - Identify the standardized variables that you would use.
  - What sample size would you have?
  - How many replications would you run?

- What type of statistics would you run?

- What type of graphs would you make?

THIS POST-LAB IS DUE AT THE NEXT LAB MEETING.

LAB EXERCISE:

The Cell Cycle and Human Genetics

Summary

Genetics testing is now being done for a multitude of reasons. One reason is fetal testing so that parents have results to make decision on continuing a pregnancy or terminating pregnancy. Parents can also make informed decisions on preparing to have a child with special needs due to chromosomal abnormalities or genetic disorders. Today you will learn all about these prenatal genetic tests by reading real pamphlets, learning about how cells divide in your body, learning what can go wrong, and learning how your DNA can affect your fetus.

Goals

- Describe the cell cycle.
- Describe mitosis.
- Describe meiosis.
- Describe the process of crossing over and resulting chromosomal abnormalities when crossing over does not occur properly.
- Describe the process of nondisjunction I and nondisjunction II and resulting gametes.
- Describe various genetic tests used to test a fetus (NIPT, amniocentesis, CVS).
- Learn to read a Karyotype.

Lab Exercises:

A. Let's pretend that you just found out that you (or your partner) are pregnant, you have Advanced Maternal Age (35 years and older) and that this is your first OB/GYN visit. Pretend that you have never taken a college level biology course.

Equipment

- Genetic Testing pamphlets
- Your instructor will lecture about this topic
- Your instructor may provide videos demonstrating the techniques

Protocol:

1. The first thing they gave you are these pamphlets to read. You are allotted 15 minutes to review the pamphlets before the OB/GYN comes in to check your embryo and order tests. You have to make a decision about running these genetic tests or not at week 10 of pregnancy.
2. Read the pamphlets and circle any concepts or words that you may not understand. Add any questions that you think you would have. Put yourself in your patient's shoes, what would they struggle understanding if they had limited biological knowledge?
3. Identify what a Noninvasive Prenatal Test (NIPT) (a screening test) is used for and how is it performed.
4. Identify what an Amniocentesis Diagnostic Test is used for and how is it performed.
5. Identify what a Chorionic Villus Sampling (CVS) Diagnostic Test is used for and how is it performed.
6. In order for you to understand this pamphlet, you must understand the cell cycle, mitosis, meiosis, nondisjunction and chromosomal abnormalities. Let's learn about these below.
7. As your postlab, put yourself in someone's shoes.
8. Why would someone want to know these results?
9. Why would someone not want to know these results?
10. As your postlab, let's pretend that you or your partner are pregnant today. Your instructor will assign you a fetal karyotype. Cut out the pieces and tape them to a separate piece of paper in order. Determine if your fetus is aneuploid and if so what is the genetic abnormality called. Determine the assigned biological sex of the fetus. You will share these results next week.

11. Think about what you would do if you found out that your fetus had an aneuploidy? What would you do if you were a carrier for a genetic disease? There is no right or wrong answer.

B. The Cell Cycle: Describe the life cycle of a cell

Equipment

- Textbook/resources
- Your instructor will lecture about this topic
- Your instructor may provide videos demonstrating this process

Protocol:

1. Label the cell cycle: Interphase (G1, S, G2), Mitotic Phase (PMAT), and Cytokinesis
2. Identify the function and major events of each phase.

C. Cellular Division: Mitosis is used to make more somatic cells

Equipment

- Textbook/resources
- Your instructor will lecture about this topic
- Your instructor may provide videos demonstrating this process
- Your instructor may provide pipe-cleaners and white boards to help you illustrate the process.

Protocol:

1. You are provided the cell at G1 phase of interphase. Draw the chromosomes going through S phase and G2 phase of interphase.
2. Now you are entering the Mitotic Phase. Draw the chromosomes going through each phase of mitosis (PMAT) and Cytokinesis
3. Identify the function and major events of each phase of mitosis.

D. Cellular Division: Meiosis is used to make gametes (sperm and eggs)

Equipment

- Textbook/resources
- Your instructor will lecture about this topic
- Your instructor may provide videos demonstrating this process
- Your instructor may provide pipe-cleaners and white boards to help you illustrate the process.

Protocol:

1. You are provided the cell at G1 phase of interphase. Draw the chromosomes going through S phase and G2 phase of interphase.

2. Now you are entering the Meiotic Phases. Draw the chromosomes going through each phase of Meiosis and Cytokinesis
3. Identify the function and major events of each phase of meiosis.

E. Crossing-over during Meiosis increases genetic diversity: a closer look at how DNA is exchanged between homologous chromosomes

Equipment

- Textbook/resources
- Your instructor will lecture about this topic
- Your instructor may provide videos demonstrating this process
- Your instructor may provide white boards to help you illustrate the process.

Protocol:

1. You are provided homologous chromosomes (one came from the mother and one came from the father) which contain various genes (gene A, gene B, gene C, gene D, etc.). Each gene has various alleles that correspond to it (for example, gene A could contain allele A which is dominant or allele a which recessive). Color the top homologous chromosome red to represent the mother and the bottom homologous chromosome blue to represent the father.
2. In the next step you will notice that the chromosomes have duplicated, and you now have a tetrad. Color the corresponding chromosomes as red for the mother and blue for the father. Label the tetrad. Label the sister chromatids.
3. In the next step you will notice that the tetrad has undergone crossing over. Color the corresponding genes as red for the mother and blue for the father to illustrate how the DNA has swapped places.

F. Chromosomal rearrangement: When Crossing-over does not occur properly

Equipment

- Textbook/resources
- Your instructor will lecture about this topic
- Your instructor may provide videos demonstrating this process
- Your instructor may provide white boards to help you illustrate the process.

Protocol:

1. You are provided a normal chromosome, which contain various genes in a specific order (gene A, gene B, gene C, gene D, gene E, gene F, gene G.).
2. Look at the following chromosomes and determine if they have a chromosomal abnormality. Label each chromosome with the proper chromosomal rearrangement: deletion, inversion, duplication, and translocation.

G. Nondisjunction I and Nondisjunction II: When Anaphase I and Anaphase II during Meiosis do not separate chromosomes properly

Equipment

- Textbook/resources
- Your instructor will lecture about this topic
- Your instructor may provide videos demonstrating this process
- Your instructor may provide white boards to help you illustrate the process.

Protocol:

1. You are provided the cell at G1 phase of interphase. Draw the chromosomes going through S phase and G2 phase of interphase.
2. Now you are entering the Meiotic Phases.
3. Complete the first figure by draw the chromosomes going through Nondisjunction I (when a tetrad does not separate during Anaphase I). Label the gametes as n (normal haploid cell), n+1 (haploid with an extra chromosome), or n-1 (haploid with missing chromosome). What percent is normal? What percent is aneuploid?
4. Complete the second figure by draw the chromosomes going through Nondisjunction II (when a pair of sister chromosomes do not separate during Anaphase II). Label the gametes as n (normal haploid cell), n+1 (haploid with an extra chromosome), or n-1 (haploid with missing chromosome). What percent is normal? What percent is aneuploid?

H. Determining if your hypothetical fetus has a genetic abnormality: Trisomy or monosomy.

Equipment

- Textbook/resources
- Your instructor will lecture about this topic
- Your instructor may provide videos demonstrating this process

- Your instructor may provide white boards to help you illustrate the process.

Protocol:

1. Look at the normal karyotype. How many pairs of chromosomes does this person have? How many chromosomes total they have? Is their assigned biological sex male or female? What is the pattern seen in the autosomal chromosomes (chromosome 1-22)? What is the difference between the X and Y sex chromosomes? Notice the different banding patterns in each pair of chromosomes.
2. Learn about Fetal Testing and Aneuploidy. These are the main trisomy and monosomy genetic abnormalities that are tested for when you have Advanced Maternal Age as they are the most commonly seen.
3. Look at each karyotype provided and determine if the fetus is normal or aneuploid. If they are aneuploidy, then determine if they have monosomy or trisomy and where. Provide two symptoms that would be seen. Also determine the fetal assigned biological sex.
4. Learn about each type of monosomy and trisomy that a fetus can be tested by completing the provided chart.
5. Visit the NIH US National Library of Medicine Genetics Home Reference website to help you (https://ghr.nlm.nih.gov/). Click on Menu then Health Conditions to search for the various topics.

Name: _____ **Score:** _____

Bio249 Lab Section: _____

Genetics Pre-Lab Assignment:

-Read the protocol before attending lab and plan accordingly.
-Understand the definitions before coming to lab. Define them.

- Chromosome
- DNA
- mRNA
- Diploid vs. Haploid
- Gametes vs. Somatic Cells
- Genes vs. Alleles
- Aneuploidy: monosomy, trisomy
- Mitosis
- Meiosis
- Interphase
- Mitosis:
- Meiosis:
- Nondisjunction I vs. Nondisjunction 2

- Crossing over
- Independent assortment
- Homologous chromosomes
- Sister chromatids
- Tetrad
- Chromosomal rearrangements
- Karyotype
- Noninvasive prenatal testing (NIPT) or Cell-free DNA screening test
- Amniocentesis diagnostic testing
- Chronic villi sampling (CVS) diagnostic testing

THIS PRE-LAB IS DUE AT THE BEGINNING OF THE LAB MEETING.

Name: _____ **Score:** _____

Bio249 Lab Section: _____

Genetics Post-Lab Assignment:

A. Let's pretend that you just found out that you (or your partner) are pregnant, you have Advanced Maternal Age (35 years and older) and that this is your first OB/GYN visit. Pretend that you have never taken a college level biology course.

1. Read and annotate your pamphlet. Attach your annotated pamphlet.
2. Identify what a Noninvasive Prenatal Test is and how it works.

3. Identify what an Amniocentesis Diagnostic test is and how it works.

4. Identify what a Chorionic Villus Sampling Diagnostic test is and how it works.

5. Why would someone want to know the genetic results of their fetus?

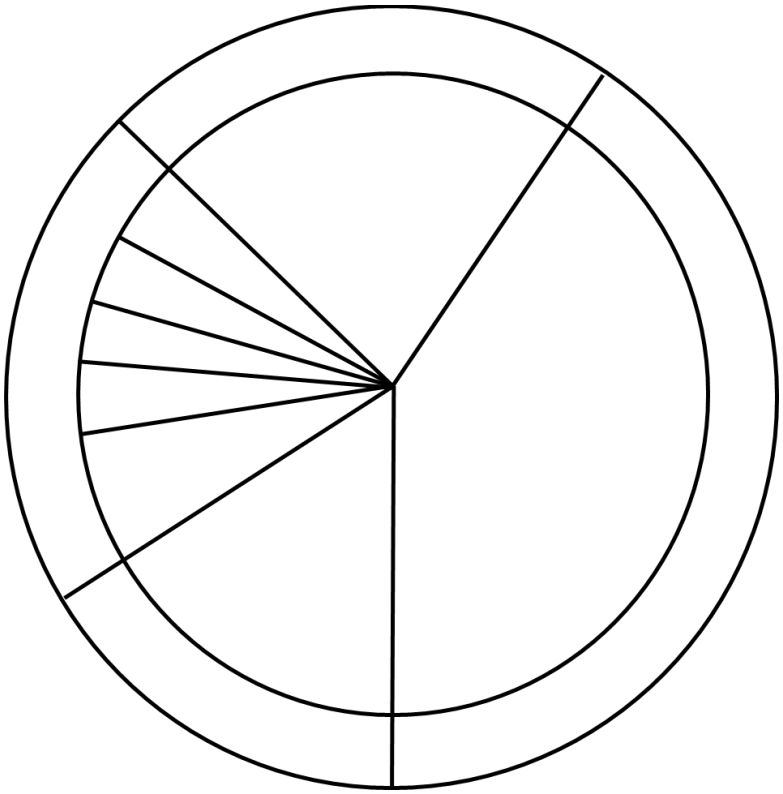
6. Why would someone not want to know the genetic results of their fetus?

7. Let's pretend that you/your partner are pregnant today. Your instructor provided you a hypothetical fetal karyotype and an example of a normal karyotype. Analyze it by cutting out the pieces and taping them on a separate piece of paper.

a. Determine the assigned biological sex of your fetus: _____
b. Determine if your fetus is aneuploid or normal. _____
c. If your fetus is aneuploidy, then determine the name of their genetic abnormality: _____
d. If you found out that your fetus was aneuploid, what would you do and what is your reasoning behind this? Remember that there is no right or wrong answer for this.

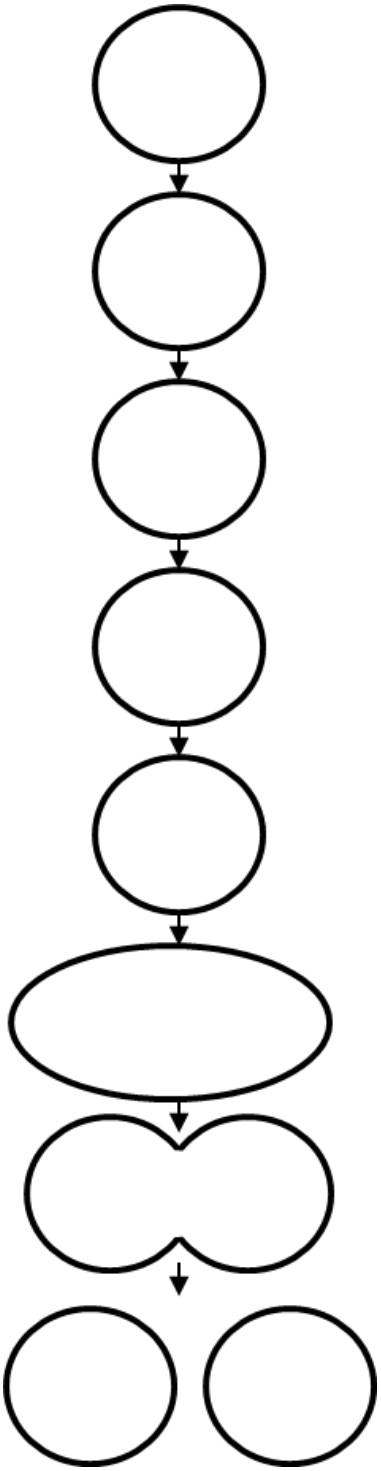
B. The Cell Cycle: Describe the life cycle of a cell

1. Label all the phases of the cell cycle on the figure below. Interphase, G1, S, G2, Mitotic phase, Prophase, Metaphase, Anaphase, Telophase, and Cytokinesis.
2. Identify the function and major events at each phase of the cell cycle.



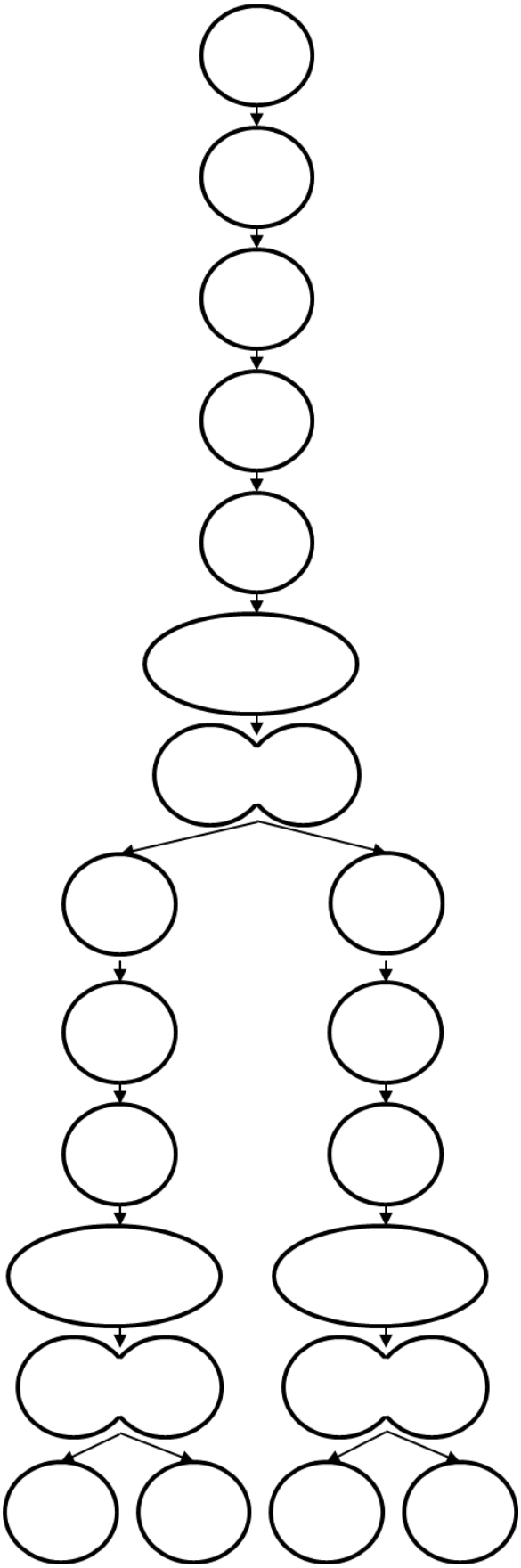
C. Cellular Division: Mitosis is used to make more somatic cells

1. Label all the phases of Interphase, G1, S, and G2 on the figure below. Then label all the Mitotic phases: Prophase, Metaphase, Anaphase, Telophase, and Cytokinesis. Identify if the cell is haploid or diploid at each phase. Identify if the DNA is duplicated or unduplicated at each phase. Identify key events.



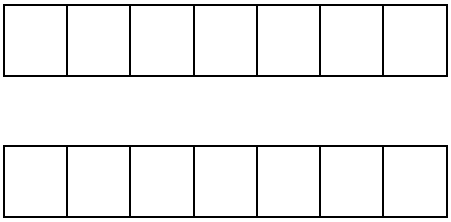
D. Cellular Division: Meiosis is used to make gametes

1. Label all the phases of Interphase, G1, S, and G2 on the figure below. Then label all the Meiotic phases: Meiosis I (Prophase I, Metaphase I, Anaphase I, Telophase I) and Meiosis II (Prophase II, Metaphase II, Anaphase II, Telophase II). Identify if the cell is haploid or diploid at each phase. Identify if the DNA is duplicated or unduplicated at each phase. Identify key events.

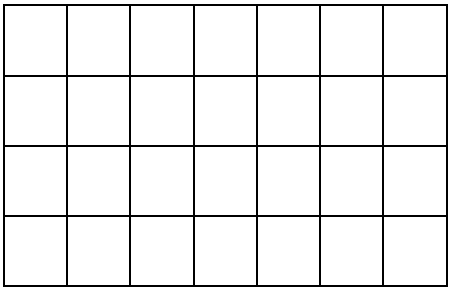


E. Crossing-over during Meiosis increases genetic diversity: a closer look at how DNA is exchanged between homologous chromosomes

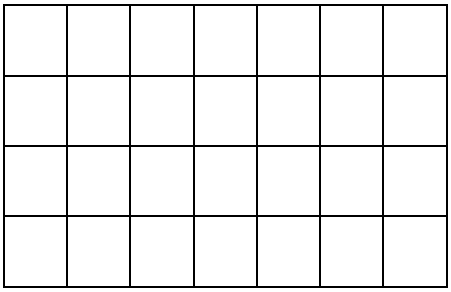
1. In the homologous chromosomes figure below color the top chromosome red to represent the DNA inherited by the mother and the bottom chromosome blue to represent the DNA inherited by the father. Label the homologous chromosomal pair.



2. In the tetrad figure below color the corresponding chromosomes as red for or blue to represent the inherited DNA. Label the sister chromatids. Label the tetrad.

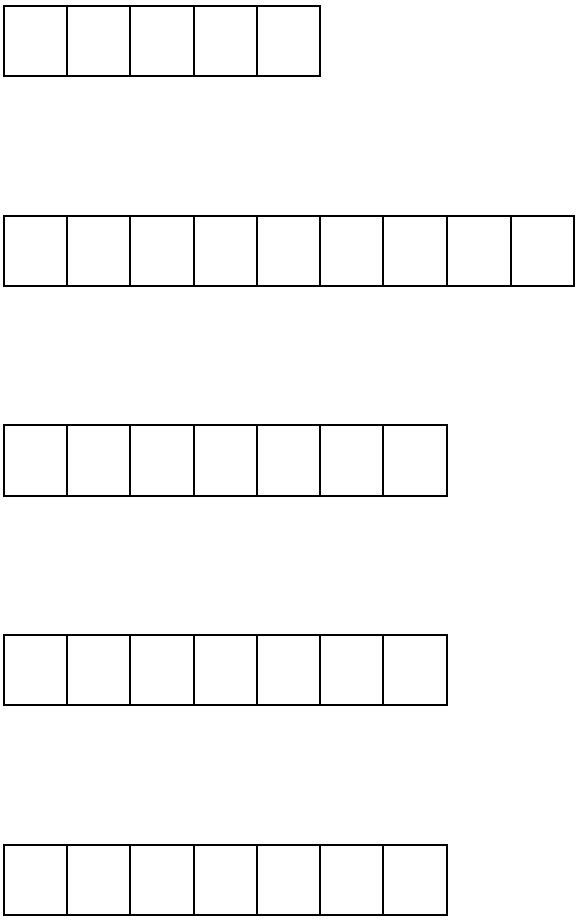


3. In the crossing over event below color the corresponding genes as red for or blue to represent the inherited DNA and to illustrate the crossing over event.



F. Chromosomal rearrangement: When Crossing-over does not occur properly

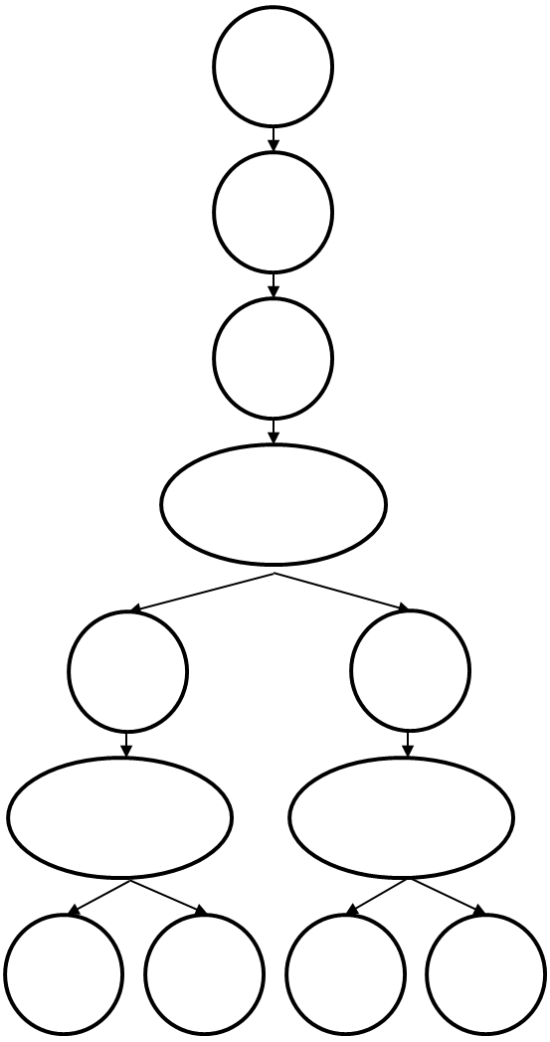
1. Label each chromosomal abnormality as either normal, deletion, inversion, duplication, or translocation.



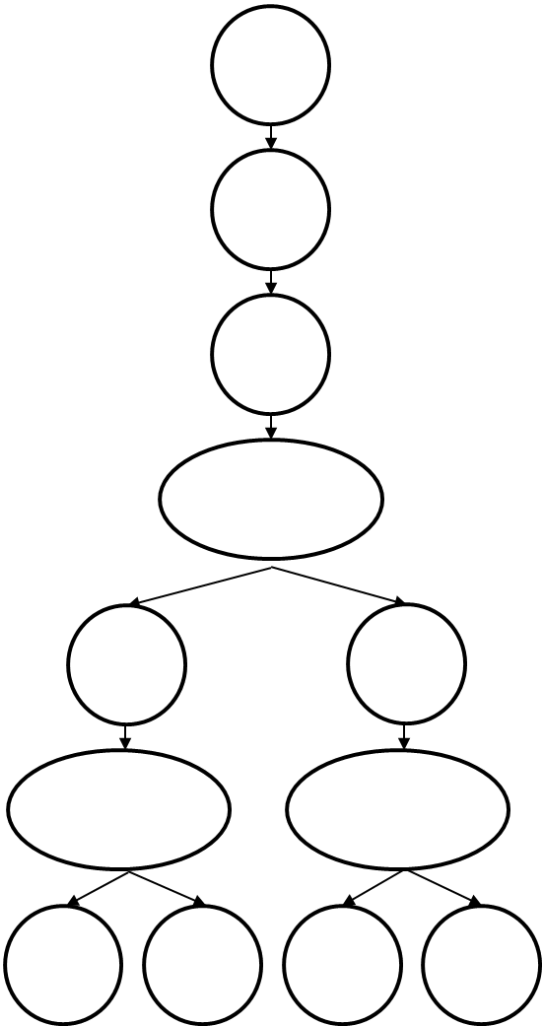
G. Nondisjunction I and Nondisjunction II: When Anaphase I and Anaphase II during Meiosis do not separate chromosomes properly

1. Complete the figure below for Nondisjunction 1 and Nondisjunction 2. Draw the cell at G1, S, G2, Telophase I and Telophase II.
2. Label the gametes as normal or aneuploidy. N, N+1, or N+2.

Nondisjunction I



Nondisjunction II



H. Determining if your hypothetical fetus has a genetic abnormality: Trisomy or monosomy.

1. Normal karyotype
    a. How many pairs of chromosomes does this person have? ____
    b. How many chromosomes total do they have? ____
    c. Is their assigned biological sex male or female? ____
    d. What is the organizational pattern seen in the karyotype? Circle your answer: Ordered from [big to small / small to big].
    e. What is the difference between the X and Y sex chromosomes?

    f. Notice the different banding in each set.

2. Complete the table below about the main genetic abnormalities that Fetal Testing results show. Visit the NIH US National Library of Medicine Genetics Home Reference website to help you (https://ghr.nlm.nih.gov/).

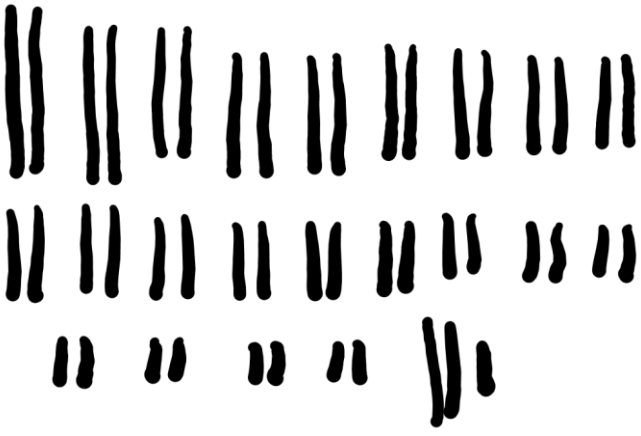
Chromosomal disorder name	Aneuploidy description on which chromosome (monosomy, trisomy or chromosomal abnormality)	Birth statistics	Symptoms
Cri-du-chat syndrome			
Patau syndrome			
Edwards syndrome			
Down syndrome			
Triple X syndrome			
Turners syndrome			
Jacobs Syndrome			
Klinefelter syndrome			
Fragile X syndrome			
Cystic Fibrosis	The parents can be carriers.		
Sickle Cell Anemia	The parents can be carriers.		

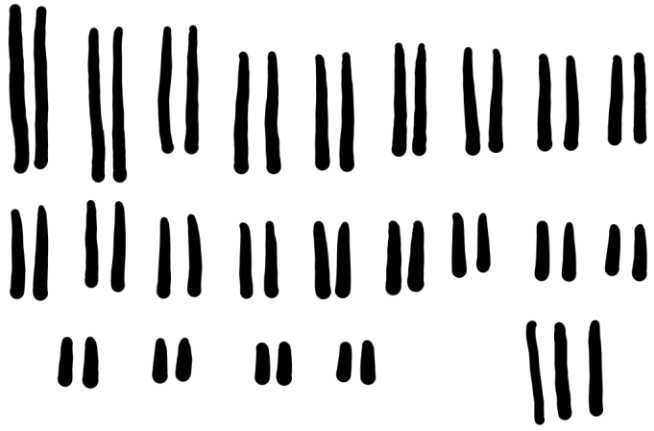
3. Determine the status of each karyotype shown and answer the following questions for each karyotype.

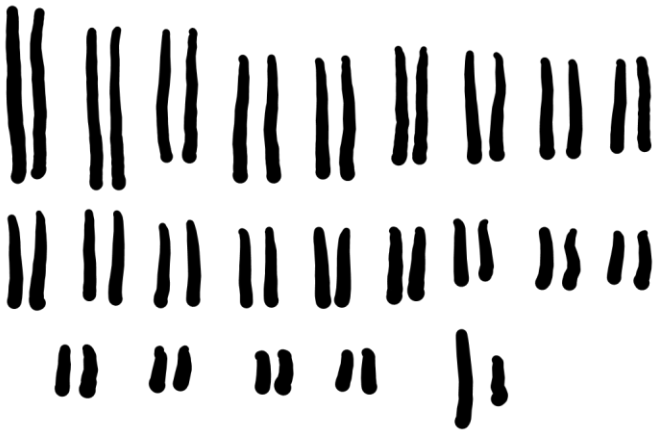
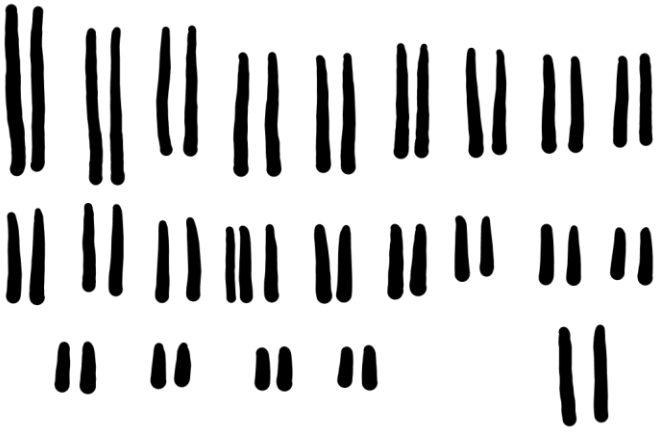
    a. Is the fetal karyotype normal or does is the karyotype abnormal?

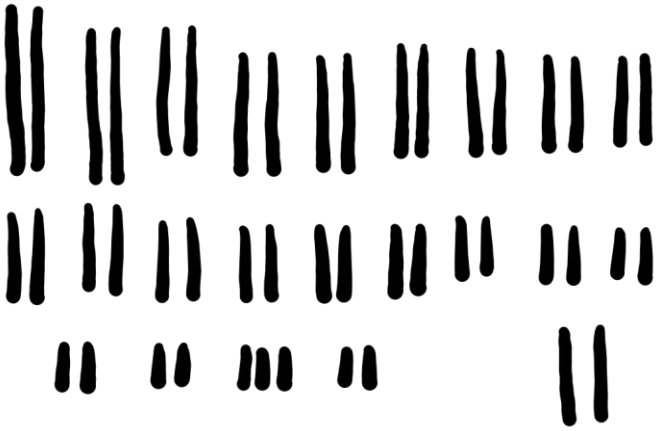
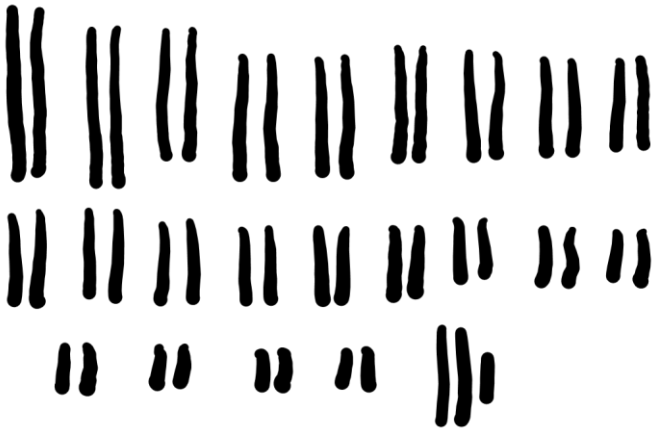
    b. If the karyotype is abnormal, circle the chromosome, monosomy or trisomy on the karyotype. Identify the syndrome name

    c. . Identify the assigned biological sex of the fetus. Male or female.

Karyotype	Answers
	
	

THIS POSTLAB IS DUE AT THE NEXT LAB MEETING.

LAB EXERCISE:

Chemistry, Solubility, Diffusion, Osmosis

Summary

Chemistry is the foundation of physiology. We need to have a good understanding of key chemical concepts to understand how the human body functions, how and why we run lab tests, and what the tests results mean. We will learn about three main chemical concepts: determining solute concentration, tonicity, and solubility.

Our bodies function best in ideal conditions: ideal pH, concentration, temperature, etc. In today's lab we will test for glucose concentration (moles/L; M) which is regulated in the body. We will use a glucose standard of known concentration to determine the concentration of an unknown sample using Beer's Law Beer's law ($C_s/A_s = C_x/A_x$).

We will also test for albumin (protein) concentration (moles/L; M), which is regulated in the body. We will create an albumin standard curve (absorbance versus albumin concentration) and fit a trendline to determine the albumin concentration of an unknown sample.

The cells in our bodies are affected by the solutions that surround them. Cells have semipermeable membranes that selectively allow certain items to cross the membrane. In general diffusion is when a solute moves passively down its concentration gradient, whereas osmosis is a very specific type of diffusion. Osmosis requires a water gradient and a semipermeable membrane; however, diffusion only requires a gradient. Water will move passively down the water gradient through a semipermeable membrane. The tonicity of a solution describes how a solution affects the final shape of the cell. The solution is said to be hypotonic if the net water movement is into the cell and the cell swells. The solution is said to be isotonic if the net water movement is zero and the cell does not change shape. The solution is said to be hypertonic if the net water movement is out of the cell and the cell will crenate. In today's lab you will determine the tonicity of intravenous (IV) solution when they are added to blood.

Solubility (property of a molecule that determines if it will dissolve into a solvent) determines various properties of chemicals in the body. For example, the solubility of a hormone can help us determine how it is carried around the body, where it will likely bind to a cell, how the cell will likely respond to the signal, and how long the hormone will remain in the body. Insoluble molecules will not dissolve in the solvent. Soluble molecules will dissolve in the solvent. We will also determine if molecules are hydrophobic (water fearing), hydrophilic (water loving), lipophobic (lipid fearing), or

lipophilic (lipid loving). Lastly, we will determine how amphipathic molecules (molecules that contain both a polar and nonpolar region) allow us to process and handle hydrophobic materials in our body. Our digestive system uses bile to help us mechanically break down fat and in today's lab we will use dish soap.

Goals

Physiology students will learn about solute concentrations, how we measure solute concentrations, diffusion, osmosis, tonicity, and solubility. Physiology students will work safely and learn to use a spectrophotometer, microliter pipet, and graduated cylinder. Physiology students will properly handle all waste materials, equipment, and chemicals.

- Use Beer's Law to calculate the concentration of an unknown solution.
- Create a standard curve, add a trendline (best fit curve), provide an equation (y=mx+b) for the trendline, and use the equation to calculate the concentration of an unknown solution.
- Understand the concepts of gradients, diffusion, osmosis, and tonicity (hypertonic, isotonic, hypotonic solution).
- Understand the concepts of solubility and amphipathic molecules.
- Learn how to use a spectrophotometer properly and will be able to demonstrate this skill to their instructor.
- Learn how to use a microliter pipet properly and will be able to demonstrate this skill to their instructor.
- Learn how to use a graduated cylinder properly and will be able to demonstrate this skill to their instructor.
- Prepare for lab, work safely and properly handle all waste materials, equipment, and chemicals.

Graphing & Calculations

- Graphs must include the following:
    - Drawn neatly and are easily read. Plan before you draw.
    - Title
    - Axis labels
    - Axis units
    - Independent and dependent variables are found on the correct axis
    - Key if appropriate (colors add another variable)
    - Scatter plot: draw a best fit line

    - Line graph: connect the points with a straight line
    - Bar graph: bar should be separate
- Calculations
    - show all work
    - box your answers
    - include units

Lab Exercises:

A. Beer's Law ($C_s/A_s = C_x/A_x$): Calculating the glucose concentration of an unknown solution (groups of 4)

Equipment

- test tubes
- cuvettes
- glucose reagent
- glucose standard
- glucose unknown
- wax pen
- DI water
- graduated cylinder
- microliter pipet
- test tube rack
- 37 ºC water bath
- parafilm

Protocol:

1. Turn on your spectrophotometer to allow it to warm up.
2. Obtain 3 test tubes and label them with a wax pen
    a. B for blank
    b. U for unknown glucose solution
    c. S for standard
3. Add 5mL of glucose reagent to each tube using a graduated cylinder.
4. Add 50µL of DI water to the BLANK test tube using a microliter pipet. Mix well.
5. Add 50µL of glucose standard to the STANDARD test tube. Mix well. Look at the bottle and record the glucose concentration.
6. Add 50µl of the unknown glucose solution to the UNKNOWN test tube. Mix well.
7. Incubate each test tube for 20min in the 37 ºC water bath . Label 3 cuvettes (B, U, S) as you are waiting.
8. After the incubation period, pour the solutions into their proper cuvette.

9. Set the wavelength of the spectrophotometer to 500nm.
10. Blank the spectrophotometer. Don't forget to mix the solution before you measure the absorbance.
11. Measure the absorbance of the standard and unknown solutions. Don't forget to mix the solutions before measuring the absorbance.
12. Using Beer's Law, calculate the concentration of the unknown glucose solution.
13. Clean-up:
    Dispose of all liquids in the chemical waste container near a sink. Place the test tubes in the test tube collection bucket. Wash the cuvettes (do not scratch) and place them upside down in the test tube rack to dry (wash with soap and water, then rinse with DI water).

B. Standard Curve: Determining the albumin (protein) concentration of an unknown solution (groups of 4)

Equipment

- test tubes
- cuvettes
- Biuret reagent
- albumin standards
- albumin unknown
- wax pen
- DI water
- graduated cylinder
- microliter pipet
- test tube rack
- water bath
- parafilm

Protocol

1. Turn on your spectrophotometer to allow it to warm up.
2. Obtain 7 test tubes and label them with a wax pen
3. B for blank
    a. U for unknown albumin solution
    b. 2 for 2g/dl albumin standard
    c. 4 for 4g/dl albumin standard
    d. 6 for 6g/dl albumin standard
    e. 8 for 8g/dl albumin standard
    f. 10 for 10g/dl albumin standard

2. Add 5mL of Biuret reagent to each tube using a graduated cylinder.
3. Add 50µL of DI water to the BLANK test tube using a microliter pipet. Mix well.
4. Add 50µL of the appropriate standard to the STANDARD test tubes. Mix well. Always look at the bottle & test tube to double check that the correct albumin concentration is being placed into the correct test tube. Always mix the bottle before pipetting any solution.
5. Add 50µl of the unknown albumin solution to the UNKNOWN test tube. Mix well.
6. Incubate each test tube for 10min at room temperature. Label 7 cuvettes (B, U, 2, 4, 6, 8, 10) as you are waiting.
7. After the incubation period, pour the solutions into their proper cuvette.
8. Set the wavelength of the spectrophotometer to 550nm.
9. Blank the spectrophotometer. Don't forget to mix the solution before you measure the absorbance.
10. Measure the absorbance of the standards and unknown solutions. Don't forget to mix the solutions before measuring the absorbance.
11. Create a standard curve and determine the albumin concentration of the unknown.
12. Practice using Beer's Law to check your standard curve results. Calculate the concentration of the unknown albumin solution using all the different standards (2, 4, 6, 8, 10) and then take the average to determine the calculated albumin concentration of the unknown solution.
13. Clean-up:
    Dispose of all liquids in the chemical waste container near a sink. Place the test tubes in the test tube collection bucket. Wash the cuvettes (do not scratch) and place them upside down in the test tube rack to dry (wash with soap and water, then rinse with DI water).

C. Diffusion, Osmosis, and Tonicity (group of 8)

Equipment

- Dialysis tubing
- 10% sucrose solution
- 30% sucrose solution
- Balance
- 500mL beaker
- Dialysis tube clips

Protocol

1. Blot the 10% sucrose dialysis bag and use the balance to determine the INITIAL WEIGHT of the dialysis bag.
2. Blot the 30% sucrose dialysis bag and use the balance to determine the INITIAL WEIGHT of the dialysis bag.
3. Label the 500mL beakers using a wax pen.
    a. 10 for 10% sucrose
    b. 30 for 30% sucrose
4. Add 250mL of tap water to each beaker.
5. Add the dialysis bags to their appropriate beaker and allow them to sit for 15min. Use a timer.
6. Remove the dialysis bag after the timer alerts you.
7. Blot the dialysis bags and use the balance to determine the weight of the dialysis bag.
8. Repeat steps 5, 6, & 7 three more times (total of 60min).
9. Draw a line graph to show your data.
10. Clean-up:
    Wash and dry the beakers. Dispose of the sucrose solutions in the sink. Wash and dry the dialysis clips and return them to your table. Dispose of the dialysis bag in the regular trash. Clean up any sucrose solution spills/drips (look like shiny drops on your desk).

D. Solubility (group of 4)

Equipment

- graduated cylinder
- serological pipet
- test tube
- hexane **caution**
- DI water
- detergent
- parafilm
- fume hood
- vegetable oil
- potassium permanganate ($KMnO_4$)

Protocol:

1. Obtain a test tube.
2. Add 2mL of water using a graduated cylinder. Draw your result.
3. Add 2mL of hexane using a serological pipet under a fume hood. Mix well. Draw your result and label each layer.
4. Add 1 crystal of potassium permanganate ($KMnO_4$). Mix well. Draw your result and label each layer.

5. Add 1mL of vegetable oil. Mix well. Draw your result and label each layer.
6. Add 1mL of detergent. Mix well. Draw your result and label each layer.
7. In detail explain what you saw at each step. Refer to the terms that you have learned (i.e. solubility, nonpolar, polar, amphipathic, micelles, etc.).
8. Clean-up: Dispose of all liquids in the Chemical Waste Container near a sink. Place the test tubes in the collection bucket.

Name: _____ **Score:** _____

Bio249 Lab Section: _____

Chemistry Pre-Lab Assignment:

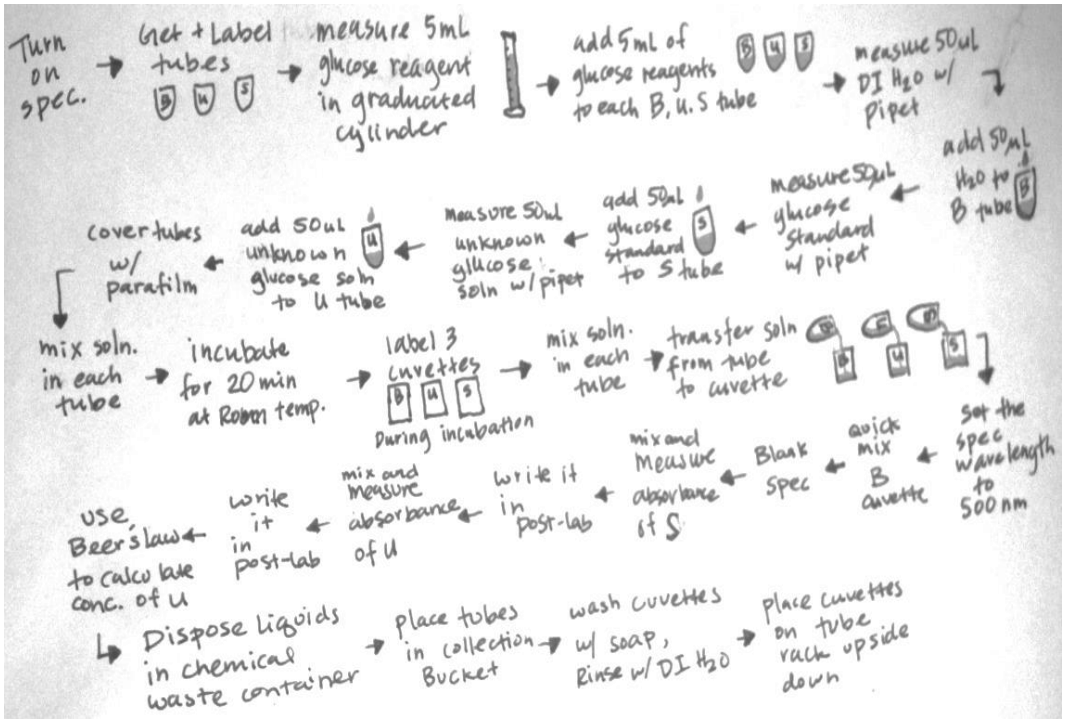
- -Read the protocol before attending lab and plan accordingly by making a flow-chart of each protocol.
- -Understand the definitions before coming to lab.
- -Understand waste management before coming to lab.
- -Bring a scientific calculator to lab.

What is a flow chart? A flow chart is a visual representation of the protocol to be followed in lab. An example is provided below for the first experiment. Notice that you can use drawings showing how you would set up each step and all critical information is shown.

Draw a flow chart for the following three protocols to prepare for each lab exercise. Add each step, volume of each solution, time intervals, and waste management. You may include drawings. Use as much space as needed.

EXAMPLE

Protocol A: Calculating the glucose concentration of an unknown



Protocol B: Calculating the albumin concentration of an unknown

Protocol C: Diffusion, osmosis and tonicity.

Protocol D: Solubility.

THIS PRELAB IS DUE AT THE BEGINNING OF THE LAB MEETING.

Name: _____ **Score:** _____

Bio249 Lab Section: _____

Chemistry Post-Lab Assignment:

A. Beer's Law: Calculating the glucose concentration of an unknown solution

- The concentration of the standard glucose solution is _____.
- Glucose reagent results:
  - Negative (glucose is absent) results will turn _____.
  - Positive (glucose is present) results will turn _____.
- The wavelength was set at _____.
- Absorbance results.
  - The absorbance of the standard glucose solution is _____.
  - The absorbance of the unknown glucose solution is _____.
- Determine the glucose concentration of the unknown glucose solution using Beer's Law. Show your work and box your final answer. Don't forget the units.

B. Standard Curve: Determining the albumin (protein) concentration of an unknown solution

- Biuret reagent results:
  - Negative (albumin is absent) results will turn the color_____.
  - Positive (albumin is present) results will turn the color_____.
- The wavelength was set at _____.
- Absorbance results.
  - The absorbance of the unknown albumin solution is _____.
  - The absorbance of the 2g/dl albumin solution is _____.
  - The absorbance of the 4g/dl albumin solution is _____.

- The absorbance of the 6g/dl albumin solution is _____.
- The absorbance of the 8g/dl albumin solution is _____.
- The absorbance of the 10g/dl albumin solution is _____.

- Create an Albumin Standard Curve using MS Excel. Label your graph properly. Print and attach your MS Excel graph to the postlab.

- Determine the albumin concentration of the unknown albumin solution using your MS Excel Standard Curve:
    - Visually: _____
        - Show your reasoning by drawing two arrows on your graph above (absorbance to best fit curve and then best fit curve to concentration).
    - Using the equation: _____
        - Show your work below:

- Determine the albumin concentration of the unknown albumin solution using Beer's Law. Show your work and box your final answer. Don't forget the units.

    - Use data for the unknown solution and the 2g/dl albumin standard solution.

    - Use data for the unknown solution and the 4g/dl albumin standard solution.

    - Use data for the unknown solution and the 6g/dl albumin standard solution.

    - Use data for the unknown solution and the 8g/dl albumin standard solution.

- Use data for the unknown solution and the 10g/dl albumin standard solution.

- Calculate the average concentration of the unknown albumin solution.

- You tried three different techniques of determining the concentration of your unknown albumin sample (visually, using the equation of the best fit curve, and using the average results from your Beer's Law calculations).
  - Which technique do you think was the best and why?

  - Which technique do you think was the worst and why?

C. Diffusion, Osmosis, and Tonicity

- Complete the table below for each dialysis bag. Enter the weight at each time interval.

Time (min)	Weight of 10% Sucrose Solution (g)	Weight of 30% Sucrose Solution (g)
0 (initial)		
15		
30		
45		
60		

- Complete the table below for each dialysis bag. Determine the percent solution left inside each dialysis bag by using this equation:

Weight (%) = (final weight /initial weight) * 100%

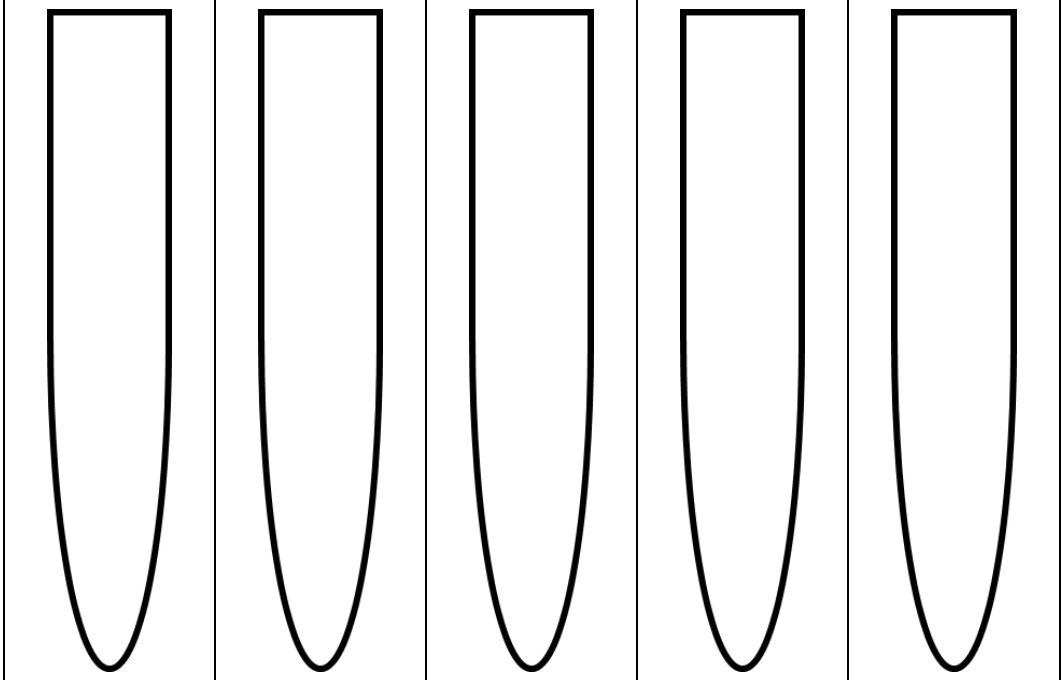
Use this table to help you create the proper graph below.

Time (min)	Weight of 10% Sucrose Solution (%)	Weight of 30% Sucrose Solution (%)
0 (initial)		
15		
30		
45		
60		

- Create a line graph using MS Excel showing the rate of weight change (%) over time (min). Label your graph properly. Print and attach your MS Excel graph to the postlab.
    - Was there an influx or efflux of water in the 10% sucrose bag? _____.
    - Was there an influx or efflux of water in the 30% sucrose bag? _____.
    - Was the 10% sucrose bag placed in a hypertonic, isotonic, or hypotonic solution? _____.
    - Was the 30% sucrose bag placed in a hypertonic, isotonic, or hypotonic solution? _____.
    - Which bag had a larger change in volume? _____.
    - Which bag had a smaller change in volume? _____.
    - Which bag showed a higher osmotic rate? _____. Explain your answer.

D. Solubility

- Draw and label your results.

#1	#2	#3	#4	#5
				

Explain your observations (focus on the why). Don't forget to explain how detergents function.

DON'T FORGET TO ATTACH THE MS EXCEL GRAPHS TO THIS POSTLAB. THIS POSTLAB IS DUE AT THE BEGINNING OF THE LAB MEETING.

LAB EXERCISE:

Digestion & Enzymes

Summary

Our GI tract is a long anatomical tube where secretions of chemicals and enzymes help us safely break down foods that we ingest. The four biological macromolecules that you ingest are: carbohydrates, proteins, fats, and nucleic acids. We use the GI tract to break down polysaccharides, proteins, fats, and nucleic acids into small units to prepare for absorption of these building blocks (nutrients). We need to have a good understanding of enzymes to understand how mechanically and chemically we digest biological macromolecules. We will learn about three main enzymatic concepts: how the presence of enzymes allows us to digest foods at a faster rate, how optimal environmental factors (specifically pH and temperature) affect the rate of enzymatic digestion, how increased surface area increases chemical digestion, and how amphipathic molecules help us digest fats.

Our bodies function best in ideal conditions: ideal pH, concentration, temperature, etc. In today's lab we will test for factors that affect carbohydrate digestion, protein digestion, and fat digestion. We will also run tests to learn how surface area affects the rate of digestion. We will use chemical indicators to test for the presence of polymers (before chemical digestion) and monomers (after chemical digestion).

Carbohydrate digestion mainly occurs in the mouth and the small intestine. The enzymes responsible for breaking down polysaccharides (complex carbohydrates) into monosaccharides (simple carbohydrates) are salivary amylase and pancreatic amylase. In today's lab you will determine how effective your salivary amylase is at breaking down starch (a polysaccharide); complete digestion, partial digestion, or no digestion. You will use two reagents to determine this. Iodine reagent will be used to determine the presence of polysaccharides; a black/dark blue color indicates a positive result and amber color indicates a negative result. Benedict's reagent will be used to determine the presence of monosaccharides; a red/green/orange/yellow color indicates a positive result and a blue color indicates a negative result.

Protein digestion mainly occurs in the small intestine. Amino acids are bound together using peptide bonds to form protein (polypeptide). The enzymes responsible for breaking down protein are called proteases (for example pepsin, trypsin, and chymotrypsin), which are secreted in the stomach and small intestine. In today's lab you will determine how temperature and pH affect the enzymatic rate of pepsin (a stomach protease). You will also determine how the presence of pepsin affects the rate of protein

(albumin) digestion. You will use Biuret reagent to determine the relative number to peptide bonds in the solution; a dark purple color indicates the presence of more peptide bonds and a light purple color indicates the presence of less peptide bonds.

Fat digestion mainly occurs in the mouth and in the small intestine. The enzymes responsible for breaking down fats into glycerol and triglycerides are called lipases (for example salivary lipase and pancreatic lipase). The amphipathic chemical that mechanically breaks down fats in order to increase the surface area for lipase to act is called bile. The liver makes bile salts and stores them in the gall bladder. The gall bladder then secretes bile into the small intestine to aid in increasing the rate of fat digestion. Today you will determine how quickly lipase acts with and without the presence of bile. You will use soap (an amphipathic molecule) to determine how amphipathic molecules like bile emulsify fats (heavy cream) to increase surface area. In the next experiment you will determine how pancreatic lipase breaks down fats (heavy cream). You will then use pancreatic lipase and bile to determine how the rate of fat digestion is influenced by both bile and pancreatic lipase. You will use litmus (a pH indicator) to determine how the fats are chemically digested; alkaline solutions are blue, neutral solutions are lavender, and acidic solutions are pink.

Goals

Physiology students will learn about chemical digestion, mechanical digestion and various factors that affect their enzymatic rates. Physiology students will work safely and learn to use graduate cylinders, serological pipet, transfer pipets, chemical indicators, and human saliva. Physiology students will properly handle all waste materials, equipment, and chemicals.

- Understand the role of mechanical digestion and how this affects the rate of chemical digestion.
- Understand the basics of enzyme kinematics by using the digestive system as a model. In other words, understand the role that environmental factors have on the rate of enzymatic digestion.
- Understand the role of amylase in carbohydrate digestion.
- Understand how optimal temperature and pH affects the rate of protein digestion by pepsin.
- Understand how bile affects the rate of fat digestion by lipase.
- Determine the positive and negative results of all chemical indicators.
- Learn how to use a transfer pipet properly and will be able to demonstrate this skill to their instructor.

- Learn how to use a serological pipet properly and will be able to demonstrate this skill to their instructor.
- Learn how to use a graduated cylinder properly and will be able to demonstrate this skill to their instructor.
- Prepare for lab, work safely and properly handle all waste materials, equipment, and chemicals.

Graphing & Calculations

- Graphs must include the following:
    - Drawn neatly and are easily read. Plan before you draw.
    - Title
    - Axis labels
    - Axis units
    - Independent and dependent variables are found on the correct axis
    - Key if appropriate (colors add another variable)
    - Scatter plot: draw a best fit line
    - Line graph: connect the points with a straight line
    - Bar graph: bar should be separate
- Calculations
    - show all work
    - box your answers
    - include units

Lab Exercises:

BEFORE YOU BEGIN WASH ALL YOUR GLASSWARE AND SEROLOGICAL PIPETS TO PREVENT CONTAMINATION.

A. Carbohydrate Digestion in the Mouth

Equipment

- test tubes
- wax pen
- graduated cylinder
- serological pipet
- transfer pipet
- test tube rack
- parafilm
- beaker

- hot plate
- test tube holder
- human saliva
- DI water
- 37 ºC water bath
- starch solution
- Iodine reagent
- Benedict's reagent

Protocol

1. Turn on the water bath and set it to 37 ºC.
2. Begin to boil a beaker of water on top of the hot plate. DO NOT LEAVE THE HOT PLATE ALONE WHEN HOT. DO NOT TOUCH HOT BEAKERS.
3. Obtain 4 test tubes and label them with a wax pen to determine if they will hold water (W) or saliva (S):
    a. W1 (negative control)
    b. W2 (negative control)
    c. S1
    d. S2
4. Add 1mL of DI water into the test tube labeled W1. Then add 1mL of DI water into the test tube labeled W2.
5. Choose one volunteer and make sure they rinse their mouth before they participate. DO NOT USE A GRADUATED CYLINDER AND DO NOT SHARE TEST TUBES. Add 1mL of human saliva to the test tubes labeled S1 and S2 by spitting into the test tubes until the tube contains about 1mL of saliva.
6. Add 2mL of starch solution to each test tube (W1, W2, S1, S2). Stretch parafilm over the opening. Mix well.
7. Incubate all the test tubes in the 37 ºC water bath for 30 minutes.
8. Remove all the test tubes from the water bath.
9. Add 5 drops of iodine indicator into the test tubes labeled W1 and S1. Stretch parafilm over the opening. Mix well. Determine the Iodine reagent results.
10. Add 2mL of Benedict's Reagent into the test tubes labeled W2 and S2. Stretch parafilm over the opening. Mix well.
11. Remove the parafilm and attach a test tube holder to each test tube.
12. Use the test tube holder to place the test tubes labeled W2 and S2 into the boiling water bath (do not let the test tube touch the bottom of the water bath). Incubate them for 5 minutes or until you see a color change. Determine the Benedict's reagent results.

13. Determine if salivary amylase digested starch. How effect was saliva as digesting starch: was it complete or incomplete digestion?
14. Clean-up:
    Dispose of parafilm in regular trash. Dispose of all liquids in the chemical waste container near a sink. Place the test tubes in the test tube collection bucket. Rinse and wash all serological pipets and graduated cylinders. Turn off and unplug your hot plate.

B. Protein Digestion in the Stomach

Equipment

- test tubes
- wax pen
- graduated cylinder
- serological pipet
- transfer pipet
- test tube rack
- parafilm
- albumin solution
- DI water
- Pepsin solution
- Biuret reagent
- 10N HCl
- 10N NaOH
- Ice bath
- 37 ºC water bath

Protocol

1. Obtain 5 test tubes and label them with a wax pen:
    a. #1
    b. #2 (positive control)
    c. #3
    d. #4 (negative control)
    e. #5
2. In test tube #1: add 2mL of albumin solution, 3mL pepsin solution, and 1 drop of DI water. Stretch parafilm over the opening. Mix well. Place test tube #1 in the 37 ºC water bath for 60 minutes making sure to mix the test tube every 10 minutes.

3. In test tube #2: add 2mL of albumin solution, 3mL pepsin solution, and 1 drop of 10N HCl. Stretch parafilm over the opening. Mix well. Place test tube #2 in the 37 ºC water bath for 60 minutes making sure to mix the test tube every 10 minutes.
4. In test tube #3: add 2mL of albumin solution, 3mL pepsin solution, and 1 drop of 10N NaOH. Stretch parafilm over the opening. Mix well. Place test tube #3 in the 37 ºC water bath for 60 minutes making sure to mix the test tube every 10 minutes.
5. In test tube #4: add 2mL of albumin solution, 5mL of DI water, 1 drop of 10N HCl. Stretch parafilm over the opening. Mix well. Place test tube #4 in the 37 ºC water bath for 60 minutes making sure to mix the test tube every 10 minutes.
6. In test tube #5: add 2mL of albumin solution, 3mL pepsin solution, and 1 drop of 10N HCl. Stretch parafilm over the opening. Mix well. Place test tube #5 in the ice bath for 60 minutes making sure to mix the test tube every 10 minutes.
7. Remove all the test tubes from the 37 ºC water bath and the ice bath.
8. Add 10 drops of Biuret reagent into each test. Stretch parafilm over the opening. Mix well.
9. Determine the Biuret reagent results Compare each test tube to the positive control and negative control. Think about the ideal environment found in the stomach. Determine how pepsin affects protein digestion. Determine how pH affects protein digestion. Determine how temperature affects protein digestion.
10. Clean-up:
    Dispose of parafilm in regular trash. Dispose of all liquids in the chemical waste container near a sink. Place the test tubes in the test tube collection bucket. Rinse and wash all serological pipets and graduated cylinders.

C. Fat Emulsification & Digestion in the Small Intestine

Equipment

- test tubes
- wax pen
- graduated cylinder
- serological pipet
- transfer pipet
- test tube rack
- parafilm
- heavy cream

- DI water
- Pancreatic lipase solution
- Litmus indicator
- Bile
- soap
- 37 ºC water bath
- Ice bath
- Glass rod
- 2% HCl
- 2% NaOH

Protocol #1l: Fat Emulsion (mechanical digestion) by the molecule bile

1. Obtain 2 test tubes and label them with a wax pen to indicate if soap (S) or bile (B) will be used as an emulsifier:
    a. S
    b. B
2. Add 20 drops of oil and 20 drops of DI water into the test tube labeled S. Stretch parafilm over the opening. Mix well. Record your results.
3. Add 2 drops of soap to the test tube labeled S. Stretch parafilm over the opening. Mix well. Record your results.
4. Add 20 drops of oil and 20 drops of DI water into the test tube labeled B. Stretch parafilm over the opening. Mix well. Record your results.
5. Add 2 drops of bile to the test tube labeled B. Stretch parafilm over the opening. Mix well. Record your results.
6. Compare your results in test tube S and test tube B. Explain how amphipathic molecules function to mechanically break down fats to increase surface area.
7. Clean-up: Dispose of parafilm in regular trash. Dispose of all liquids in the sink while running water. Place the test tubes in the test tube collection bucket. Rinse and wash all serological pipets and graduated cylinders.

Protocol #2: Mechanical and Chemical Fat Digestion in the Small Intestine

1. Obtain 4 test tubes and label them with a wax pen:
    a. #1
    b. #2 (positive control)
    c. #3
    d. #4 (negative control)

CAUTION for steps 2-5: To mix the following test tube ingredients you may need a glass rod. Make sure to wash and dry the rod between uses. Do not use a serological pipet to mix the tubes.

2. Add 1.5mL of Heavy Cream, 2.5mL DI water, 0.5mL bile, and 1.5mL litmus into the test tube labeled #1. Stretch parafilm over the opening. Mix well.
3. Add 1.5mL of Heavy Cream, 2.5mL pancreatic lipase, 0.5mL bile, and 1.5mL litmus into the test tube labeled #2. Stretch parafilm over the opening. Mix well.
4. Add 1.5mL of Heavy Cream, 2.5mL pancreatic lipase, 0.5mL DI water, and 1.5mL litmus into the test tube labeled #3. Stretch parafilm over the opening. Mix well.
5. Add 1.5mL of Heavy Cream, 3mL DI water and 1.5mL litmus into the test tube labeled #4. Stretch parafilm over the opening. Mix well.
6. Show your test tubes to your instructor. They should all have a neutral pH (pH 7) and should show the same lavender color. You instructor may adjust the pH of your solutions to get the correct pH in each test tube by adding 2% HCl or 2% NaOH as needed.
7. Incubate all the test tubes in the 37 ºC water bath. Observe how the pH of each test tube changes every 3 minutes for 15 minutes by looking at the color change.
8. Remove the test tubes from the 37 ºC water bath after the incubation period.
9. Compare your test tube results to the positive control and negative control. Explain how bile affects the rate of chemical digestion by pancreatic lipase. Explain the role of bile and pancreatic lipase in fat digestion. Explain why the pH changes when fats are digested.
10. Clean-up:
    Dispose of parafilm in regular trash. Dispose of all liquids in the chemical waste container. Place the test tubes in the test tube collection bucket. Rinse and wash all serological pipets, graduated cylinders, and glass rods.

Name: _____ **Score:** _____

Bio249 Lab Section: _____

Digestion Pre-Lab Assignment:

- -Read the protocol before attending lab and plan accordingly.
- -Understand the definitions before coming to lab.
- -Understand waste management before coming to lab.
- -Bring a scientific calculator to lab.
- -Draw a flow chart of the protocol to prepare for lab. Be prepared to use your flow chart in lab. A flow chart is a visual representation of the protocol to prepare for each lab exercise. Add each step, volume of each solution, time intervals, and waste management. You may include drawings. Use as much space as is needed. Include drawings.

A. Carbohydrate Digestion in the Mouth

B. Protein Digestion in the Stomach

C. Fat Emulsification (mechanical digestion) by the molecule bile.

Mechanical and Chemical Fat Digestion in the Small Intestine

THIS PRELAB IS DUE AT THE BEGINNING OF THE LAB MEETING.

Name: _____ **Score:** _____

Bio249 Lab Section: _____

Digestion Post-Lab Assignment:

A. Carbohydrate Digestion in the Mouth

- The polysaccharide that we are using is called _____ and salivary amylase will break it down into _____ (a monosaccharide).
- Is this an example of chemical or mechanical digestion? _____
- Iodine reagent results:
  - Negative (starch is absent) results will be the color _____.
  - Positive (starch is present) results will be the color _____.
- Benedict's reagent results:
  - Negative (glucose is absent) results will be the color _____.
  - Positive (glucose is present) results will be the color _____.
- Circle your answers: If complete digestion occurs the results will show a [positive / negative] iodine result and a [positive / negative] benedicts result. This will indicate the [presence / absence] of starch and the [presence / absence] of glucose.
- Circle your answers: If incomplete digestion occurs the results will show a [positive / negative] iodine result and a [positive / negative] benedicts result. This will indicate the [presence / absence] of starch and the [presence / absence] of glucose.
- Circle your answers: If no digestion occurs the results will show a [positive / negative] iodine result and a [positive / negative] benedicts result. This will indicate the [presence / absence] of starch and the [presence / absence] of glucose.
- Complete the data table below:

Tube	Contents	Reagent	Results	Is starch present?	Is glucose present?	Did digestion occur?
W1	Water	Iodine				
S1	Saliva	Iodine				
W2	Water	Benedict's				
S2	Saliva	Benedict's				

- Which tubes are your negative controls? Explain why they are considered negative controls?

- What type of carbohydrate digestion (mechanical or chemical) occurred when salivary amylase was used? Explain your results thoroughly.

B. Protein Digestion in the Stomach

- The protein that we are using is called _____ and pepsin will break it down into _____ (a monomer).
- Is this an example of chemical or mechanical digestion? _____
- Biuret reagent results:
  - Circle your answers and fill in the blank. Negative results will be the color _____ indicating [many / few] peptide bonds and thus [a lot of / a little] protein digestion.
  - Circle your answers and fill in the blank. Positive results will be the color _____ indicating [many / few] peptide bonds and thus [a lot of / a little] protein digestion.
- Circle your answers: The stomach has an [acidic / basic / neutral] pH therefore the optimal pH of pepsin is [acidic / basic / neutral] pH. Pepsin will have the highest enzymatic activity in an [acidic / basic / neutral] pH and anything varying of that pH will denature pepsin.
- Circle your answers: The stomach's temperature is [very hot / ~ 37 ºC / very cold] therefore the optimal pH of pepsin is [very hot / ~ 37 degrees C very cold]. Pepsin will have the highest enzymatic activity in [very hot / ~ 37 degrees C / very cold] and anything varying from that temperature will denature pepsin.

- Complete the data table below:

Tube	Albumin added?	Pepsin added?	Water added?	HCl added?	NaOH added?	Temperature (°C)	Results	Did protein digestion occur?
1								
2								
3								
4								
5								

- Which tube is your negative control? Explain why it is considered the negative control?

- Which tube is your positive control? Explain why it is considered the positive control?

- Explain how optimal pH and optimal temperature affect optimal enzymatic rates. Draw figures if needed.

- Did protein digestion occur in Tube 1? Explain why or why not.

- Did protein digestion occur in Tube 2? Explain why or why not.

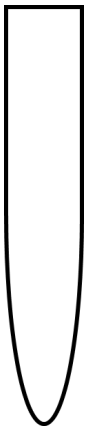
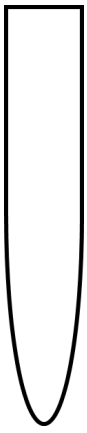
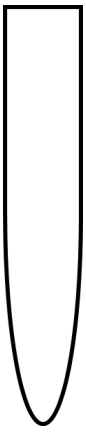
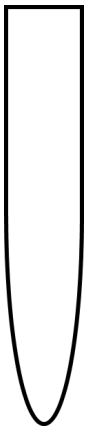
- Did protein digestion occur in Tube 3? Explain why or why not.

- Did protein digestion occur in Tube 4? Explain why or why not.

- Did protein digestion occur in Tube 5? Explain why or why not.

C. Fat Emulsification & Digestion in the Small Intestine
Fat Emulsion (mechanical digestion) by the molecule bile.

- Draw and label your results in the data table below:

Tube S before soap.	Tube S after soap.	Tube B before bile.	Tube B after bile.
			

- Do oil and water mix? Explain why or why not.

- What is an amphipathic molecule?

- What is fat emulsification?

- How does soap affect the layers seen in tube S? Explain.

- How does bile affect the layers seen in tube B? Explain.

- Explain how micelles help emulsify fats (mechanical digestion) to increase the surface area of fat.

Mechanical and Chemical Fat Digestion in the Small Intestine

- Litmus indicator results:
  - Circle your answers and fill in the blank. Results will be the color _____ indicating acidic pH and thus [a lot / a little] fat chemical digestion occurred.
  - Circle your answers and fill in the blank. Results will be the color _____ indicating neutral pH and thus no fat chemical digestion occurred.
  - Fill in the blank. Results will be the color _____ indicating basic pH.

- Circle your answer: Bile will [mechanically / chemically] digest fat.
- Circle your answer: Pancreatic lipase will [mechanical / chemically] digest fat.
- Circle your answer: The fastest rate of digestion will occur in the presence of [both bile and pancreatic lipase / bile only / pancreatic lipase only].
- Circle your answers: The small intestine's temperature is [very hot / ~37 ºC / very cold] therefore the optimal temperature of pancreatic lipase is [very hot / ~37 ºC / very cold]. Pancreatic lipase will have the highest enzymatic activity in [very hot / ~37 ºC / very cold] and anything varying from that temperature will denature pancreatic lipase.

- Test tube set up:

Tube	Heavy cream present?	Pancreatic lipase present?	Water present?	Bile present?	Litmus present?
1					
2					
3					
4					

- Results:

Tube	0 min	3 min	6min	9 min	12 min	15 min	pH of solution	Did fat digestion occur?
1								
2								
3								
4								

- Which tube is your negative control? Explain why it is considered the negative control?

- Which tube is your positive control? Explain why it is considered the positive control?

- Did fat chemical digestion occur in Tube 1? Did fat mechanical digestion occur in Tube 1? Explain why or why not.

- Did fat chemical digestion occur in Tube 2? Did fat mechanical digestion occur in Tube 2? Explain why or why not.

- Did fat chemical digestion occur in Tube 3? Did fat mechanical digestion occur in Tube 3? Explain why or why not.

- Did fat chemical digestion occur in Tube 4? Did fat mechanical digestion occur in Tube 4? Explain why or why not.

- Is pancreatic lipase necessary for fat chemical digestion? _____.

- Is bile necessary for fat chemical digestion? _____.

- Will pancreatic lipase function without bile? _____. Explain your answer.

- Which tube had the highest rate of fat digestion? Why do you think so?

DO NOT FORGET TO ATTACH THE MS EXCEL GRAPHS TO THIS POSTLAB. THIS POSTLAB IS DUE AT THE NEXT LAB MEETING.

LAB EXERCISE:

Endocrine, Hyperglycemia, Hypoglycemia, Diabetes

Summary

The body uses two systems to communicate with the body: the endocrine system and the nervous system. The endocrine system is a communication system that utilizes signaling molecules (hormones and neurohormones) to send information from one part of the body to another. Hormones are chemical signals that are synthesized and secreted by endocrine cells/glands. Neurohormones are chemical signals that are synthesized and secreted by neurons. Hormones/neurohormones travel via diffusion or via the blood stream to their target cells. Target cells contain specific receptors for the hormone/neurohormone. Receptors may be located on the cell surface, cytoplasm, or in the nucleus. Receptors on the cell surface will use second messenger systems to cause a rapid, non-genomic effect. Receptors in the cytoplasm and nucleus will influence transcription/translation causing a slower genomic effect. If a cell does not contain the receptor that is specific for that hormone/neurohormone, then it will not respond to the hormone/neurohormone.

The classification of a hormone can help us understand how they affect the target cell. Hormones can be classified in three different classes: peptide hormones, steroids, and amino acid derived hormones. Your job today is to memorize the hormones/neurohormones of the body.

Peptide hormones are synthesized through transcription and translation and are stored by the cell in storage vesicles until the cell receives a signal to secrete the hormone. Peptide hormones (most hormones) are protein signals therefore they tend to be 1) hydrophilic and are soluble in bodily fluids, 2) their receptors are on the cell surface causing a rapid non-genomic effect, and 3) their half-life is relatively short.

Steroid hormones (corticosteroids and sex hormones) are derived from cholesterol. Steroid hormones tend to be 1) hydrophobic therefore they can easily cross membranes, 2) they require a carrier protein to travel through the blood stream, 3) their receptors are cytoplasmic or nuclear causing a slow genomic effect, and 4) their half-life is relatively long.

Amino acid derived hormones are derived from amino acids. Catecholamines (dopamine, norepinephrine, epinephrine) are derived from one amino acid, tryptophan. Melatonin and serotonin are also derived from tryptophan. Thyroid hormones (Triiodothyronine (T_3) and thyroxine (T_4)) are derived from tyrosine. Histamine is derived

from glutamic acid. Amino acid derived hormones tend to be 1) hydrophilic and soluble in bodily fluids, 2) their receptors are on the cell surface causing a rapid non-genomic effect, and 3) their half-life is relatively short.

Tropic hormones are hormones that regulate the secretion of other hormones. Tropic hormones can be classified as either releasing hormones or inhibiting hormones. For example, trophic hormones synthesized by the hypothalamus (neurons) travel through the portal system to the anterior pituitary gland (endocrine cells) and affect the secretion of various hormones. Trophic hormones are hormones that have a growth effect on the target tissue resulting in the tissue cells increasing in size and number (thyroid stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), and gastrin).

Today you will study two endocrine pathways to understand how hormones help maintain homeostasis in the body. You will study how the body regulates plasma (blood) glucose levels by using a goldfish model and by using human volunteers. We will administer human insulin to goldfish to decrease their plasma glucose levels and then determine how the goldfish react to the stimulus. We will also ask human volunteers to ingest glucose pills to increase their plasma glucose levels and then we will determine how humans react to the stimulus. You will then review how diabetes mellitus affects the body.

Plasma glucose levels are constantly regulated in the body to maintain homeostasis. If a person cannot regulate their plasma glucose levels, then they may have diabetes mellitus (Type 1 or Type 2) and will need to change their life style habits and may need to take medication. An adult should have a fasting (have not eaten/drank for 8 hours) blood glucose level of <100mg/dL and a random (after a meal) blood glucose level of <200mg/dL. A person can be diagnosed as prediabetic if their fasting blood glucose is between 100-125mg/dL. A person can be diagnosed as diabetic if their fasting blood glucose is >125mg/dL.

Hyperglycemia results when plasma glucose levels increase above the setpoint. The beta cells of the pancreas sense the increased blood glucose levels and they secrete insulin (a protein hormone). Insulin affects 1) the cells of the body by increasing the number of glucose transporters on the cell membrane thus increasing facilitated diffusion of glucose into the cell, 2) increasing the rate of glycogenesis (synthesis of glycogen) in the liver, 3) increasing the uptake of amino acids and increasing the rate of protein synthesis, 4) increasing lipogenesis (synthesis of fatty acids), 5) slowing the rate of glycogenolysis (breakdown of glycogen into glucose), and 6) slowing the rate of gluconeogenesis (forming glucose from lactic acid and amino acids). The secretion of insulin results in a decrease in plasma glucose levels.

Hypoglycemia results when plasma glucose levels decrease below the setpoint. The alpha cells of the pancreas sense the decreased blood glucose levels and they secrete glucagon (a protein hormone). Glucagon affects hepatocytes (cells of the liver) and causes an 1) increase in the rate glycogenolysis (breakdown of glycogen into glucose), 2) increase in the rate of gluconeogenesis (synthesizing glucose from lactic acid, glycerol and amino acids), 3) slowing the rate of glycogenesis (synthesis of glycogen) in the liver, and 4) slowing the rate of lipogenesis (synthesis of fatty acids). The secretion of glucagon results in an increase of plasma glucose levels.

Diabetes mellitus can result when plasma glucose levels are not regulated properly and the body cannot maintain homeostasis. Diabetes mellitus type 1 results when the beta cells of the pancreas fail to produce enough insulin therefore resulting in high plasma glucose levels. Diabetes mellitus type 2 results when the cells of the body do not respond to insulin properly (insulin resistance) therefore resulting in high plasma glucose levels.

Goals

Physiology students will learn about the endocrine system by studying how plasma glucose is regulated in the body; hypoglycemia and hyperglycemia. Students will use a goldfish model to study hypoglycemia and they will observe two student volunteers to study hyperglycemia.

- Compare hormones and neurohormones.
- Compare hydrophilic and hydrophobic hormone mechanisms.
- Compare non-genomic and genomic responses.
- Compare peptide hormones, amino acid derived hormones, and steroid hormones.
- Compare tropic hormones and trophic hormones.
- Understand the basic mechanism of tropic hormones.
- Draw the feedback mechanisms used to regulate plasma glucose levels (hyperglycemia and hypoglycemia).
- Explain the symptoms shown during hyperglycemia and hypoglycemia.
- Explain the difference between Diabetes Type 1 and Diabetes Type 2.
- Understand how to safely and appropriately use a glucose-meter to measure blood glucose levels.
- Students will review all hormones (where they are secreted, target tissue, primary actions, hormone classification, hormone half-life, and basic hormone mechanisms).

Lab Exercises:

A. Blood glucose regulation-Insulin shock (using a live Goldfish) decreases glucose levels

Equipment

- Fishnet
- An active goldfish (you may take them home after lab)
- Beaker holding freshwater.
- Beaker holding human insulin solution.
- Beaker holding 5% glucose solution
- Stopwatch (cellphone)

Protocol:

1. Use your textbook. Draw the feedback loop that explains what happens with there is low blood glucose levels.
2. Take the beaker holding freshwater to the provided fish tank.
3. Using your fish net, choose an active goldfish and place it in the beaker holding freshwater.
4. Transfer the goldfish to your table and begin timing.
5. For every minute (<5min), observe how the goldfish behaves and determine the metabolic rate by counting the number of opercula beats per minute (beats/min).
6. Place your goldfish into the beaker holding human insulin solution and begin timing.
7. For every minute (<5min), observe how the goldfish behaves and determine the metabolic rate by counting the number of opercula beats per minute (beats/min) every minute.
    a. BE CAREFUL WHEN YOU SEE LETHARGIC BEHAVIOR: Your goldfish is expected to show a decrease in its metabolic rate. However, your goldfish may go into a comatose state if you are not paying close attention and the goldfish may die if left in human insulin solution for too long. If your goldfish becomes lethargic, turns to its side, and begins to float, then IMMEDIATELY put it into the 5% glucose solution.
    b. FOOD-SEEKING BEHAVIOR: Your goldfish may increase its metabolic rate and begin to actively look for food. However, this behavior does not persist for very long and the goldfish soon decreases its metabolic rate and becomes lethargic.

8. Place your goldfish into the beaker holding 5% glucose solution and begin timing.
9. For every minute (<5min), observe how the goldfish behaves and determine the metabolic rate by counting the number of opercula beats per minute (beats/min) every minute.
10. CLEAN-UP:
    Place your goldfish back in the fish tank. You may take your fish home (ask your instructor for details). Place all the materials back where you found them. Wipe up any water on the tables.

B. Blood glucose regulation-Consuming glucose tablets increases glucose levels

Equipment (2 students volunteers per course)

- Two student volunteers who are non-diabetic and willing to run the glucose tests on themselves
- 20g of glucose (form of glucose tablets)
- 200mL bottled water
- Glucose-meter and test-strips
- Lancets
- Alcohol swabs
- Band-Aids
- Stopwatch (cellphone)

Protocol

Use your textbook. Draw the feedback loop that explains what happens with there is high blood glucose levels.

1. Turn on the glucose-meter.
2. Place the empty test-strip into the glucose-meter.
3. Clean your finger with an alcohol swab and wait to dry.
4. Lance your finger using the lancet.
5. Place a drop of blood into the test-strip.
6. Clean the site and put on a Band-Aid.
7. Allow the glucose-meter to read the initial blood glucose level.
8. Clean the glucose-meter with an alcohol swab.
9. The student should now consume 20 grams of glucose and 200mL of bottled water. Begin timing.
10. Determine their blood glucose levels after 15, 30, 45, and 60 minutes by repeating steps 1-8 at each time interval. Take a reading at 120 minutes if time allows.
11. Share your results with the class in real-time.
12. CLEAN-UP:

The lancets and test-strips should go in the Sharps Biohazard waste container. The alcohol swabs with blood, tissue paper with blood, and Band-Aids with blood should go in the Soft Biohazard waste containers. Water cups should go in the regular trash.

C. Study Time: Endocrine System Overview- Hormone Regulation, Stimulus, Sensor, Controller, Secretion, Target(s), Action(s), Feedback Response, Hormone Types, and Endocrine Case Studies

Equipment

- Textbooks (hormones chapter)
- Online resources

Protocol:

1. Use your textbook/resources to learn all the hormones from lecture. Use this time to study. You may get questions about hormones in the lab practical.

Name: _____ **Score:** _____

Bio249 Lab Section: _____

Endocrine Pre-Lab Assignment:

- -Read the protocol before attending lab and plan accordingly.
- -Understand the definitions before coming to lab.
- -Understand waste management before coming to lab.
- -Draw a flow chart of the protocol to prepare for lab. Be prepared to use your flow chart in lab. A flow chart is a visual representation of the protocol to prepare for each lab exercise. Add each step, volume of each solution, time intervals, and waste management. You may include drawings. Use as much space as is needed. Include drawings.
    A. Blood glucose regulation-Insulin shock (using a live Goldfish) decreases glucose levels.

    B. Blood glucose regulation-Consuming glucose tablets increases glucose levels.

- Complete the feedback mechanisms used when plasma glucose levels are low.

Stimulus	
Sensory	
Controller	
Efferent Pathway	
Effector(s)	
Response(s)	

- Draw the feedback mechanism used when plasma glucose levels are high.

Stimulus	
Sensory	
Controller	
Efferent Pathway	
Effector(s)	
Response(s)	

THIS PRELAB IS DUE AT THE BEGINNING OF THE LAB MEETING.

Name: _____ **Score:** _____

Bio249 Lab Section: _____

Endocrine Post Lab Assignment:

A. Blood glucose regulation-Insulin shock (using a live Goldfish) decreases glucose levels

- Refer to the glucose-meter pamphlet. Determine the normal fasting blood glucose levels for humans:
  _____mg glucose/dL to _____mg glucose/dL

- Complete the data tables below.

TREATMENT: FRESHWATER

Time (min)	Metabolic Rate (operculum beats/min)	Behavior Description
1		
2		
3		
4		
5		

TREATMENT: HUMAN INSULIN SOLUTION

Time (min)	Metabolic Rate (operculum beats/min)	Behavior Description
1		
2		
3		
4		
5		

TREATMENT: 5% GLUCOSE SOLUTION

Time (min)	Metabolic Rate (operculum beats/min)	Behavior Description
1		
2		
3		
4		
5		

- Did your fish behave as expected after being placed in the insulin solution? _____ Would you expect insulin or glucagon to be secreted when the goldfish was placed in insulin? _____
- Did your fish behave as expected after being placed in the 5% glucose solution? _____ Would you expect insulin or glucagon to be secreted when the goldfish was placed in glucose? _____
- Use your textbook/resources to identify some symptoms of hypoglycemia in humans.

- Use your textbook/resources to identify some reasons that a human may experience hypoglycemia.

B. Blood glucose regulation-Consuming glucose tablets increases glucose levels

- Determine the normal fasting blood glucose levels for humans: _____mg glucose/dL to _____mg glucose/dL
- Complete these data tables.

STUDENT #1 NAME:

Time (min)	Blood Glucose (mg/dL)	Feelings/Behavior changes?
1		
2		
3		
4		
5		

STUDENT #2 NAME:

Time (min)	Blood Glucose (mg/dL)	Feelings/Behavior changes?
1		
2		
3		
4		
5		

- Use MS Excel to draw an appropriate graph of the blood glucose level data for both students. Label properly and add a key. Attach the graph to this post-lab.
- Does Student #1 data accurately represent the feedback loop mechanism when there is an increase in blood glucose levels?

_____

- Does Student #2 data accurately represent the feedback loop mechanism when there is an increase in blood glucose levels? _____
- Was this experiment an example of hyperglycemia or hypoglycemia? _____ Would you expect insulin or glucagon to be secreted after ingesting a meal? _____
- Use your textbook/tools to identify some symptoms of hyperglycemia in humans.

- Use your textbook to identify some reasons that a human may experience hyperglycemia.

C. Study time. Endocrine System Overview- Hormone Regulation, Feedback Response, and Hormone Types.

- Review your Lecture Endocrine notes and readings.
  - Identify the following:
    - The hormone type (peptide, steroid, or amine)
    - Stimulus
    - Specific gland/endocrine cell/neuron that secretes the hormone
    - Target(s)
    - Action(s)
  - **Do not need to turn anything in for Study Time.**

DO NOT FORGET TO ATTACH THE MS EXCEL GRAPHS TO THIS POSTLAB. THIS POSTLAB IS DUE AT THE NEXT LAB MEETING

LAB EXERCISE:

Skeletal Muscle, Electromyography, Stretch Reflexes

Summary

We use our skeletal muscles to move our bodies, maintain posture, stabilize joints, and generate heat. We usually control skeletal muscle consciously; however, stretch reflexes can control our skeletal muscle subconsciously. We must understand how skeletal muscles respond to stimuli in order to understand how our nervous system and our skeletal muscle function as a unit. We must also understand how skeletal reflexes help us prevent bodily injury. Today you will explore how your skeletal muscles function by electrically stimulating your wrist flexors and by testing your skeletal reflexes.

Let's review our skeletal muscle anatomy. Skeletal muscle is highly organized and follows the following morphology: 1) Skeletal muscle is striated and is composed of muscle fibers (cells), 2) These muscle fibers contain myofibrils, 3) muscle fibers are grouped together to form muscle fascicles, 4) muscle fascicles are grouped together for form muscle bundles, 5) the functional unit is the sarcomere, and 6) myosin (thick) and actin (thin) filaments are highly organized which results in striations. The neuromuscular junction is the site where a motor neuron communicates with a skeletal muscle fiber.

Let's review our skeletal muscle physiology. The central nervous system (CNS) and skeletal muscle communicate to cause skeletal muscles to contract. Motor neurons receive an action potential from the CNS. When the action potential (AP) reaches the synaptic bulb, voltage gated calcium channels open and calcium ions diffuse into the synaptic bulb to trigger exocytosis of the neurotransmitter acetylcholine (ACh). ACh diffuses across the synaptic cleft and binds to the ACh-gated channels on the motor end plate (MEP) of the sarcolemma (plasma membrane of the muscle fiber). The ACh-gated channels open allowing sodium ions and potassium ions to diffuse across the MEP. A rush of sodium ions crosses into the muscle fiber and depolarizes the membrane resulting in an end-plate potential (EPP). If the EPP reaches threshold, then the action potential travels across the sarcolemma and into the transverse tubules (t-tubules). The voltage-sensing receptors (L-type calcium channels called dihydropyridine receptor, DHP) on the t-tubule change conformation and open the calcium ion release channels (ryanodine receptors, RyR) that are on the sarcoplasmic reticulum (SR; the smooth ER of the muscle cell). Calcium ions diffuse into the cytosol of the muscle fiber and bind to troponin. Troponin changes conformation and moves tropomyosin away from the myosin-binding site on actin. Myosin and actin can now interact to form cross-bridges. Cross-bridge cycling will continue until ACh is removed

from the synaptic cleft by acetylcholine esterase (AChE) or via diffusion. Once ACh is removed, ACh-gated channels on the MEP close resulting in a loss of the AP on the muscle fiber. The SR then closes its calcium ion channels and the calcium ion pumps return the calcium into the SR.

Force production is dictated by many factors and processes. Motor units are composed of somatic motor neuron(s) and all the muscle fibers they innervate. Motor units range from small to large. The smaller motor units have a lower threshold while the larger motor units have a higher threshold. You can recruit different motor units to produce the force that you require. A muscle fiber twitch is affected by the length of the muscle. Muscles at optimal length produce optimal force because there is an optimal amount of cross bridges being formed. Muscles that are shortened produce less than optimal force because the myosin filaments run into the Z disks inhibiting them from sliding and produce less tension. Muscles that are too long produce less than optimal force because there is not enough overlap for optimal cross bridging resulting in less sliding and less force production. Single twitches, summation, unfused tetanus (incomplete tetanus) and fused tetanus (complete tetanus) are all different ways to produce tension. A twitch is when a single action potential (voltage) causes contraction and full relaxation of the muscle. The tension a twitch creates can vary depending on the voltage of the action potential. The duration of a twitch can vary depending on the duration of the action potential. Summation is when the frequency of the action potential is shortened, and full relaxation cannot be reached. Therefore, each subsequent action potential creates a larger tension. If summation reaches maximal tension, then tetanus has been reached. Unfused tetanus is when there is partial relaxation at maximal tension (shaky muscle contraction) while fused tetanus is when there is no relaxation at maximal tension (smooth muscle contraction). Today you will experiment with a stimulation machine (Stim machine) to study twitches, summation and tetanus in one of your partners.

Let's review stretch reflexes. Stretch reflexes involve: stimulus (stretching the muscle), stretch receptors (muscle spindles), the CNS, somatic motor neurons (alpha motor neurons), and skeletal muscle fibers (extrafusal muscle fibers). Muscle spindles are found throughout skeletal muscle. They consist of small muscle fibers called intrafusal fibers wrapped in sensory nerve endings. The purpose of muscle spindles is to constantly monitor muscle length. At rest, muscle spindles communicate tonically to the CNS and results in a resting level muscle contraction (i.e. muscle tone). When a muscle is stretched rapidly the embedded muscle spindles are also stretched rapidly. Muscle spindles will then fire at a higher frequency and it will result in a stronger muscle contraction (i.e. muscle jerk). Stretch reflexes are used by the body to prevent damage from over stretching skeletal muscles. A good example of a stretch reflex is the patellar tendon reflex (knee jerk). When you tap the patellar tendon, you rapidly stretch the

muscle causing the quadriceps and therefore the muscle spindles within the quadriceps to stretch. The muscle spindles sense the stretching of the muscle and fire more rapidly. The sensory neuron sends action potentials to the spinal cord and results in two responses: 1) the sensory neuron synapses with somatic motor neuron within the ventral horn of the spinal cord causing the somatic motor neuron to send action potentials to the quadriceps resulting in contraction and 2) the sensory neuron synapses with an interneuron in the grey matter of the spinal cord which results in inhibiting the somatic motor neuron of the hamstrings causing them to relax. When the quadriceps contract and the hamstrings relax, the knee will extend (knee jerk). Today you will study your own stretch reflexes.

Goals

- Review the anatomy and physiology of skeletal muscle.
- Understand the concepts of: a twitch, summation, unfused tetanus (incomplete tetanus), and fused tetanus (complete tetanus).
- Understand the patellar tendon reflex (our model stretch reflex feedback loop).
- Understand the normal and abnormal results of stretch reflex tests.
- Understand the relationship between stimulus voltage and tension produced.
- Understand the relationship between stimulus frequency and tension produced.
- Understand the relationship between stimulus duration and tension produced.
- Understand some of the consequences of nerve injuries on stretch reflexes.

Lab Exercises:

- **DO NOT PARTICIPATE IN THE STIMULATOR EXPERIMENTS IF YOU HAVE A PACEMAKER OR OTHER HEALTHRELATED ELECTORNIC DEVICE.**
- **REMOVE ALL YOUR JEWELRY DOUBLECHECK ALL OF YOUR SETTINGS BEFORE YOU BEGIN.**

A. Stretch Reflexes

Equipment

- Textbook/resources
- Reflex hammer (quickly and firmly strike with either the blunt or proper end)

- Relaxed student (have them close their eyes and count down from 100 for all jerk reflexes)

Protocol

1. Draw a representative stretch reflex (feedback loop). Look up the knee jerk in your textbook/resource and draw the feedback loop. Label the stimulus, sensor, afferent pathway, controller, efferent pathways, and responses.
2. Jaw Jerk
    a. Tell the volunteer to relax their jaw and open slightly.
    b. Place your pointer and middle finger in front of the mandible and place your ring finger below the chin to support the jaw.
    c. Firmly strike your pointer finger in a downward motion using the wide end of the reflex hammer.
    d. Normal response: Elevation of the mandible.
3. Biceps Jerk
    a. Face the volunteer and cradle their right arm by placing their right elbow in your right palm and placing their right hand on top of your forearm. Their elbow should be slightly bent.
    b. Jiggle their arm to make sure they are relaxed.
    c. Use your fingers to firmly press in the cubital fossa to look for the tendon.
    d. Firmly strike the tendon using the pointed end of the reflex hammer.
    e. Repeat steps 1-4 with the left arm.
    f. Normal response: The biceps should twitch, and the elbow should flex.
4. Triceps Jerk
    a. Stand next to your patient and cradle their elbow. Their humerus should almost be parallel to the floor.
    b. Jiggle their arm to make sure they are relaxed.
    c. Use your fingers to firmly press to look for the triceps tendon.
    d. Firmly strike the tendon using the wide end of the reflex hammer.
    e. Repeat steps 1-4 with the left arm.
    f. Normal response: The triceps should twitch, and the elbow should extend.
5. Knee Jerk

a. Ask the volunteer to sit on the table. Make sure their feet dangle from the side of the table.
b. Use your fingers to firmly press under the right patella and look for the patellar ligament.
c. Firmly strike the ligament using the wide end of the reflex hammer.
d. Repeat steps 1-3 for the left side.
e. Normal response: extension of the knee.
f. Repeat steps 1-3, however this time compare the response when using a soft versus a firm strike.

6. Ankle (Achilles) Jerk
    a. Ask the volunteer to sit on the table. Make sure their feet dangle from the side of the table.
    b. Support the right heel of the foot by cupping it in your hand.
    c. Locate the calcaneal (Achilles') tendon.
    d. Firmly strike the tendon using the wide end of the reflex hammer.
    e. Repeat steps 1-3 for the left side.
    f. Normal response: plantar flexion.
7. Plantar (Babinski) Reflex
    a. Ask the volunteer to sit on the table. Make sure their feet dangle from the side of the table.
    b. Using the metal handle, firmly and swiftly trace the right foot in the following pattern. Begin by tracing the lateral side of the plantar surface from the heel towards the toes and then continue medially across the ball of the foot.
    c. Repeat steps 1-2 for the left side.
    d. Normal ADULT response: plantar flexion. Recorded as a negative Babinski sign.
    e. Normal INFANT response: plantar extension (dorsiflexion) of the great toe, great toe extends upward, and lesser toes fan apart. Recorded as a positive Babinski sign. If this is seen in an adult, then they may have damage in the corticospinal tract.

B. Stimulator Experiment (Electrode Stimulation Experiment)

Equipment

- Electrodes
- Electrolyte gel
- Elastic bands

- Stimulator with variable stimulation settings (duration, voltage, and frequency)
- Alcohol swabs
- Follow directions and ask for help in necessary.

Protocol:

1. Draw a figure displaying a twitch, summation, incomplete (unfused) tetanus, and complete (fused) tetanus. Label the axes and the stimuli.
2. Locate the motor points on the forearm (finger flexor muscle group).
3. Place a droplet of electrolyte gel on each electrode.
4. Place the electrodes on the motor points and secure them with the elastic bands.
5. Initial Twitch experiment
    a. INITIAL STIMULATOR SETTINGS:
        i. Duration of the stimulus = 2msec
        ii. Voltage = 20V
        iii. Frequency = single pulse
    b. Look at the fingers and deliver a stimulus.
    c. Determine if a twitch was seen. If so, then this is your threshold setting. If not, then continue to the next step.
    d. Change the Voltage to 30V and deliver a stimulus. Determine if a twitch was seen. If so, then move on to the next experiment. If not, adjust the electrode placement and repeat this experiment until you see a twitch.
6. Threshold experiment
    a. Begin by using the settings that you determined in the Initial Twitch experiment.
    b. For each test, lower the VOLTAGE in a stepwise manner until you determine threshold.
    c. Determine the lowest voltage needed to stimulate a twitch.
7. Duration experiment
    a. Begin by using the settings that you determined in the Initial Threshold experiment.
    b. Lower the DURATION to 0.1msec.
    c. Determine how the twitch results change as duration is varied.
8. Voltage experiment

a. Begin by using the settings that you determined in the Initial Threshold experiment.
b. Increase the VOLTAGE by 5V and deliver a stimulus.
c. Repeat step 2 *however you should not surpass 30V. If your volunteer does not want to proceed to a higher voltage, then you should move on to the next experiment.*
d. Determine how the twitch results change as voltage is varied.

9. Frequency experiment
    a. Begin by using the settings that you determined in the Initial Threshold experiment.
    b. Increase the Frequency in a stepwise manner until summation occurs. Record these settings.
    c. Continue to increase the Frequency in a stepwise manner until incomplete (unfused) tetanus occurs. Record these settings.
    d. Continue to increase the Frequency in a stepwise manner until complete (fused) tetanus occurs. Record these settings.

Name: _____ **Score:** _____

Bio249 Lab Section: _____

Muscle Prelab Assignment:

-Read the protocol before attending lab and plan accordingly.
-Understand the definitions before coming to lab.
-Draw a flow chart of the protocol to prepare for Stimulator Experiment. Be prepared to use your flow chart in lab. A flow chart is a visual representation of the protocol to prepare for each lab exercise. Add each step, volume of each solution, time intervals, and waste management. You may include drawings. Use as much space as is needed. Include drawings.

A. Draw the knee jerk and label this feedback loop.

B. Flow Chart of Stimulator Experiment

THIS PRELAB IS DUE AT THE BEGINNING OF THE LAB MEETING.

Name: _____ **Score:** _____

Bio249 Lab Section: _____

Muscle Post Lab Assignment:

A. Stretch Reflex Results

Stretch Reflex	Result (normal or abnormal)
Jaw Jerk	
Biceps Jerk	
Triceps Jerk	
Knee Jerk	
Ankle Jerk	
Babinski Reflex	

- How did the knee jerk reflex results change when you varied the strike force (soft versus firm)?

B. Electrode stimulation

- Draw figures displaying a twitch, summation, incomplete (unfused) tetanus, and complete (fused) tetanus. Label the figures well.

Single Twitch	Summation
Incomplete Tetanus	Complete Tetanus

- Stimulator results:
  - Initial Twitch experiment
    - Duration of the stimulus = _____
    - Voltage = _____
    - Frequency = _____
  - Threshold experiment
    - Duration of the stimulus = _____
    - Voltage = _____
    - Frequency = _____
  - Duration experiment
    - Duration of the stimulus = _____
    - Voltage = _____
    - Frequency = _____
    - Determine how the twitch results change as duration is varied.

  - Voltage experiment
    - Duration of the stimulus = _____
    - Voltage = _____
    - Frequency = _____
    - Determine how the twitch results change as voltage is varied.

  - Frequency experiment
    - Summation results
      - Duration of the stimulus = _____
      - Voltage = _____
      - Frequency = _____
      - Describe how the contraction changes.

    - Incomplete (unfused) tetanus results
      - Duration of the stimulus = _____
      - Voltage = _____
      - Frequency = _____
      - Describe how the contraction changes.

- Complete (fused) tetanus results
  - Duration of the stimulus = _____
  - Voltage = _____
  - Frequency = _____
  - Describe how the contraction changes.

THIS POSTLAB IS DUE AT THE NEXT LAB MEETING

LAB EXERCISE:

Immunohistochemistry & Competitive Inhibition

Summary

The plasma membrane of a cell is composed of a lipid bilayer, proteins and carbohydrates. Plasma membrane proteins can be embedded across the membrane (transmembrane proteins) or anchored to the membrane on the extracellular or intracellular surface (peripheral proteins). Transmembrane proteins are often bound to oligosaccharide chains (sugar chains) on the extracellular side of the plasma membrane (glycoproteins). Many of these glycoproteins are used as means for cell communication/recognition and are referred to as cell-surface receptors. For example, red blood cells (RBC/erythrocytes) can contain the glycoproteins A and/or B or neither that make up the A, B, O system used to determine blood type. When RBCs with glycoprotein A (type A blood) are mixed with antibodies against glycoprotein A, the cells clump together (agglutinate). The agglutination (clumping) process is helped by lectins, proteins that bind to oligosaccharide chains on glycoproteins on the plasma membrane of RBCs.

Immunohistochemistry is a technique that physiologists can use to help them determine the types of carbohydrates found on the cell membrane. Glycolipids and glycoproteins have specific carbohydrate chains that help create receptors. Immunohistochemistry allows us to use lectins (a protein molecule that will bind to specific carbohydrates and tends to be isolated from plants) to determine the carbohydrate make-up of the cell surface receptors of a cell.

The lectin you will be investigating today is named Concanavalin A (Con A). Today, you will determine if cheek epithelial cells express cell surface receptors that bind Con A. By adding various free carbohydrates (monosaccharides), we can determine if these free carbohydrates compete with the receptors on the cell surface. If the free carbohydrates are competitive inhibitors, then lectin (Con A) will bind to both the free carbohydrate and the receptor and we can conclude that the cell surface receptor contains the same structure as the free carbohydrate. If the free carbohydrates are not competitive inhibitors, then the lectin will only bind to the cell surface receptor and we can conclude that the receptor does not contain the same structure as the free carbohydrate. You will use two ways to visualize the lectin (Con A) binding to the cell surface receptors: 1) colorimetric (color change) and 2) hemagglutination (seeing red blood cells clump/stick together). Your objective is to identify the type of sugar chains found on Con A cell surface receptors that allow Con A (lectin) to bind.

Goals

Physiology students will learn about the cell membrane, cell surface receptors, antibodies, lectins, antigens, competitive inhibition, and immunohistochemistry. Physiology students will work safely and learn to use a microliter pipet, a microscope and learn to follow precise protocols. Physiology students will properly handle all waste materials, equipment, and chemicals.

- Understand the structure and function of the cell membrane (Fluid Mosaic Model).
- Understand how to determine if competitive inhibition has occurred analyzing hemagglutination results and colorimetric results.
- Understand how to determine the types of carbohydrates found on an animal cell (human or sheep) by using competitive inhibition tests using lectins.
- Properly and safely handle sheep's blood and human cheek cells (dissociated simple squamous epithelium).
- Learn how to use a microliter pipet properly and will be able to demonstrate this skill to their instructor.
- Prepare for lab, work safely and properly handle all waste materials, equipment, and chemicals.

Lab exercises:

A. Antigen-Antibody interaction simulation

Equipment

- Textbook/resources
- Immunohistochemistry demo kits

Protocol:

1. Instructor will walk you through a demo for cheek cell experiment (Lab Exercise B).
2. Run the demo for the red blood cell experiment (Lab Exercise C).
3. Clean-up:
   Return all the Immunohistochemistry demo kits; make sure all pieces are inside the bag.
4. After this demo you should be able to explain the following concepts: specificity, competitive inhibition, antibody, antigen, lectin, binding site, ligand, substrates, and hemagglutination.

B. Immunohistochemistry-Cheek Cells

Equipment

- Wax pen
- Microscope
- 2 Slides
- 4 Cover slips
- Tooth picks
- Ethanol
- Microliter pipets
- Transfer pipets
- Small beakers
- Microcentrifuge tubes
- Con-A Buffer (pH 6.8)
- Con-A Peroxidase (Con-A peroxidase + Con-A buffer)
- 1M Galactose (Galactose + Con-A buffer)
- 1M Mannose (mannose + Con-A buffer)
- Peroxidase Substrate Solution (hydrogen peroxide + chloronapthol + Tris buffer)

Protocol:

1. You will place two samples on each slide.
2. Label each side of the slide either: #1, #2, #3, or #4.
3. Using a wax pen, draw a circle next to each label. This is where you will create your cheek cell smear.
4. Rinse your mouth with DI water to remove bacteria, food, mucus, etc. Take a clean toothpick and gently scrape the inside of your cheek to remove simple squamous epithelial cells.
5. Smear the cells on the slide labeled #1 and allow it to air dry.
6. Repeat step 7 for slide #2, #3, and #4.
7. Fix (makes cells permeable to macromolecules and causes them to adhere to the slide) the cheek cells onto the slide by using 1 drop of ethanol on the cheek cells and allowing the slide to air dry.
8. Repeat step 7.
9. Hold the slide at an angle and slowly rinse each slide with 1mL of Con-A Buffer. Remove excess buffer by taping the slide on its side.
10. Repeat step 9.
11. Label 4 microcentrifuge tubes: #1, #2, #3, and #4.
12. Fill each microcentrifuge tube accordingly and mix well.

Tube	Con-A Buffer (µL)	Con-A Peroxidase (µL)	1M Galactose (µL)	1M Mannose (µL)
1	3	0	0	0
2	10	25	0	0
3	0	25	10	0
4	0	25	0	10

13. Place the slides on the bench top and dispense accordingly.
    a. Dispense 15µL of solution #1 onto slide #1.
    b. Dispense 15µL of solution #2 onto slide #2.
    c. Dispense 15µL of solution #3 onto slide #3.
    d. Dispense 15µL of solution #4 onto slide #4.
14. Wait for 10 minutes and repeat step 13 and wait 10 minutes.
15. Rinse each slide with 5mL of Con-A buffer to remove any unbound Con-A peroxidase. Remove excess buffer by tapping the slide on its side. Allow to air dry on its side.
16. Place the slides on the bench top and dispense 4 drops of Peroxidase Substrate Solution onto each set of cells.
17. Wait 5 minutes and repeat step 16.
18. Gently rinse with DI water. Remove excess moisture by tapping the slide on its side. Allow to air dry.
19. Place a cover slip over slide #1, #2, #3, and #4. Look at the cells under the microscope.
20. Record your results: No color, Light purple, Dark purple.
21. Clean-Up:
    Dispose of your slides in the Biohazards Sharps container. Dispose of your toothpicks and disposable microliter pipet tips in the Biohazards Sharps container. Dispose of any chemicals in the Chemical Waste container. Dispose of any tissue/paper towel with absorbed chemicals in the Biohazards Softs container. Dispose of transfer pipets and microcentrifuge tubes in the Biohazards Softs container.

C. Immunohistochemistry-Red Blood Cells

Equipment

- Sheep blood
- Wax pen
- Microscope
- 2 Slides
- 4 Cover slips

- Ethanol
- Microliter pipets
- Transfer pipets
- Small beakers
- Microcentrifuge tubes
- Con-A Buffer (pH 6.8)
- Con-A solution (Con-A lectin and Con-A buffer)
- 1M Galactose (Galactose and Con-A buffer)
- 1M Mannose (mannose and Con-A buffer)
- Erythrocyte suspension (2mL of Con-A buffer and 2 drops of sheep blood)

Protocol

1. Your instructor will prepare the Erythrocyte Suspension (2mL Con-A buffer and 2 drops of sheep blood). An aliquoted of 0.2mL of the suspension will be given to each group. ALWAYS MIX THIS SUSPENSION BEFORE USING.
2. Label 4 microcentrifuge tubes: #1, #2, #3, and #4.
3. Fill each microcentrifuge tube accordingly and mix well.

Tube # & Solution #	Erythrocyte Suspension (µL)	Con A Buffer (µL)	Galactose (µL)	Mannose (µL)
1	15	20	0	0
2	15	10	10	0
3	15	0	10	0
4	15	0	10	10

4. Incubate at room temperature for 30 minutes and shake the microcentrifuge tubes every 5 minutes during this incubation period.
5. You will place two samples on each slide.
6. Label each side of the slide either: #1, #2, #3, or #4.
7. Using a wax pen, draw a circle next to each label. This is where you will place your solutions.
8. Dispense 10µL of the proper solution to each slide. Don't forget to mix the solution before you transfer it to the slide.
9. Place a cover slip over each solution and examine 100 RBCs and using a microscope. Record the following: hemagglutination, no hemagglutination and % of RBC contact.
10. Clean-Up:
    Dispose of your slides in the Biohazards Sharps container. Dispose of your disposable microliter pipet tips in the Biohazards Sharps container. Dispose of any chemicals in the Chemical Waste

container. Dispose of any tissue/paper towel with absorbed chemicals in the Biohazards Softs container. Dispose of transfer pipets and microcentrifuge tubes in the Biohazards Softs container.

Name: _____ **Score:** _____

Section: _____

Immunohistochemistry Pre-Lab Assignment:

-Read the protocol before attending lab and plan accordingly.
-Understand the definitions before coming to lab.
-Understand waste management before coming to lab.
-Draw a flow chart of the protocol for lab exercises B & C to prepare for each lab exercise. Add each step, volume of each solution, time intervals, and waste management. You may include drawings.

Flow Chart for Protocol B: Immunohistochemistry-Cheek Cells

Flow Chart for Protocol C: Immunohistochemistry-Red Blood Cells

THIS PRE-LAB IS DUE AT THE NEXT LAB MEETING.

Name: _____ **Score:** _____

Section: _____

Immunohistochemistry Post-Lab Assignment:

A. Immunohistochemistry-Demo using models

- Draw three examples for the cheek cell demo. Label the cell, antibody, and antigen. Determine if there is specificity and/or competitive inhibition. Determine if the cells will bind the antigen/lectin and result in the cells turning purple.

- Draw three examples for the red blood cell demo. Label the cell, antibody, and antigen. Determine if there is specificity and/or competitive inhibition. Determine if the cells will bind the antigen/lectin and result in hemagglutination.

B. Immunohistochemistry-Cheek Cells

- Draw your results for Slide #1.

  - What color were the cells? _____
  - Did competitive inhibition occur? _____

- Draw your results for Slide #2.

    - What color were the cells? _____
    - Did competitive inhibition occur? _____

- Draw your results for Slide #3.

    - What color were the cells? _____
    - Did competitive inhibition occur? _____

- Draw your results for Slide #4.

    - What color were the cells? _____
    - Did competitive inhibition occur? _____

- Explain the mechanism behind the purple color reaction.

- Which sugar inhibited Con A binding to cheek epithelial cells? _____

C. Immunohistochemistry-Red Blood Cells

- Draw your results for Slide #1.

    - Did hemagglutination occur? _____
    - Did competitive inhibition occur? _____

- Draw your results for Slide #2.

    - Did hemagglutination occur? _____
    - Did competitive inhibition occur? _____

- Draw your results for Slide #3.

    - Did hemagglutination occur? _____
    - Did competitive inhibition occur? _____

- Draw your results for Slide #4.

    - Did hemagglutination occur? _____
    - Did competitive inhibition occur? _____

- Which sugar inhibited Con A from agglutinating red blood cells? _____

THIS POST-LAB IS DUE AT THE NEXT LAB MEETING.

LAB EXERCISE:

Blood Cell Counts, Hemoglobin, Hematocrit, Blood Typing

Summary

Blood tests are laboratory analyses performed on blood samples, usually from a vein in the arm. Blood tests can provide useful information about a persons' health. Two sets of blood tests are routinely used in the hospital setting: comprehensive metabolic panel and complete blood count. Today you will test your own blood and measure hematocrit level, hemoglobin concentration, determine red blood cell count, calculate various variables, determine your blood type, and learn to use various equipment.

A comprehensive metabolic panel (CMP) is a blood chemistry profile that provides a quick check for a wide variety of problems, such as high glucose, electrolyte imbalances, and markers of kidney or liver problems.

A complete blood count (CBC) is a blood panel that provides information about the patient's blood, such as the cell count for each cell type and the concentration of various proteins and minerals. CBC tests are a valuable tool for a variety of disorders such as anemia, infection, and blood diseases.

Blood is composed of plasma (composed of mainly water, proteins, & electrolytes) and formed elements (cells). The cells that circulate in the blood include leukocytes (white blood cells), erythrocytes (red blood cells), and thrombocytes (platelets). Abnormally high or low blood cell counts may indicate a variety of diseases and is the main reason why CBCs are routinely performed. Blood cells counts can be counted manually using a hemocytometer. A hematocrit provides the fraction, or percent, of red blood cells in the blood. The amount of hemoglobin in blood (expressed in g/dL) can be measured with a hemoglobinometer; a low level of hemoglobin can be a sign of anemia. Mean corpuscular hemoglobin concentration (MCHC), is the average amount of hemoglobin in red blood cells and can be calculated using hemoglobinometer and hematocrit results. Mean corpuscular volume (MCV) is the average volume of red blood cells.

Goals

- Learn how to perform a subset of the CBC panel of tests and determine their blood type using immunohistochemistry.
- Work safely and learn to use a hemocytometer, hemoglobinometer & hematocrit, and learn to follow precise protocols.

- Learn to calculate MCHC & MCV.
- Properly handle all waste materials, equipment, and chemicals.

Calculations

***A tutorial is provided for you online.

- Red blood cell count (10^6 RBCs/µL/mm^3) (RBCs/µL/ mm^3)
- Hemoglobin concentration of blood (g/dL)
- Hematocrit (% RBCs)\Mean corpuscular hemoglobin (MCH) (µg/cell)
- Mean corpuscular hemoglobin concentration (MCHC) (%)
  =([hemoglobin] * 100%) / hematocrit
- Mean corpuscular volume (MCV) (um^3)
  =(Hematocrit * 10) / (RBC count)

Lab Exercises:

*****Students must work with their own blood only. If you have a medical condition that prevents you from participating, please tell your instructor for accommodations.**

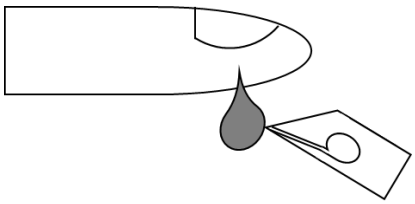
A. Hemoglobin concentration

Equipment

- Placemat
- Hemoglobinometer
- Test strips
- Lancet
- Alcohol swab
- Band Aid

Protocol:

1. Set up your placemat and materials. Read through the entire set of instructions before you begin.
2. Use an alcohol swab to clean the area that you will lance. Allow the area to dry.
3. Lance your finger and discard the first drop of blood.
4. Quickly and completely fill test strip. Capillary action will easily fill the test strip. If you take too long, then it will coagulate. See the following figure for guidance.



5. Clean the outside of the test strip with an alcohol swab making sure you do not touch the blood sample. Allow to dry.
6. Turn on the hemoglobinometer. Read the provided instructions card.
7. Insert the test strip and record your results. Wipe the machine with an alcohol swab after use. Allow to air dry.
8. CLEAN-UP:
   Test strips and lancets should go in the biohazard sharps waste. Alcohol swabs, used Band-Aids, and any other material that touched blood should go in the biohazard soft waste. Other clean waste should go in the regular trash.

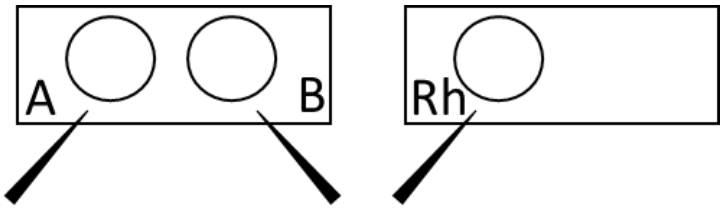
D. Blood typing

Equipment

- Placemat
- Lancet
- Alcohol swab
- Band-Aid
- 2 slides
- 4 cover slips
- ABO blood typing kit (A antibody, B antibody)
- Rh blood typing kit (D antibody)
- 3 Toothpicks
- Wax pencils

Protocol:

1. Set up your placemat and materials. Read through the entire set of instructions before you begin.
2. Use an alcohol swab to clean the area that you will lance. Allow the area to dry.
3. Draw two circles on a slide and label A and B.Draw one circle on a slide and label Rh. See the following figure for guidance.



4. Add one drop of A antibody on the circle labeled A.
5. Add one drop of B antibody on the circle labeled B.
6. Add one drop of D antibody on the circle labeled Rh.
7. Lance your finger and discard the first drop of blood. Place one drop in each circle on the slides.
8. Mix the blood and antibody using a clean toothpick. Use a clean toothpick for each circle/antibody.
9. Wait 1 minute.
10. Put a coverslip over each circle and look under the microscope.
11. Record any hemagglutination. Remember that hemagglutination is considered a positive result.
12. CLEAN-UP:
    Lancets and slides should go in the biohazard sharps waste. Alcohol swabs, used Band-Aids, and any other material that touched blood should go in the biohazard soft waste. Other clean waste should go in the regular trash.

D. Hematocrit Level

Equipment

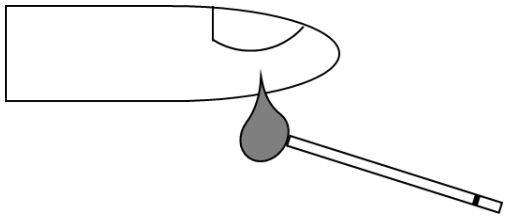
- Placemat
- Hematocrit tube (Heparinized capillary tube)
- Clay sealant
- Hematocrit centrifuge
- Hematocrit reader

Protocol:

1. Set up your placemat and materials. Read through the entire set of instructions before you begin.
2. Use an alcohol swab to clean the area that you will lance. Allow the area to dry.
3. Identify the side of the hematocrit tube that has the red ring around it. This side is the “clean” side of the hematocrit tube

and this side will go into the clay sealant. BLOOD CAN ONLY TOUCH THE SIDE WITHOUT THE RED RING.

4. Lance your finger and discard the first drop of blood.
5. Quickly and completely fill the heparinized capillary tube with fresh blood (must fill 75% of the tube). Capillary action will easily fill the tube. If you take too long, then it will coagulate.
   See the following figure for guidance.



6. Once you have filled the hematocrit tube, stick the “clean” side of the hematocrit tube in the clay sealant (do this at least two times). Blood will not run out of the hematocrit tube if sealed properly.
7. Clean the outside of the hematocrit tube with an alcohol swab making sure you do not touch the blood sample. Allow to dry.
8. Place the hematocrit tube into the hematocrit centrifuge making sure that the clay sealant is facing outward. If placed incorrectly, then the blood will flow out of the hematocrit tube. Make note of the location of your hematocrit tube.
9. Properly close the hematocrit centrifuge and spin at high speed for 5 minutes.
10. Remove the hematocrit tube and use a hematocrit reader to read the hematocrit level. Your instructor will show you how to use the hematocrit reader. Record your results.
11. CLEAN-UP:
    Hematocrit tubes and lancets should go in the biohazard sharps waste. Alcohol swabs, used Band-Aids, and any other material that touched blood should go in the biohazard soft waste. Other clean waste should go in the regular trash.

D. RBC (erythrocyte) Count

*****Student will only get to try this experiment one time.**

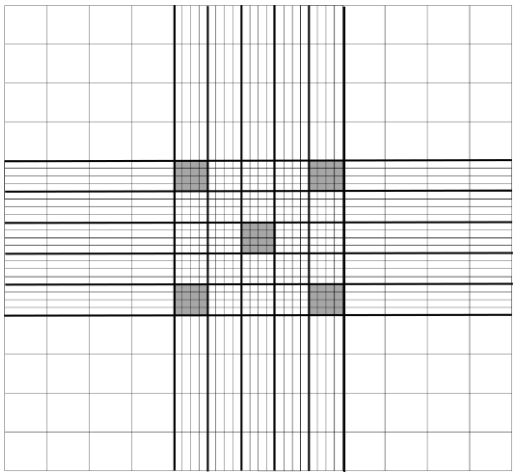
Equipment

- Placemat

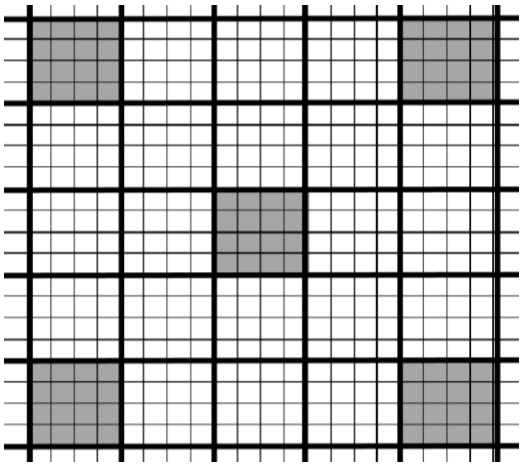
- Lancet
- Band-Aid
- Alcohol swab
- Hemocytometer
- Unopette reservoirs (small microcentrifuge tube with fluid)
- Small capillary tube for unopette reservoir
- Counter
- Micropipette

Protocol:

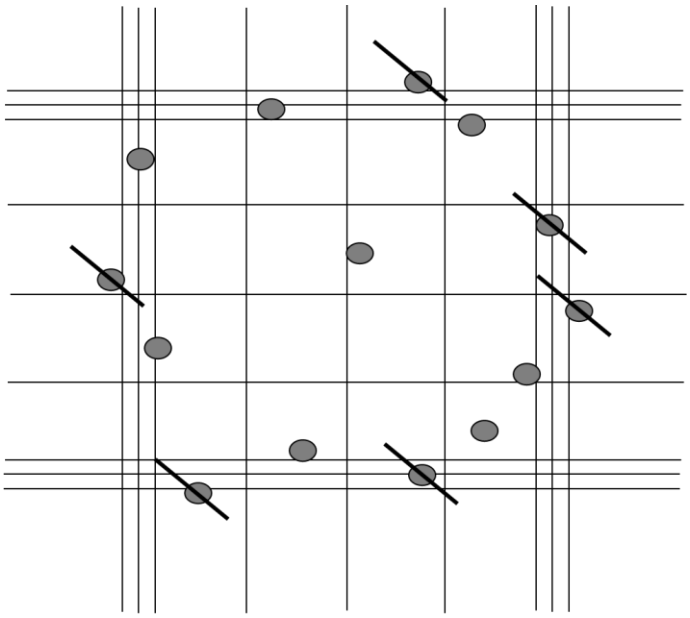
1. Set up your placemat and materials. Read through the entire set of instructions before you begin.
2. Use an alcohol swab to clean the area that you will lance. Allow the area to dry.
3. Lance your finger and discard the first drop of blood.
4. Quickly and completely fill the small capillary tube with fresh blood. Capillary action will easily fill the tube. If you take too long, then it will coagulate.
5. Immediately place the filled small capillary tube into the unopette reservoir and shake well to release the blood into solution.
6. Clean your hemocytometer with alcohol and lens paper only. Allow to air dry. Never use soap, alcohol swab, or a paper towel as it will scratch the surface of the slide.
7. Place a cover slip over the reflective surface of the hemocytometer.
8. Invert your unopette reservoir to resuspend the RBCs.
9. Use a micropipette to add 10µL of your RBC suspension into the V-shaped groove. Capillary action will move the suspension over the reflective surface.
10. Use a compound microscope to view the grid on the reflective surface of the hemocytometer. counting View the hemocytometer at the highest magnification that allows you to see an entire quadrant. Remember that the RBCs are not stained and therefore you should use high contrast by closing the iris diaphragm.
11. Using the Scanning Lens look for a cross (see the figure below) and focus on the middle of the cross. Put the pointer on the middle of the cross and then move to the Low Power Lens.



12. Look for the 5x5 grid. You will be counting the RBC in squares found at each corner and the center square. In the figure below, we have shaded the square for you. Place the pointer on one of the required squares and then move to the High Power Lens to count the RBCs.



13. Use a counter to count the RBCs in the 5 required quadrants (center, upper right, lower right, upper left, lower left) and report your results. You will count all of cells within the large quadrants on the hemocytometer. Count all of the cells within each quadrant except those on the far right edge and lower bottom edge. Cell that touch the middle line of the boundary on the right edge and lower edge will not be counted. See the figure below to learn how to count the RBCs; cells that are crossed out are not counted.



14. CLEAN-UP:

    Unopette reservoirs, disposable pipet tips, cover slips, and lancets should go in the biohazard sharps waste. Alcohol swabs, used Band-Aids, and any other material that touched blood should go in the biohazard soft waste. Other clean waste should go in the regular trash. Rinse your hemocytometer with DI water and then with ethanol. Blot dry with lens paper. Allow to air dry.

Name: _____ **Score:** _____

Section: _____

Pre-Lab Assignment:

- Read the protocol before attending lab and plan accordingly.
- Understand the definitions before coming to lab.
- Understand waste management before coming to lab.

Draw a flow chart of the protocol for lab exercises A-D to prepare for each lab exercise. Add each step, volume of each solution, time intervals, and waste management, etc. You may include drawings.

A. Hemoglobin concentration

B. Blood typing

C. Hematocrit Level

D. RBC (erythrocyte) Count

THIS PRE-LAB IS DUE AT THE NEXT LAB MEETING

Name: _____ **Score:** _____

Section: _____

Blood Post-Lab Assignment:

- **RBC (erythrocyte) Count**

    - RBC count for Square 1: _____
    - RBC count for Square 2: _____
    - RBC count for Square 3: _____
    - RBC count for Square 4: _____
    - RBC count for Square 5: _____
    - Total RBC count for Squares 1-5: _____
    - Multiply the above number by 10,000: _____
    - Normal RBC counts vary for males and females:
        - Males: 4,200, 000 – 6,900,000 µL/mm^3
        - Females: 3,900, 000 – 5,600,000 µL/mm^3
    - Are you within normal range? _____

- **Hematocrit**

    - Draw your hematocrit tube before and after it was centrifuged. Label the plasma, buffy coat if visible, and hematocrit. Label the clay sealant.

    - What is the purpose of using a hematocrit centrifuge?
    - What is your hematocrit level? _____
    - Normal hematocrit levels vary for males and females:
        - Males: 45 – 62%
        - Females: 37 - 48%
    - Are you within normal range? _____

- **Hemoglobin concentration**

  - What is your hemoglobin concentration? _____
  - Normal hemoglobin concentration varies for males and females:
    - Males: 13 – 18 g/dL
    - Females: 12 – 16 g/dL
  - Are you within normal range? _____

  - Calculate your Mean Corpuscular Hemoglobin Concentration.
    - Mean corpuscular hemoglobin concentration (MCHC) in (%)
      - ([hemoglobin] * 100%) / hematocrit

  - Normal MCHC values: 32 – 36% hemoglobin / cell
  - Are you within normal range? _____

  - Calculate your Mean Corpuscular Volume.
    - Mean corpuscular volume (MCV) in (um^3)
      - (Hematocrit * 10) / (RBC count)

  - Normal MCV values: 76 – 100 um^3
  - Are you within normal range? _____

- **Blood typing**
    - Draw your results:

    - What blood type are you? _____
    - List the blood types could you accept during a blood transfusion:

    - List the blood types could you not accept during a blood transfusion:

    - Explain why a universal donor can donate to anyone but can only accept from one blood type.

THIS POST-LAB IS DUE AT THE NEXT LAB MEETING

LAB EXERCISE:

Cardiac Cycle, Electrocardiograms, Heart Sounds, Heart Rate, Blood Pressure, and Exercise

Summary

In the clinical setting, vital signs are routinely measured to keep a record of the body's most basic functions. Pulse and blood pressure are two of the main vital signs routinely monitored. Pulse is the rate at which the heart beats (measured as beats per minute, bpm); this is also known as heart rate. Pulse measurements not only provide rhythm information, they can also provide information on the strength of the heartbeat. Every time the heart beats, it produces a sound often referred to as "lub-dub". The "lub" is the first heart sound caused by turbulence caused by the closure of the atrioventricular valves. The "dub" is the second heart sound caused by the closure of the semilunar valves. Since the bicuspid valve closes slightly before the tricuspid valve, and the aortic semilunar valve closes slightly before the pulmonary valve, each individual valve sound can be distinguished by auscultating (listening with a stethoscope) different regions of the thorax (chest). Today you will learn to measure heart rate and use a stethoscope to listen to your partner's heart sounds.

The heart can also be monitored by electrocardiogram (ECG), a machine that measures the hearts electrical activity as line tracings. Being able to read and interpret the peaks and dips in an ECG recording can allow one to determine if there is abnormal or unusual electrical activity. Blood pressure is not considered a vital sign; however it is often measured with vital signs. Today you will look at an ECG (three lead) of one of your group members and determine how exercise changes their ECG. You will also see a demo on how to use a clinical ECG machine (12 lead).

Blood pressure measures the force exerted on the walls of your arteries as your blood pumps through your body. Blood pressure provides information about your general health and can provide insight on having a higher risk for heart health problems in the future. Today you will measure the blood pressure of your group members using a sphygmomanometer.

Goals

- Learn how to check for a pulse at various pulse points & monitor heart rate.

- Learn to measure blood pressure.
- Learn to interpret ECG recordings.
- Students will learn to use a stethoscope, sphygmomanometer, & ECG, and learn to follow precise protocols.
- Students will properly handle all waste materials, equipment, and chemicals.

Graphs & Calculations

- All calculations must be shown & be legible.
- Graphs must include the following:
    - Drawn neatly and are easily read. Plan before you draw.
    - Title
    - Axis labels
    - Axis units
    - Independent and dependent variables are found on the correct axis
    - Key if appropriate (colors add another variable)
    - Scatter plot: draw a best fit line
    - Line graph: connect the points with a straight line
    - Bar graph: bar should be separate

Lab Exercises:

NOTE: For those of you that wish to participate in the ECG experiments, it is recommended that you wear comfortable exercise clothes with stretch, including undergarments (sports bra), & that skin be relatively hairless or depilated (shaved). Some of our ECG machines have more than 3 electrodes that need to be placed on other regions of the body, such as the chest region.

A. Assessment of Heart Sounds

Equipment

- Stethoscope
    - ear tips (earpieces should face forward)
    - ear tubes (binaural)

    - tubing
    - diaphragm (flat-shaped, high-pitched sounds)
    - bell (cup-shape, low-pitched sounds)

- Auscultation method chart.

Protocol

1. Listen to the heart sounds of your patient at the “4 corners”.

B. Pulse Points and Heart Rate

Equipment

- Pulse point chart

Protocol

1. Record your patient’s heart rate at various pulse points.

C. Assessment of Blood Pressure

Equipment

- Sphygmomanometer
    - inflatable cuff (contains a rubber bladder)
    - air release valve
    - inflation bulb
    - pressure gauge (mmHg)

- Stethoscope

Protocol

1. Place the inflatable cuff around the patient’s left upper arm.
2. Using alcohol swabs clean the ear pieces on the stethoscope and diaphragm. Let them air dry and place them in your ears facing forward.
3. Close the air release valve and put on the stethoscope. Gently tap the diaphragm to make sure you can hear sound.

4. Hold the pressure gauge in your left hand and the inflation bulb in your right hand. Use your left hand to position the stethoscope over the brachial artery.
5. Quickly inflate the cuff until the pressure is 200mmHg. You should hear no sound.
6. Slowly open the air release valve to steadily release the pressure in the cuff. Release at 2-3mmHg per second.
7. Listen carefully as you watch the gauge.
    a. The systolic blood pressure (~120mmHg) is measured when you hear the first Korotkoff sound.
    b. The diastolic blood pressure (~80mmHg) is measured when you stop hearing the Korotkoff sound.
8. Quickly open the air valve when you have measured the diastolic blood pressure.

D. Heart Rate, Blood Pressure, and Exercise

Equipment

- Sphygmomanometer
- Stethoscope
- Alcohol swabs
- Metronome

Protocol (Work as a table, each group will test one person)

1. Create a hypothesis.
2. Assign jobs: Must be reliable.
    a. Recorder of all measurements.
    b. HR reader will read HR on the right arm.
    c. BP reader will read BP on the left arm.
    d. “No Weekly Cardio” patient will exercise. This patient does not do any exercise on a regular basis.
    e. “Weekly Cardio” patient will exercise. This patient does 1 hour cardio workouts (running, swimming, biking, etc.) at least 2 times per week.
3. Pick patients that can safely run the exercise lab. Standardize your patients as much as possible (age, gender, weight, height, etc.).
4. Using alcohol swabs clean the ear pieces on the stethoscope and diaphragm.

5. Place the sphygmomanometer and stethoscope on your patient's left upper arm. Your patient will not remove the sphygmomanometer until the experiment is completed.
6. RESTING EXEPERIMENT: Measure the patient's resting HR and BP every minute for 5 minutes to determine baseline measurements for your patient.
7. EXERCISE EXPERIMENT: Your instructor will have a metronome playing. Your patient will need to exercise (jump) at this pace for 5 minutes. Measure their exercise HR and BP every minute for 5 minutes with minimal rest.
   *HINT: Have them quickly stop while you take readings and then quickly have them return to jumping.
8. RECOVERY EXPERIMENT: When you have completed the exercise measurements, have your patient immediately rest and take their HR and BP every minute for 10 minutes.
9. Graph and interpret your data using MS Excel. Attach your graph to your post-lab.

E. Practice Measuring Electrocardiograms

Equipment

- Practice ECG handouts
- Computer
- Normal values for Duration (and V) of different phases of an ECG complex. Exercise 3: ECG and Exercise Aim: To determine the effects of exercise on an ECG
    1. P wave 0.1sec (0.2mV)
    2. QRS complex (lead II) 0.08-0.12sec (1V)
    3. T wave 0.16-0.27sec (0.2-0.3mV)
    4. PR interval 0.13-0.16sec
    5. QT interval 0.3-0.34sec
    6. PR segment 0.03-0.06sec
    7. ST segment 0.08sec

Protocol:

1. Amplitude of waves/complexes
    - Measure the amplitude of the P wave

- Measure the amplitude of the QRS complex
- Measure the amplitude of the T wave

2. Duration of waves
    - Measure the duration of the P wave
    - Measure the duration of the QRS complex
    - Measure the duration of the T wave
3. Duration of segments
    - Measure the PR segment
    - Measure the ST segment
4. Duration of intervals
    - Measure the RR interval
    - Measure the PR interval
    - Measure the QT interval

F. Electrocardiograms and Exercise

Equipment

- Metronome
- PowerLab hardware
- LabChart Software on class laptops
- 3 Electrodes
- Electrode gel
- Alcohol swabs
- Patient-Student volunteer who will rest for 5 minutes, exercise for 5 minutes, and recover for 10 minutes.

Protocol:

1. Pick a patient who can safely run the exercise lab and have them remove all of their jewelry.
2. Using alcohol swabs, clean the area where the electrodes will be placed and allow to air dry.
3. Attach the electrodes to the wires. Do not remove the backing yet.
4. Remove the backing for each electrode and place a small amount of electrode gel to the middle of each electrode. Place them accordingly on your patient.
    - Red = right wrist
    - Black = left wrist
    - Green = left ankle

5. While your patient is at REST, click on Start and record their ECG. Make sure that the ECG waveforms look normal. If not, tell your instructor and they will help you troubleshoot.
6. Once you have a good ECG reading, click on Stop.
7. RESTING EXEPERIMENT: Measure the patient's resting ECGs every minute for 5 minutes to determine baseline measurements for your patient.
8. EXERCISE EXPERIMENT: Your instructor will have a metronome playing. Your patient will need to exercise (jump) to this pace for 5 minutes. Measure their exercise ECGs every minute for 5 minutes with minimal rest.

    WARNING:

    a. Make sure your patient is aware of the wires and does not trip.
    b. Make sure that the ECG waveforms look normal and readable. If not, you will need to re-do this experiment after they have completely rested for 30 minutes.

10. RECOVERY EXPERIMENT: When you have completed the exercise measurements, have your patient immediately rest and take their ECG readings every minute for 10 minutes or until they have fully recovered.
11. Data Analysis: Click on the Marker and Cursor Icon and practice moving the vertical cursors (black lines on the software) to measure change in time and change in amplitude for the various waveforms.
12. Clean-Up: Throw away used electrodes.

G. Cardiac Arrhythmias

Equipment

Practice Cardiac Arrhythmias ECG Handout

Protocol: Use your knowledge of ECGs to determine what is wrong with each patient's ECG reading. Explain your answers.

Name: _____ **Score:** _____

Section: _____

Cardiovascular Pre-Lab Assignment:

- Read the protocol before attending lab and plan accordingly.
- Understand the definitions before coming to lab.
- Understand waste management before coming to lab.

Draw a flow chart of the protocol for lab exercises C, D & F to prepare for each experimental lab exercise. Add each step, time intervals, and waste management, etc. You may include drawings. Lastly draw a figures of a normal ECG recording and label it.

C. Assessment of Blood Pressure

D. Heart Rate, Blood Pressure, and Exercise (add your hypotheses)

F. Electrocardiograms and Exercise (add your hypotheses)

Draw and label a normal ECG recording. Label the waves, segments and intervals.

THIS PRE-LAB IS DUE AT THE NEXT LAB MEETING.

Name: _____ **Score:** _____

Section: _____

Cardiovascular Post-Lab Assignment:

A. Assessment of Heart Sounds

Did you hear the individual valve closing?

- Aortic semilunar valve: _____
  Name the location: _____
- Pulmonary semilunar valve: _____
  Name the location: _____
- Bicuspid valve: _____
  Name the location: _____
- Tricuspid valve: _____
  Name the location: _____
- Define “heart murmur”.

What would you hear if your patient had a heart murmur?

Did you patient have a heart murmur? _____

B. Pulse Points and Heart Rate

Did you feel the pulse at various pulse points? If so, what heart rate did you measure?

- Temporal: _____
- Common carotid: _____
- Brachial: _____
- Radial: _____
- Popliteal: _____
- Dorsalis pedis: _____

What happened to the pulse rate (heart rate) as you moved away from the heart? Why didn't the pulse rate change?

What happened to the pulse pressure as you moved away from the heart? Why did the pulse pressure change?

C. Assessment of Blood Pressure

- Your BP: _____
- When using the brachial artery, do you have a normal BP? _____

Calculate Pulse Pressure (systolic – diastolic)

What would happened to BP as you moved further away from the heart?

D. Heart Rate, Blood Pressure, and Exercise

Design a question(s) for this experiment:

Design a hypothesis(ses) (testable and falsifiable) for this experiment.

Identify the control(s) for this experiment.

Identify the experimental variables for this experiment.

Complete the table for Rest, Exercise and Recovery.

	Time (min)	HR (BPM)	Systolic BP (mmHg)	Diastolic BP (mmHg)	Pulse Pressure (mmHg)
Rest	1				
	2				
	3				
	4				
	5				
Exercise	6				
	7				
	8				
	9				
	10				
Recovery	11				
	12				
	13				
	14				
	15				
	16				
	17				
	18				
	19				
	20				

Graph your data using MS Excel to compare both patients. You should have one graph per variable (HR, Systolic BP, Diastolic BP, and Pulse Pressure). Write a conclusions paragraph for each table. Remember that the conclusions differ from the results. The results literally state what the graph shows, and the conclusions explain why you see these patterns.

Does your data support or reject your hypotheses? Explain why:

E. Practice Measuring Electrocardiograms

Attach the handout your received in lab.

F. Electrocardiograms and Exercise

Design a question(s) for this experiment:

Design a hypothesis(ses) (testable and falsifiable) for this experiment.

Identify the control(s) for this experiment.

Complete the table for Rest, Exercise and Recovery.

	Time (min)	R-R Interval (sec)	HR (BPM)	P-wave Amplitude (mV)	QRS complex Amplitude (mV)
Rest	1				
	2				
	3				
	4				
	5				
Exercise	6				
	7				
	8				
	9				
	10				
Recovery	11				
	12				
	13				
	14				
	15				
	16				
	17				
	18				
	19				
	20				

Graph your data using MS Excel to compare both patients. You should have one graph per variable (HR, Systolic BP, Diastolic BP, and Pulse Pressure). Write a conclusions paragraph for each table. Remember that the conclusions differ from the results. The results literally state what the graph shows, and the conclusions explain why you see these patterns.

Does your data support or reject your hypotheses? Explain why:

G. Cardiac Arrhythmias

Attach the handout your received in lab.

DO NOT FORGET TO ATTACH THE MS EXCEL GRAPHS TO THIS POST-LAB. THIS POST-LAB IS DUE AT THE NEXT LAB MEETING

LAB EXERCISE:

Ventilation, Spirometry, Volumes & Capacities, Pulmonary Function, pH Regulation

Renal Experiment Assignments: Your instructor will randomly assign you an experimental variable for your Renal Group Project. Write it down in your Post-Lab.

Summary

Spirometry is a commonly used test in the clinical setting that measures lung function, specifically it measures the amount of air (volume) and/or speed of air (air flow) that can be inhaled or exhaled (pulmonary ventilation). Spirometry can be useful in identifying conditions that affect the lungs such as asthma, pulmonary fibrosis, cystic fibrosis and chronic obstructive pulmonary disease (COPD). Spirometry is often used after surgery to facilitate recovery and prevent respiratory complications. After surgery, people often feel weak and taking deep breaths can be painful, however, if they don't take deep breaths they may develop post-operative pulmonary complications, including pneumonia, bronchitis, and/or hypoxemia (low oxygen levels in the blood). Breathing plays a major role in acid-base balance in the body. Changes in pulmonary ventilation (breathing rate, depth of respiration) can help adjust pH when disturbances in pH occur.

Goals

- Learn how to check for lung sounds.
- Learn how to measure (or calculate) lung volumes.
- Learn how to titrate solutions and check for changes in pH.
- Will work safely and learn to use a stethoscope & spirometer and learn to follow precise protocols.
- Will properly handle all waste materials, equipment, and chemicals.

Graphing

- Graphs must include the following:

- Drawn neatly and are easily read. Plan before you draw.
- Title
- Axis labels
- Axis units
- Independent and dependent variables are found on the correct axis
- Key if appropriate (colors add another variable)
- Scatter plot: draw a best fit line
- Line graph: connect the points with a straight line
- Bar graph: bar should be separate

Lab Exercises:

A. Respiratory Rate

Equipment

- Stop watch (cell phone)

Protocol:

1. Record the respiratory rate of a patient in breaths per minute. Repeat 3 times and take the average.
2. Average respiratory rate is 12-20 breaths per minute. Is your patient normal?

B. Auscultation and Lung Sounds

Equipment

- Stethoscope
- Alcohol swab
- Reference sheet

Protocol:

1. Use a cleaned stethoscope to listen to the anterior surface of the superior lobes, middle lobe, and inferior lobes of your patient.
2. Listen to the posterior surface of the superior lobes, middle lobe, and inferior lobes of your patient.
3. Listen to the lateral surface of the superior lobes, middle lobe, and inferior lobes of your patient.
4. Are the sounds you here normal? Do you hear any wheezing, crackling, or other unusual sounds?

C. Pulmonary Volumes, and Capacities using a Spirometer

Equipment

- Spirometer
- Disposable mouthpiece

Protocol:

1. SAFETY NOTES FOR ALL SPIROMETER TESTS:
    a. Assign a coach. The coach will cheer you on as these tests are difficult to perform. Don't allow the patient to remove their lips from the mouth piece (they must seal their lips around the mouth piece) as small leaks will measure a lower volume. This spirometer only measures how much air is exhaled.
    b. Before each trial, hold your nose and breathe normally for 1 minute. You should only mouth-breathe for these experiments.
    c. Before each experiment, set the dial to 1000mL. This is your baseline, therefore 1000mL is equal to 0mL exhaled. For example, if after exhalation the needle is at 2500mL, then you exhaled 1500mL of air.
2. TIDAL VOLUME: Inhale normally and then exhale normally into the spirometer. Repeat 3 times and take the average.
3. EXPIRATORY RESERVE VOLUME: Inhale normally, exhale normally, and then take a deep exhalation until you cannot exhale any longer into the spirometer. Repeat 3 times and take the average.
4. VITAL CAPACITY: Take a deep inhalation and then take a deep exhalation until you cannot exhale any longer into the spirometer. Repeat 3 times and take the average.
5. ESTIMATE RV: RV=VC * RV factor
    a. RV factors based on age:
        i. 16-34 years old = 0.250
        ii. 35-49 years old = 0.305
        iii. 50-69 years old = 0.445
6. CALCULATE ALL OTHER VOLUMES AND CAPACITIES: IRV, IC, RV, FRC, TLC.
7. Using measured and calculated volumes, label and fill in lung volume chart.

8. EFFECTS OF EXERCISE: Jump up and down for 3 minutes and then immediately measure your TV, ERV, and VC. You do not need to repeat these measurements. Calculate your IRV, IC, RV, FRC TLC. How did these values change?
9. CLEAN-UP:
   Mouthpieces should go in the biohazard softs. Wipe the spirometer with alcohol swabs and allow to dry.

D. Pulmonary Function-Percent Predicted Vital Capacity

Equipment

- Spirometer

Protocol:

1. Inhale normally, exhale normally, and then take a deep exhalation into the spirometer. Repeat 3 times and take the average.
2. Predicted VC (in L):
   a. Male = 0.052(H) – 0.022(A) – 3.6
   b. Female = 0.041(H) – 0.018(A) – 3.6
   c. (H) = height in cm
   d. (A) = Age in years
   e. (VC) = predicted vital capacity in L
3. Calculate their Percent Predicted Vital Capacity.
   % predicted VC = (measured VC in mL/predicted VC in mL)*100
4. CLEAN-UP:
   Mouthpieces should go in the biohazard softs. Wipe the spirometer with alcohol swabs and allow to dry.

E. Pulmonary Function-Forced Expired Volume

Equipment

- Spirometer
- Disposable mouthpiece

Protocol:

1. During these tests you must breathe “fast and hard” which can be tiring. Take a break between each trial to let your accessory muscles rest.
2. FORCED EXPIRED VOLUME FOR 1 SECOND: Take a deep inhalation and then take “fast and hard” deep exhalation for 1 second. Repeat 3 times and take the average.

3. FORCED VITAL CAPACITY: Take a deep inhalation and then take “fast and hard” deep exhalation until you cannot exhale any longer. Repeat 3 times and take the average.
4. CLEAN-UP:
   Mouthpieces should go in the biohazard softs. Wipe the spirometer with alcohol swabs and allow to dry.

F. Buffered and Unbuffered Solutions

Equipment

1. pH paper
2. 2 Transfer pipet
3. 4 50mL beaker
4. Buffer at pH 7
5. DI water at pH 7
6. 10% HCl
7. 10% NaOH

Protocol:

1. Set up 2 beakers with 40mL DI water and 2 beakers with 40mL Buffer solution.
2. Titration using 10% HCl: Add 1 drop of acid to a beaker of water; mix solution. Test the pH after each drop and determine how many drop it took for the pH to become more acidic. Repeat with the buffer solution.
3. Titration using 10% NaOH: Add 1 drop of base to a beaker of water; mix solution. Test the pH after each drop and determine how many drop it took for the pH to become more basic. Repeat with the buffer solution.
4. CLEAN-UP:
   All solutions must go in the chemical waste container. Wash your beakers with soap and water.

G. Bicarbonate Blood Buffer Simulation

Equipment

1. 2 100mL beaker
2. 10mL graduated cylinder
3. Straw
4. DI water at pH 7

5. 0.1N NaOH
6. Saturated phenolphthalein solution (pH indicator; clear=acid, pink=basic)

Protocol:

1. Set up both beakers with 100mL DI water and 2mL of 0.1N NaOH. Add drops of phenolphthalein solution to each beaker until it turns and remains pink. Make sure that each beaker received the same number of drops and that you mix the solution after each drop.
2. RESTING: Prepare your timer to determine how long it will take to turn the solution clear. Then take a clean straw and blow into the water beaker until it turns clear.
3. EXERCISE: Jump for 3 minutes. Prepare your timer to determine how long it will take to turn the solution clear. Then take a clean straw and blow into the water beaker until it turns clear.
4. Why was there a time difference between rest and exercise?
5. CLEAN-UP:
   All liquid waste should go in the chemical waste container. Wash your beakers. Straws should go in the biohazard softs.

Name: _____ **Score:** _____

Section: _____

Respiratory Pre-Lab Assignment:

- Read the protocol before attending lab and plan accordingly.
- Understand the definitions before coming to lab.
- Understand waste management before coming to lab.

Draw and label a Volumes and Capacities Chart. Label the axes, volumes, and capacities.

.

Make a flow chart for experiments D-G.

Draw a flow chart of the protocol for lab exercises D, E, F, & G to prepare for each experimental lab exercise. Add each step, time intervals, and waste management, etc. You may include drawings.

D. Pulmonary Function-Percent Predicted Vital Capacity

E. Pulmonary Function-Forced Expired Volume

F. Buffered and Unbuffered Solutions

G. Bicarbonate Blood Buffer Simulation

THIS PRE-LAB ASSIGNMENT IS DUE AT THE NEXT LAB MEETING

Name: _____ **Score:** _____

Section: _____

Respiratory Post-Lab Assignment:

Renal Experiment Assignment: _____

A. Respiratory Rate

- Average respiratory rate is 12-20 breaths per minute. Is your patient normal?

B. Auscultation and Lung Sounds

- Are the sounds you here normal? Do you hear any wheezing, crackling, or other unusual sounds?

C. Pulmonary Volumes, and Capacities using a Spirometer

- TIDAL VOLUME:
  - Trial 1:
  - Trial 2:
  - Trial 3: Average:
- EXPIRATORY RESERVE VOLUME:
  - Trial 1:
  - Trial 2:
  - Trial 3: Average:
- VITAL CAPACITY:
  - Trial 1:
  - Trial 2:
  - Trial 3: Average:
- ESTIMATE RV:
  - RV factors based on age:
    - 16-34 years old = 0.250
    - 35-49 years old = 0.305
    - 50-69 years old = 0.445
  - RV=VC * RV factor

- CALCULATE ALL OTHER VOLUMES AND CAPACITIES: IRV, IC, EC, TLC.
    - IRV
    - IC
    - RV
    - TLC
    - FRC

- EFFECTS OF EXERCISE: What where your exercise values and how did they change (increase, decrease, stayed the same)?
    - TV
    - ERV
    - VC
    - IRV
    - IC
    - RV
    - TLC
    - FRC

D. Pulmonary Function-Percent Predicted Vital Capacity

- PREDICTED VITAL CAPACITY:

- PERCENT VITAL CAPACITY:

% predicted VC=(measured VC in mL/predicted VC in mL)*100

E. Pulmonary Function-Forced Expired Volume

- FEV1
    - Trial 1:
    - Trial 2:
    - Trial 3:
    - Average:
- FORCED VC:
    - Trial 1:
    - Trial 2:
    - Trial 3:
    - Average:

F. Buffered and Unbuffered Solutions

- How many HCl drops did it take for it to turn acidic:
    - Water:
    - Buffer:
- How many NaOH drops did it take for it to turn basic:
    - Water:
    - Buffer:
- Identify the buffer system(s) that are found in blood. Explain why it is important that blood is buffered.

G. Bicarbonate Blood Buffer Simulation

- How much time did it take to the solution to turn clear after REST: ___
- How much time did it take for the solution to turn clear after EXERCISE: _____
- Explain why there is a difference:

THIS POST-LAB IS DUE AT THE NEXT LAB MEETING

MULTIPLE DAY LAB EXERCISE

Renal Feedback Loops and Mechanisms Focusing on the Regulation of Blood Volume, Blood Osmolarity, Blood pH, and Blood Glucose. The Effects of Diuretics.

Summary

Urine tests are laboratory analyses performed on urine samples. Urine analysis can provide useful information about a persons' health. They are routinely used are regular doctor appointments and hospital settings to determine if patients have infections, diabetes, renal issues, liver issues, metabolic issues, and other conditions. Students will run a urine analysis on their own urine. In addition, for the next few days groups will run an experiment on a group member to determine how the human body regulates certain variables (blood volume, blood osmolarity, blood pH, blood glucose & caffeine).

Physiology students will be research scientists. They will conduct learn how to analyze urine samples, ask a question, develop hypotheses, research renal feedback loops and renal mechanisms, perform an experiment to test the hypotheses, analyze data, develop a conclusion, write a scientific abstract and give a scientific presentation. Groups will choose one person to run a two day experiment; Day 1 the participant will drink the control drink and Day 2 the participant will drink the experimental drink that was randomly assigned to the group. Your group will randomly be assigned an experimental drink to research and test. On Day 3 students will formally present their findings.

DAY 1: CONTROL DRINKS

All group members will run urine analysis experiments on their personal urine. Groups will choose a participate. The participant will empty their bladder and test it. Then they will drink the CONTROL DRINK and test their urine at specific time intervals (don't forget that the participant cannot drink/eat any additional drink/food during the lab meeting). The group may want to take pictures along the way to document your experiment. The group will present a quick review of the proper feedback loops and mechanisms that they used to create their hypotheses. Your instructor may discuss with you or make suggestions to guide to towards more specific background research to make better hypotheses. The group should begin to create their scientific presentation by working on their title slide, introduction, hypotheses, and methods. In addition, they should talk about how they will analyze their data, and which graphs their will create and present (no raw data should be presented).

DAY 2: EXPERIMENTAL DRINKS
Urine analysis will only be conducted on the participant. The participant will empty their bladder and test it. Then they will drink the EXPERIMENTAL DRINK and test their urine at specific time intervals (don't forget that the participant cannot drink/eat any additional drink/food during the lab meeting). The group may want to take pictures along the way to document your experiment. The group will review and work on their scientific presentations and plan accordingly. Ask for guidance if you or your group is in need.

DAY 3: SCIENTIFIC ABSTRACTS & PRESENTATIONS
Group project requires a group abstract (250 words max) and group scientific presentation (10 minutes max). The group project is due on the last week of lab.

Goals:

- Students will study and understand renal feedback loops and renal mechanisms that help regulate blood volume, blood osmolarity, blood pH, & blood glucose. Students will also learn about the effects of diuretics.
- Students will work safely and follow precise protocols to run urine analysis experiments.
- Students will properly handle all waste materials, equipment, and chemicals.
- Students will work together to design a good experiment, take and analyze data, create conclusion, write an abstract, write an abstract, create a scientific presentation, and participate in their group scientific presentation.
- Students will listen attentively during group presentations and ask good questions.

Lab Exercise:

****NOTE: your instructor will assign your group an experimental drink. If you have any health issues (diabetic, high blood pressure, etc.., then please do not volunteer to be the participant for the experiment). Run your assigned experiment only. Experimental drinks will be randomly assigned as follows: Drinking a lot of water (hypotonic & increases blood volume), 1.5% sodium chloride solution (hypertonic), 0.9% sodium chloride solution (isotonic), 5% glucose solution, Orange juice (acid and sugar; focus on acid), Sodium bicarbonate (base and sodium; focus on base), filtrate diuretic (black coffee), & Gatorade.*

A. Personal Urinalysis Tests

Equipment

- Gloves
- Placemat
- 500mL of DI water as your control
- 500mL of your assigned experimental drink
- Urine analysis reagent strips (urobilinogen , glucose, ketone,- bilirubin, protein, nitrite, pH , blood, specific gravity, leukocytes)
- Urinometer (graduated cylinder and hydrometer, specific gravity, urine concentration)
- Graduated cylinder/Urine cup (urine volume)
- Chloride ion testing station
- Transfer pipets
- Test tubes
- 20% potassium chromate
- 3% silver nitrate

Protocol: Urine analysis:

1. Using the urine cup/graduated cylinder determine the urine volume.
2. Using a urine analysis reagent strip determine the pH, glucose concentration, specific gravity, and protein concentration. Take a sample of urine in a transfer pipet and add one drop to each test pad. Allow to sit for the allotted time and then check results. To prevent contamination, DO NOT touch the test strip to the bottle.
3. Using the urinometer, measure the specific gravity. Use a transfer pipet to fill the glass graduated cylinder about 75% full. Gently add the hydrometer and make sure it is floating freely in the middle of the graduated cylinder. Take your reading at the meniscus.
4. Using the chloride testing station determine the chloride ion concentration. Add 10 drops of urine into a test tube. Add one drop of 20% potassium chromate and mix. Add 3% silver nitrate and mix. Continue adding one drop of 3% silver nitrate at a time until the solution turns brown. Calculate the chloride ion concentration
    a. # drops * 61mg Cl- / 100mL
    b. Convert to Molarity (g/L; M)
    c. Convert to millimolarity (mM)

5. CLEAN-UP:
   Return your urine to your urine cup and **dispose of it by flushing it down the toilet**. All items that have or may have urine on them must go in the biohazard softs. Chloride testing liquids should go in the chemical waste container and the test tubes should go in the test tube collected bucket.

B. Group Project: Control Drink (Day 1) &. Experimental Drink (Day 2) Tests

Equipment

- Same as section A
- Choose one student per group to be the participant. On Day 1 they will drink the control. On Day 2 they will drink the experimental.
- Control drink: 500mL of drinking water
- Experimental drink: 500mL of an assigned experimental drink
  - A lot of water (NOTE: 1000mL)
  - ½ teaspoon Baking Soda
  - Orange juice
  - 0.9% NaCl
  - 1.5% NaCl
  - 5% glucose
  - Gatorade
  - Caffeine/Black coffee

General Rules:

1. Completely empty your bladder and run a urine analysis before you drink the experimental drink.
2. Always measure your urine volume and bring back a sample for analysis.
3. Rinse and dry your urine cup after each use.
4. You have to attempt to urinate at each interval even though you may not feel the need to urinate.
5. Follow all safety and waste management procedures.
6. If you are studying pH then you should also measure breathing rate.

Protocol:

1. Time 0min: Have the students empty their bladder as soon as the lab begins. They should measure their urine volume

and bring back a sample. When they return, have them “chug” their assigned drink and begin the timer (20min interval). STUDENTS CANNOT EAT NOR DRINK ANYTHING FROM THIS POINT ON. Run a complete urine analysis on their time 0min urine.

2. Time 20min: When the timer rings, have the student empty their bladder. They should measure their urine volume and bring back a sample. When they return begin the timer (20min interval). Run a complete urine analysis on their time 20min urine.
3. Time 40min: When the timer rings, have the student empty their bladder. They should measure their urine volume and bring back a sample. When they return begin the timer (20min interval). Run a complete urine analysis on their time 40min urine.
4. Time 60min: When the timer rings, have the student empty their bladder. They should measure their urine volume and bring back a sample. When they return begin the timer (20min interval). Run a complete urine analysis on their time 60min urine.
5. Time 80min: When the timer rings, have the student empty their bladder. They should measure their urine volume and bring back a sample. When they return begin the timer (20min interval). Run a complete urine analysis on their time 80min urine.
6. Time 100min: When the timer rings, have the student empty their bladder. They should measure their urine volume and bring back a sample. When they return begin the timer (20min interval). Run a complete urine analysis on their time 100min urine.
7. OPTIONAL Time 120min: When the timer rings, have the student empty their bladder. They should measure their urine volume and bring back a sample. When they return begin the timer (20min interval). Run a complete urine analysis on their time 120min urine.
8. STUDENTS MAY NOW EAT & DRINK WHATEVER THEY WISH OUTSIDE OF THE LAB.
9. CLEAN-UP: dispose of urine and waste appropriately. Points will be lost if waste is mismanaged.

SCIENTIFIC PRESENTATION & ABSTRACT: work on a scientific presentation and abstract (a short description of your experiment and finds). Determine who will post your work online and when you will get together to practice your talk with your group. You will present your findings at the last lab meeting of the semester. Your instructor will provide a rubric for the abstract and presentation.

You must include each section of a scientific presentation (10 minutes max):

- Title slide: title, names, semester, year, course, lab time and day.
- Introduction: include appropriate mechanisms and/or feedback loops, terms, and other concepts needed to prepare the audience for your experimental findings.
- Hypothesis: include your "if, then" statement. Make sure to point out the independent and dependent variables.
- Methods: explain how you ran your experiment and how you collected data.
- Results: Use line graphs to plot your data. Explain the trends/patterns that you found. Does this data support/reject your hypothesis?
- Conclusion: Explain your results using the mechanism and/or feedback loops that you presented in your introduction. Where there any issues during data collection/analysis? Explain why you think so and how you would improve your methods.
- Clinical Application: How does this apply in the real world? Present at least 2 examples.
- Citations.
- Use PowerPoint (or a similar program) to create your slide show.

Write an Abstract to summarize your work. This is due on the day of your presentation. Include the following: (250 words max).

- Group names and lab section.
- Title: provide a good title.
- Introduction: 1-2 sentences
- Methods: 1-2 sentences
- Results: 1-2 sentences
- Discussion: 2-4 sentences.
- Figures: Attach your final figures and tables at the end of your abstract. Resize appropriately.

Name: _____ **Score:** _____

Bio249 Lab Section: _____

Renal Pre-Lab Assignment: Due Day 1

A. Read the protocol before attending lab and plan accordingly.
B. Understand the definitions before coming to lab.
C. Understand waste management before coming to lab.

A. Personal Urinalysis Preparation

- Hydrate two days prior to the renal lab.
- Eat a bland diet the day of the renal lab (i.e. no sweets, caffeine, or salty food).
- You will urinate as soon as you come into the lab. Make sure that you do not empty your bladder before you come to lab.

B. Group Project: Control Drink &. Experimental Drink Tests

Who is your volunteer participant? _____

What drink was your group assigned to test:

Experimental: _____

Control: _____

- Make a flow chart below to illustrate how you will perform your CONTROL TESTS ONLY. Add each step, time intervals, and waste management, etc. You may include drawings.

THIS PRE-LAB IS DUE AT THE NEXT LAB MEETING.

Name: _____ **Score:** _____

Bio249 Lab Section: _____

Renal Pre-Lab Assignment: Due Day 2

- Read the protocol before attending lab and plan accordingly.
- Understand the definitions before coming to lab.
- Understand waste management before coming to lab.
- Pace yourself: Before day 2 you should have your title slide, methods, and part of your introduction completed for you scientific talk. You should also know what graphs/tables you would like to create once you have all the data. You should have also completed your background research.

B. Group Project: Control Drink &. Experimental Drink (Day 2) Tests (Due Day 2)

- Make a flow chart below to illustrate how you will perform your EXPERIMENTAL TESTS ONLY. Add each step, time intervals, and waste management, etc. You may include drawings.

- Background information (NOTE: this section must be complete for the group to run their experiment). Before coming to lab, think about group what renal mechanisms or feedback loops are the most relevant to your experiment? Be ready to discuss these with your group & instructor for guidance/approval. Bring your textbook and notes.

  Below is a list of feedback loops, mechanisms, and hormone related to the renal system. Circle the topics that will use to explain your results.
    - Stimuli:
      - Increased blood volume
      - Increased blood pressure
      - Increased blood osmolarity
      - Decreased blood osmolarity
      - Increased blood pH
      - Decreased blood pH
      - Diuretics
      - Increased blood glucose
      - Increased atrial stretch
    - Mechanisms:
      - Filtration barriers in the renal corpuscle
      - Glomerular filtration rate (GRF)
      - Renal threshold for glucose
      - PCT Reabsorption/Secretion
      - PCT-Reabsorption/Secretion
      - Loop of Henle-Reabsorption/Secretion
      - DCT-Reabsorption/Secretion
      - Collecting Duct-Reabsorption/Secretion
      - Countercurrent exchange systems
      - Renal countercurrent multiplier
      - Buffer systems for pH regulation
        - Bicarbonate ions
        - Ammonia & phosphate ions
        - Secrete hydrogen ion/bicarbonate ion
        - Reabsorb hydrogen ion/bicarbonate ion
      - Type A intercalated cells
      - Type B intercalated cells
    - Feedback loops & Hormones:
      - Vasopressin
      - Aldosterone

        - Natriuretic peptides
            - Atrial natriuretic peptide (ANP)
            - Brain natriuretic peptide (BNP)
        - Renin-Angiotensin Pathway (RAS pathway)
            - Angiotensin I & Angiotensin II
            - Angiotensin converting enzyme (ACE)
            - Renin (an enzyme)
        - Cardiovascular responses
            - Baroreceptor reflex
                - Increases TPR
                - Decreased TPR
                - Increased HR
                - Decreased HR
                - Increase BP
                - Decrease BP
        - Respiratory responses
            - pH regulation via ventilation
                - hyperventilation
                - hypoventilation
        - Sympathetic response
        - Parasympathetic response
    - Others: list anything else you would like to research

- Create a question(s) for your experiment:

- Create hypotheses(sis) for your experiment:

THIS PRE-LAB IS DUE AT THE NEXT LAB MEETING.

Name: _____ **Score:** _____

Bio249 Lab Section: _____

Renal Post-Lab Assignment for Day 1:

A. Personal Urinalysis Results

- Record your personal results:

Variable	Results
Volume (mL)	
Leukocytes	
Nitrite	
Urobilinogen	
Protein	
pH	
blood	
Specific gravity (test strip)	
Ketone	
Bilirubin	
Glucose	
Chloride ions	
Specific gravity (urinometer)	
Specific gravity (hydrometer)	

- What does your urine look like? Color, cloudy, clear, etc.

- What was your specific gravity when using the:
    - urinometer? _____
    - hydrometer? _____
    - test strip? _____
    - Which piece of equipment do you think is the most accurate?

- Calculate your chloride ion concentration (Mm). Show your work below:

- Were your values normal? Why or why not?

THIS POST-LAB IS DUE AT THE NEXT LAB MEETING.

Name: _____ **Score:** _____

Bio249 Lab Section: _____

Renal Post-Lab Assignment for Day 2:

Who is your volunteer participant? _____

B. Group Project: Control Drink (Day 1) &. Experimental Drink (Day 2) Tests

Record your group results: Draw your data tables below and record your results.

CONTROL DRINK:_____

Record your group results: Draw your data tables below and record your results.

EXPERIMENTAL DRINK:_____

Analyze your data. Draw appropriate graphs for your data using MS Excel. Label properly. Attach them to this post-lab.

- Graphing
  - Title
  - Axis labels
  - Axis units
  - Independent and dependent variables are found on the correct axis
  - Key if appropriate (colors add another variable)
  - Line graph: connect the points with a straight line
  - Add captions to each figure.

THIS POST-LAB IS DUE AT THE NEXT LAB MEETING.