Exercise 1
The Scientific Method

OBJECTIVES:
- Students will familiarize themselves with lab policies and practices.
- Students will know where all emergency/safety equipment are and why they are needed in the lab setting.
- Students will identify, understand, and apply the elements of a scientific experimental plan.
- Students will determine what types of statistics are needed to make appropriate conclusions at the end of an experiment.
- Students will interpret data and make conclusions about the results of the experiment.
- Students will be able to construct a graph using Microsoft Excel.

Task 1 - SAFETY: Learn about safety in a biology laboratory.

Introductory Notes:
All tasks in this laboratory manual are designed to have students work as a team to accomplish the learning objectives. Students must come in to each laboratory session having thoroughly read the laboratory exercise(s) of the day. The instructor will provide directions or demonstrate procedures in the lab prior to the start of any experiment. Students are encouraged to asked questions about the experiments. Always make note of any additional safety requirements before the start of any experiment. When in doubt ask!

Safety Procedure
1. Review the safety handout with your instructor.
2. Note the location and give a brief explanation of the use and importance of each of the following safety and emergency equipment/items:
   - Shower:
   - Eyewash:
   - Medical emergency procedure/contacts:
   - Fire extinguisher:
   - First Aid Kit:
   - Emergency gas cut-off valve:
   - Spill kits:
   - Fume Hood:
   - Emergency Phone:
   - AED:
Task 2 - THE SCIENTIFIC METHOD

The term science refers to the body of knowledge gained through the process of systematic study. We use the process of science to discover novel and fascinating pieces of knowledge. The scientific method enables us to make observations, pose questions, and solve problems about the natural world that can be effectively and efficiently tested through repeated and unbiased experiments. It is an approach to solving problems of all types that is practical, reliable, and logical; with the ultimate goal of obtaining new knowledge.

Figure 1.1. The Scientific Method

The origin of the modern scientific method (Figure 1.1) can be traced back to the works of the Greek philosopher Aristotle, where principles of the natural world must be extrapolated from the collection and studies of observations. We all make observations of the world around us, but when dealing with science the key is to formulate informative observations that look for repeated or unusual patterns. The majority of scientific works come from trying to understand these patterns. In fact, many great scientific discoveries stem from observations of unusual events that they did not expect. These patterns are crucial to creating
informative and insightful observations. For example, consider the following observations: (1) All the plants I try to grow on the right side of my backyard ultimately die. (2) Despite the fact that throughout the rest of my backyard the plants prosper, the plants on the less shaded right side of my backyard ultimately yellow and die. Both statements may be true, but it is the latter of the two statements that informs us of relevant features and potential relationships. By utilizing informative observations of the natural world or by reading the works of others who have reported their observations and conclusions, a scientist can then ask questions based on this preliminary information.

Questions are the basis of our thinking processes. These questions, when using the scientific method, should be based on informative observations and lead you to a proper scientific hypothesis and suggests a pathway for investigation. The better the quality of the preliminary observations the better the quality of the questions and ultimately the hypotheses proposed. Initially questions tend to be too general, which is not necessarily a bad thing. For example, “Under what conditions do enzymes work optimally?” This might be the question we want to ultimately understand, but within the context of a scientific experiment the question is too vague. Proper questions for the process of science need to be specific enough to be answered clearly through experimentation. For example, “What pH and temperature does amylase work optimally?” Typically questions at this stage still need to be refined and further classified to lead to a more specific scientific hypothesis. For example, “Does amylase catabolize starch optimally in higher, lower, or neutral pH?” This question clearly refers to a testing subject (organism, biomolecule, etc.), variables, and processes. If at this point a clear question cannot be written, then the observations need to be reevaluated. It is very difficult to write good questions without having a proper understanding of the subject at hand first.

A hypothesis is a candidate explanation for an observed phenomenon. This explanation must be stated in a testable and falsifiable manner. This statement, also, must not be anthropomorphized. A scientific hypothesis clearly states the relationship between scientific variables and must provide a mechanistic explanation for the relationship that is being systematically tested. For example, when determining the relationship between precipitation and secondary succession an appropriate scientific hypothesis would be: Heavy precipitation during the rainy season in South Florida increases the amount of woody and herbaceous fuels, eliminating the constraints on natural brush fires, which result in secondary succession.

This statement is not to be confused with statistical hypotheses nor predictions. The purpose of statistical hypotheses is to identify or clarify patterns typically through the use of quantitative statistical data analysis. A good statistical hypothesis identifies two general components: (1) the relationship between the tested variables and (2) the process(es), molecule(s) or organism(s) under scrutiny. Experimentation and its resultant data analysis is used to be able to determine whether the statistical hypothesis should be rejected or not. There are two types of statistical hypotheses when performing statistical analysis in experiments on the natural world: (1) Null Hypothesis (H₀) and (2) Alternative Hypothesis (Hₐ). The null hypothesis states that there is no significant determined relationship between two observed phenomena (variables). The alternative hypothesis states that there is a significant determined relationship between two observed phenomena.

These statistical hypotheses are considered concurrently when performing data analyses. Just like a scientific hypothesis the statistical hypothesis must be written in a way that it can be falsifiable. As a rule these hypotheses are not accepted as an absolute truth, but are considered to be tentatively not rejected. With that in mind, one can reject or fail to reject a statistical hypothesis in an experiment. This is the case because there remains the possibility of another experiment proving the relationship written in the statement false. It is only the hypotheses that stand the test of time and cannot be determined false through repeated experimentation which are then deemed theories (i.e., Theory of Evolution, Theory of Gravity, etc.).

Once the hypotheses have been stated the next step is the make appropriate predictions. A prediction is a statement that foretells what is believed to most likely occur, based on observations. This is not to be confused with a hypothesis. A prediction does not provide a possible mechanistic explanation to the relationship depicted in the statement. Instead, it simply provides a statement that anticipates the outcome of an experiment in the lab or in nature. Statements like, “I need to buy ice cream, so I will go directly to the freezers instead of searching through the entire store,” are not considered true scientific predictions.

Creating an experimental plan is a process that requires very meticulous thought. You need to be aware of all possible factors and account for their influence on the subject at hand. To be able
to test hypotheses all variables must be under the control of the individual conducting the experiment. The variable that is manipulated is considered the independent variable, while the response variable is termed the dependent variable. The dependent variable is the measurable observed response seen in the experiment. For example, if the objective is to determine the effect of exercise on heart rate, then the measured value, heart rate, is the dependent variable because the value is dependent on the manipulation in the experiment, the exercise (independent variable).

For an ideal experiment to occur, only the independent variable can vary in the experiment during the course of multiple trials. Ensuring that this occurs is essential in standardizing the experiment plan. Part of this experimental process is the determination of an appropriate control group for the purposes of comparison. It is important to determine how the experimental plan will influence the subject being investigated under normal conditions before it can be determined that a relationship truly exists between the dependent and independent variables. Again, the experimental groups being investigated should only differ from the control group by a single factor, the independent variable. All experiments must be replicable. When it comes to replication of the experimental treatments try to repeat at least three times. When appropriate for the experiment it is typically best to reach at least a statistically significant sample size (n = 30). The data is then collected, put through statistical analysis and compared to the predictions.

Hypotheses at this point, once the statistical analysis has been reviewed and a summary of the data is available, can be evaluated for their validity. These conclusions are discussed along with the potential ramifications of these newly formed observations. It is always important to put your scientific work through peer-review with the ultimate aim of helping one another examine experimental design and interpret results. Once this occurs, these new observations can be reported. However, this is not the end. The process of scientific thought is cyclic. Once new results are obtained it should lead to the creation of new questions, which leads to a new experimental plan.

**Procedure:**

As per the instructor’s directions, read and discuss the scenario, below, that has been assigned to your class.

**Scenario 1:** Neuromuscular reaction time can be influenced by a number of different factors. Design an experiment where neuromuscular reaction time and the factors that influence it are tested among different individuals. This can be tested by measuring how quickly a person can grasp a falling meter stick.

**Scenario 2:** Termites are social insects that live in colonies, which can have millions of termites. Termites have a structured social system with the ability to communicate between individuals and groups using pheromone signals. Design and conduct an experiment where you test the termites’ ability to communicate with each other.

**Scenario 3:** Blackworms, *Lumbriculus variegatus*, is a freshwater oligochaete worm (related to earthworms and leeches). Blackworms have a large dorsal blood vessel that is easy to observe under a microscope. Design and conduct an experiment that tests the effect of chemical exposure on the cardiovascular system of the blackworms.

**Scenario 4:** The house cricket, *Gryllus domesticus*, like many social animals have structure dominance hierarchies, which is based on the individuals’ strength and influence over other crickets. Design and conduct an experiment where you test the benefits and costs to these individuals.
Making Observations

1. The first step in the scientific method is making observations. To begin exploring this scenario, as a group, summarize the group’s collective knowledge about the scenario at hand. For example, what factors might influence the subject being tested? As the discussion proceeds, make notes below that summarize the group’s knowledge and observations about the characteristics of the scenario and anything that could influence the scenario at hand.

Posing Questions

2. Scientific questions must be based on observations previously made. These newly developed questions are further refined and one of these questions is chosen to construct hypotheses. Use the observations gathered above and design scientifically answerable questions based on the assigned scenario. What criteria can be used to judge the quality of a “good question”? Propose questions based on these criteria and the gathered observations. These questions will then be presented and discussed as a class. Write the criteria points and proposed questions in the space below.

When defining a question to ask there are a lot of factors that must be considered including the funding needed to back the research. The scientific community looks to public and private institutions to help fund their research. To receive grant money there needs to be a proper justification for the questions being asked in the research project. For the proposed questions justify your reasoning for attempting to answer these question.

Developing Hypotheses and Predictions

3. A problem/question cannot be scientifically solved without reducing the problem into an appropriate hypothesis. The hypothesis should be identified before any research experiment is conducted. Remember this statement needs to be a clear statement of the intentions of the investigation and can be reasonably tested. Discuss the proposed questions, using the criteria of a “good question”, and as a class, decide on the best question to formulate an appropriate scientific hypothesis. Write the question in the space provided below.
4. Formulate a scientific hypothesis based on the chosen question above. Discuss this hypothesis as a class. This must be a testable hypothesis through experimentation. Write the hypothesis below.

5. Develop an appropriate null hypothesis (H₀) and alternative hypothesis (Hₐ) based on the scientific hypothesis written above. These hypotheses must be testable through experimentation. Write the hypotheses below.

   Null Hypothesis (H₀):

   Alternative Hypothesis (Hₐ):

6. Translate your question-based hypothesis into a prediction. Write the prediction below. Make sure to also discuss the reasoning for your predictions.

   **Experimental Design**

   In the process of developing an experiment all the factors that could influence the results of an experiment must be considered before developing a protocol. To test the predictions and hypotheses, control and variables must be defined and taken into account. As the group discusses the potential factors that influence the experiment keep the overall design of the experiment in mind.

   7. What are the factors involved in the experiment?

   - Which of the factors would be considered the independent variable(s)?

   - Which of the factors would be considered the dependent variable(s)?
• What factors would need to be controlled during the experiment and how will they be controlled?

• How will the experiment be standardized so that results can be comparable across all treatments to be able to collect and analyze data as a class? Why is setting standards for your experiment important?

8. Decide on the number of trials the experiment will include? Explain your reasoning.

9. Discuss your initial experimental plans with the class. Decide on an experiment plan which inquires about the developed hypotheses. Takes notes on the experiment plan in the space provided below.

Protocol Development

10. Write a step by step procedure that depicts how the experiment is to be conducted. The class will use this consensus protocol to conduct the experiment.
Organizing Data

11. Before performing any experiment, once the protocol has been identified there needs to be a plan in place to be able to clearly collect all the data necessary to be able to test the proposed hypotheses. Create a table in which the data will be recorded within the group. Also, create a data table that summarizes the class data. What statistical analyses should be included in the table, if any? Is qualitative or quantitative data being collected? Explain.

Group Data Table

Class Data Table

Once all aspects of the experiment have been explored and are clearly understood perform the experiment. Always record any observations you make during the experiment. Tabulate the collected data in the respective tables above. Write those non-quantitative observations below in an organized manner.

Data Analysis

12. Translate the class data onto an Excel spreadsheet. This file will be shared electronically with the class for the purposes of creating appropriate graphs. When graphing the independent variable needs to be graphed against the dependent variable.
13. What trends do you see in the data and the differences observed between groups.

**Making Conclusions**

14. Return to the hypotheses (H₀ and H₁) that were formulated at the beginning of the experiment. Compare them to the experimental results. Do you reject or fail to reject the hypothesis? Why? Cite the data used in making the decision. NOTE: When writing a scientific paper you NEVER simply write “I reject my null hypothesis because...” so avoid writing it here.

15. Provide an explanation as to why the results came out the way they did. Make sure to relate it back to the hypotheses and predictions.

16. As the experiment was conducted and the results were analyzed, additional questions probably came to mind. As a result of this inquiry and the results of this experiment, what would be some interesting questions to test if another experiment were to be conducted?

17. Evaluate the experiment design. Be critical of every aspect of the experiment. After having conducted the experiment is there anything else that should have been considered during the planning of the experiment. What errors could have occurred during the experiment? How can these errors be reduced in future experiment? Errors are inevitable in experiments. It can be defined as the difference between an observed measure of a quantity and its actual value. This is not always considered a mistake. Variability in data is part of analyzing collected data.