Lab 1: Tools for Biologists

Scientists use a variety of tools to carry out laboratory experiments and to understand and manipulate data. Students need to develop familiarity with simple tools like pipettes as well as complex machinery such as spectrophotometers. Learning to use lab equipment simply requires time and practice. Mathematical manipulation and presentation of data are just as important as good lab technique. They require attention to detail and a basic understanding of mathematical principles.

**Objectives**

When you have successfully completed this lab, you should be able to:

1. Use and understand scientific notation.
2. Explain the basics of experimental design
3. Use pipettes and pipette pumps to measure volumes.
4. Understand and perform serial dilutions to measure concentrated solutions.
5. Calculate the mean and standard deviation for a dataset. Understand the significance of each.
6. Use a spectrophotometer.
7. Understand the course structure and student expectations in Biology 107.

**Procedures**

There are several skills to practice in this lab. The laboratory classroom has been divided into sections as described below. Read through the entire lab and then start with the area which is least crowded.

Section A: Scientific notation; do this section on your own before lab begins.

Section B: Experimental design

Section C: Use of pipettes and balances to gather data

Section D: Serial dilutions of a colored solution and use of a spectrophotometer to read absorbances

Section D: Small group discussions with the lab assistant or instructor

Section E: Data analysis (Calculate mean and standard deviation.)

You must complete this section on SI (System International) units and scientific notation before class.

A number expressed in scientific notation is $a \times 10^n$ where $a$ is a number between 0 and 9 and $n$ (the exponent) is a whole number. For example, $3.4 \times 10^4$ can also be written as 34,000 and $5.88 \times 10^{-3}$ can be written as 0.00588.

Express the numbers below as decimals.

<table>
<thead>
<tr>
<th>Decimal</th>
<th>Scientific Notation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2 x $10^{-4}$</td>
<td></td>
</tr>
<tr>
<td>1.7 x $10^2$</td>
<td></td>
</tr>
<tr>
<td>9.84 x $10^6$</td>
<td></td>
</tr>
<tr>
<td>5.43 x $10^{-3}$</td>
<td></td>
</tr>
</tbody>
</table>

Express the decimals below using scientific notation.

<table>
<thead>
<tr>
<th>Decimal</th>
<th>Scientific Notation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>0.000026</td>
<td></td>
</tr>
<tr>
<td>3,000,000</td>
<td></td>
</tr>
<tr>
<td>5.45</td>
<td></td>
</tr>
<tr>
<td>0.000000002</td>
<td></td>
</tr>
<tr>
<td>236,000</td>
<td></td>
</tr>
</tbody>
</table>

None of these exercises requires a calculator.
In Biology 107, all data will be expressed in SI units. Meters, seconds, grams, and liters are the SI units for length, time, mass, and volume, respectively. Each unit may be combined with prefixes that represent powers of ten. Some examples are given in the table below.

<table>
<thead>
<tr>
<th>Unit</th>
<th>Number of grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>kilogram (kg)</td>
<td>1 x 10^3 or 1000</td>
</tr>
<tr>
<td>gram (g)</td>
<td>1</td>
</tr>
<tr>
<td>milligram (mg)</td>
<td>1 x 10^{-3} or 0.001</td>
</tr>
<tr>
<td>microgram (μg)</td>
<td>1 x 10^{-6}</td>
</tr>
<tr>
<td>nanogram (ng)</td>
<td>1 x 10^{-9}</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Unit</th>
<th>Number of liters</th>
</tr>
</thead>
<tbody>
<tr>
<td>liter (l)</td>
<td>1</td>
</tr>
<tr>
<td>milliliter (ml)</td>
<td>1 x 10^{-3}</td>
</tr>
<tr>
<td>microliter (μl)</td>
<td>1 x 10^{-6}</td>
</tr>
</tbody>
</table>

Using this information, fill in the boxes below.

38 mg = ________ g
2760 g = ________ kg
17 mg = ________ μg
0.3 ml = ________ μl
120 μl = ________ ml
0.001 liters = ________ ml
0.002 g = ________ mg
0.21 g = ________ μg
6.1 x 10^{-3} ml = ________ μl
23 ml = ________ μl
0.002 liters = ________ ml = ________ μl

When writing scientific reports for Biology 107 (and for many other classes) you will need to use superscripts and subscripts in molecular formulae, to represent exponents, and for other reasons. If you are using recent versions of Microsoft Word, you can press the Control/Shift/+ keys together to activate superscript mode. Then type the text you would like to superscript. Press the same three keys together to exit superscript mode. Pressing the Control/+ keys together activates and deactivates subscript mode. If you have a different word processor, consult your manual or help screen for instructions. Do not hand-write superscripts and subscripts in your lab report.

Section B

In the first lecture, we discussed experiments and hypotheses. Designing an experiment to test your hypothesis is the most creative aspect of science. Many times scientists think of interesting questions that can’t be answered or experiments that are impractical. Examples: “Does heat increase immorality” (What is immorality?) or “What percent of all women over 25 have tumors observable by CAT scan?” (How could we scan all women?) Other ideas might never receive approval from the animal rights committees. “Does sacrificing a bull by throwing it into a volcano decrease the lava flow during the eruption?”

Scientists usually design, critique, and modify an experiment before they commit the time and resources to perform it. Designing an experiment begins with defining variables. The investigator generally manipulates one or more variables, minimizes variation in other variables, and measures responses in a third set of variables. Variables that are manipulated by the investigator are **independent variables**. Variables that are held constant are known as **fixed variables**, and variables that measure responses to the experiment are **dependent variables**.

**Dependent variables** are the responses to the experiment. For example, if a scientist investigates the ability of a new fertilizer to increase growth of soybeans, the height of the plants or the weight of the seeds produced by the plants are dependent variables.

**Independent variables** are controlled by the investigator, and are changed to evaluate how the dependent variables will respond. In the soybean experiment, the amount of fertilizer applied to the plant is the independent variable. It is the factor that you, the experimenter, manipulated. Frequently, inde-
dependent variables can be divided into two subsets: the control treatment, and the experimental treatment(s). The control is usually a standard or baseline treatment to which the experimental treatment is compared. Often the control treatment differs from the experimental treatment by the complete absence of the independent variable in the control condition. Thus, in the soybean experiment, plants with no fertilizer applied would be the control. The experimental treatments would be treatments in which the variable is present at some level. In this case, the amount of fertilizer applied might be 5, 10 and 15 units per plot.

In all experiments, there are many fixed variables. These are variables that might affect the dependent variable, but are not of interest in the hypothesis. For example, in the soybean experiment, the scientist wants to know how a new fertilizer affects growth of soybeans. The experimenter is not interested in how watering affects growth of soybeans, but because it is well known that the amount of water given to a plant strongly affects the growth of the plant, it is important for each plant to receive the same amount of water. Thus, water is a fixed variable in this example.

Prepare for class discussion by thinking about these questions:

1. What other fixed variables should the soybean scientist consider?

2. What is an example of a variable that is completely irrelevant, and is neither fixed nor dependent nor independent?

3. What does a fixed variable become if the scientist decides to also manipulate its conditions?

4. What is a placebo?

Then, analyze the following experiment. Identify independent, dependent, and fixed variables. Is there a problem in the experimental design?

Dr. Chaleb intends to study the effects of household bleach on various items of laundry. He plans to wash clothing items in a Maytag washer without bleach, and compare their color to other items of clothing washed in a Kenmore washer which automatically adds bleach. The items will be compared to determine whether bleach reduces brightness of colors.

Identify:

- Independent variable
- Dependent variable
- Fixed variables

Is there a problem in the experimental design? Explain your answer.

One of the most difficult questions to answer when designing an experiment is how many samples, or replicates, to take. Generally, the same procedure will not produce exactly the same results each time it is run. If you take too many samples, you may be wasting time, materials and money. On the other hand, if you take too few samples, it may be difficult to draw any meaningful conclusions from your results. Sampling is a compromise between accuracy and effort, and scientists often find that they do not take enough samples because of their failure to plan ahead. In our biology laboratory you will find that the sample sizes of experiments will be greatly influenced by lab time, materials, space and whether or not you have taken the time to read your instructions thoroughly ahead of time. In Biology 107 experiments, a minimum of three trials is required. Students are encouraged to improve their lab report, and therefore their grade, by using more than three trials.

Section C

Measuring volumes with pipettes is an important technique in biology and chemistry labs. Each group of two students may use 1 ml, 5 ml and 10 ml pipettes, pipette bulbs, and a flask of distilled water. Detailed instructions on the use of pipettes and suction bulbs are given in the Lab Manual Appendix, page C1. Read this section carefully before you begin.
Practice measuring the following volumes. You may dispense the liquid into the waste beaker provided.

0.1 ml using a 1 ml pipette
2 ml using a 10 ml pipette

After practicing, take your flask of water and your pipettes and move to a work table which contains the electronic top-loading balances. Place a weighing boat on the balance and press the button labeled zero to tare the balance to 0.000 g. Using the volumes below, weigh the specified amounts of water into the boat. Repeat each volume listed 4 (four) times and enter the mass of the liquid in the spaces provided. Use the zero button to reset the balance to zero before each addition. There is no need to remove liquid from the boat between weighings as long as the balance is set to zero each time. For now, just gather the data. You will calculate means and standard deviations in Section E.

0.1 ml from the top of a 1 ml pipette (between the 0.0 and 0.1 mark) weighed:

Use the same 1.0 ml pipette to weigh 0.75 ml from the top and bottom of the pipette.

Top
Bottom

Use a 5.0 ml pipette to measure 1.5 ml from two areas of the pipette.

Top
Bottom

Use a 10.0 ml pipette to measure 1.5 ml.

Top
Bottom

Section D

Concentration measurements are common in biology and chemistry. Since the color intensity of a solution is often related to its concentration, we can measure color intensity and calculate concentration. Some solutions are too concentrated to measure directly. When working with concentrated solutions, we dilute by a known amount, measure the concentration of the dilute solution, and calculate the concentration of the original solution.

In many studies, it is important to know the concentration (how many) of microorganisms in a sample. Milk and water are regularly examined by public health departments, and molecular biologists frequently count the bacteria they use to manipulate DNA and protein molecules.

Take your blue test tube rack to a table with a spectrophotometer. You will learn more about the spectrophotometer in another lab experiment, but today you will use it as an aid to help understand serial dilutions.

Place 4.5 ml of untinted distilled H₂O in each of three tubes labeled 10⁻¹, 10⁻² and 10⁻³. Take 0.5 ml of the undiluted stock (original) solution from its tube and place this 0.5 ml into the tube marked 10⁻¹. Since the total volume in the tube is now 5.0 ml, you have made a ten-fold dilution with this step. Cover the tube with parafilm and mix it by inverting it several times. Remove 0.5 ml of this dilution and place it in the tube marked 10⁻². Cover, mix and make one more ten-fold dilution into the last tube.

Adjust the wavelength on the spectrophotometer to 592 nm. Using a blank tube of distilled water, zero the absorbance reading with the assistance of a lab helper or the instructor. Then, place a tube with 5 ml of undiluted dye solution into the machine, close
the lid and try to read the absorbance. Is this an accurate measurement of the level of color in the tube? Remove the tube and follow the same procedure for all three of your dilutions. A lab assistant has been stationed near the spectrophotometer to assist you.

### Absorbance at 592 nm x Dilution Calculated Absorbance of Undiluted Tube

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Calculation</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-1}$</td>
<td>$x 10$</td>
<td>$______$</td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>$x 100$</td>
<td>$______$</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>$x 1000$</td>
<td>$______$</td>
</tr>
</tbody>
</table>

When you are finished, empty all of the tubes, and clean up the area, but do not turn off the spectrophotometer. Take your test tube rack to the sink and discard your diluted samples. **Do not discard your original undiluted dye solution.** The dye will not harm you or the environment. Rinse your test tubes several times with tap water and invert to drain so that they are ready for the next lab group.

Imagine that your undiluted tube contained $4.3 \times 10^9$ bacteria per ml and you made the same 1:10 dilutions as you did with the tubes of blue dye. That would mean:

- **Undiluted bacteria** = $4.3 \times 10^9$ bacteria per ml
- **10\(^{-1}\) dilution** = $4.3 \times 10^8$ bacteria per ml
- **10\(^{-2}\) dilution** = $4.3 \times 10^7$ bacteria per ml
- **10\(^{-3}\) dilution** = $4.3 \times 10^6$ bacteria per ml

How many more 1:10 dilutions would you need to make to produce a suspension that contained $4.3 \times 10^3$ bacteria per ml? How would you set up the dilutions if you wanted to dilute the original solution to $4.3 \times 10^3$ bacteria per ml in two steps?

**Section E**

Your lab helper will meet with groups of four to show you how to use the course web site.

**Section F**

In Section C, you measured volumes of water from the top and bottom of various pipettes. You could add the four values you found for each volume and divide by four to get a mean number of grams of water, but that would not give you enough information. You also need to know how much the individual weights varied from the mean. Were all of the values close together or was there a wide variation? You need to calculate the standard deviation (s. d.), which is a measurement of the spread of data from the mean. In Biology 107 lab reports, you will need to report the mean +/- standard deviation. The formula for standard deviation is below.

$$
S. D. = \sqrt{\frac{\sum (x_i - \bar{x})^2}{(n - 1)}}
$$

$n =$ the number of trials or samples taken (you used 4 trials for each experiment)

$\sum =$ the summation of all trials

$\bar{x} =$ the mean or arithmetic average

$x_i =$ each observed value that you measured ($x_1$, $x_2$, $x_3$, $x_4$)

Do not use your calculator to determine the first standard deviation. Do the addition and subtraction steps by hand and when you get to the point where you need to find the square root, use your calculator or the one on the instructor’s desk for this step only. Show all of your work. An example of how to calcu-
late the standard deviation is provided for you at the
top of page 14. After you have studied the example
provided, enter your experimental pipette volume
values under the headings “mass 1” (found in the
second box at the bottom of the page), “mass 2”, etc.
Use the values obtained when you weighed 1.5 ml
using a 5.0 ml pipette using the “top” of the pipette.

After you have calculated your first mean and stan-
dard deviation and understand the necessary steps,
you may use your calculator for the remaining means
and standard deviations for the data you gathered in
section B. Enter your results in the table on page 13.

When calculating mean and standard deviation,
your calculator or spreadsheet program will often
return values with many numerals to the right of the
decimal. Reporting all of these numerals in your lab
report is known as false precision, and is incorrect.
Knowing how many decimal places to report in your
answer is an important skill that you will develop as
you work with data in biology, chemistry and phys-
ics classes. For now, report your answers using the
number of decimal places that you were able to accu-
ately measure. For example, the mass of 1.5 ml of
water may have been measured as 1.455 grams on
the digital balance. During measurement, though,
the student notices that the thousandths digit on the
balance display fluctuates during the measurement,
so she enters a mass of 1.45 grams. All further means
and standard deviations calculated using this mass,
then, should not have more than two places to the
right of the decimal point.
Lab Assignment - Exercise 1
Hand in this page next week in lab with typed answers to the questions below stapled behind it. Each student must complete this page, including answers to the questions, individually.

Name ___________________________________      Lab Section _______

A. On page 14, show the work you did to calculate a standard deviation. Use the data from the 1.5 ml dispensed from the top of the 5.0 ml pipette (bold text in the table below). Remember to turn in page 14.

B. You and your partner collected data on page 10. Enter those data in the table below and calculate the mean +/- standard deviation for each category.

<table>
<thead>
<tr>
<th>Pipette</th>
<th>Volume (ml)</th>
<th>Measurements (g)</th>
<th>Mean Mass (g)</th>
<th>+/- SD (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Example</td>
<td>(data from page 12)</td>
<td>15, 12, 8, 13</td>
<td>12</td>
<td>2.9</td>
</tr>
<tr>
<td>1.0 ml</td>
<td>0.1 ml from top</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 ml</td>
<td>0.1 ml from bottom</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 ml</td>
<td>0.75 ml from top</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 ml</td>
<td>0.75 ml from bottom</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.0 ml</td>
<td><strong>1.5 ml from top</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.0 ml</td>
<td>1.5 ml from bottom</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.0 ml</td>
<td>1.5 ml from top</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.0 ml</td>
<td>1.5 ml from bottom</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

C. Look at your data on page 11. From these data, decide how to best compute your one best estimate of the absorbance of the undiluted tube. You have several estimates. Should they all produce similar answers? Why did you choose the one you did?

D. Questions: **Type your answers and staple them to this sheet.**

1. Which pipette would you use to measure 1.5 ml? Would you choose a 5 ml or a 10 ml pipette? Why?

2. Your instructor gives your group 15.0 ml of a 8.0 x 10^9 cells per ml bacterial suspension and asks you to prepare 1.0 ml of 2.0 x 10^6 cells per ml suspension using the tools from today’s lab. Use a drawing (can be hand drawn) to indicate how you would make this suspension.

3. In the table above, you were making four measurements of the same mass, so the standard deviation gives you an indication of the accuracy of your pipetting. In other cases, the standard deviation might represent something different. Imagine that we collected data on the credit-card balance of each student in the class. What would the standard deviation tell us for this data set? Why is that different from the meaning of standard deviation for the pipetting data?
<table>
<thead>
<tr>
<th>Activity</th>
<th>Mass 1 (g)</th>
<th>Mass 2 (g)</th>
<th>Mass 3 (g)</th>
<th>Mass 4 (g)</th>
<th>Sum</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enter Measurements</td>
<td>15</td>
<td>12</td>
<td>8</td>
<td>13</td>
<td>48</td>
<td>12</td>
</tr>
<tr>
<td>Find Sum and Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculate Differences</td>
<td>3</td>
<td>0</td>
<td>-4</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(measurement - mean)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Square Differences</td>
<td>9</td>
<td>0</td>
<td>16</td>
<td>1</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Find Sum of Squares</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Determine Sample Size</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n; number of measurements)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample Size - 1</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n-1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculate Variance</td>
<td>8.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(sum of squared differences</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>divided by (n-1))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculate Standard Deviation</td>
<td>2.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(square root of the variance)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

S.D. = \( \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}} \)

- \( n \) = number of measurements in your dataset
- \( \Sigma \) = the summation of (add the following values across all measurements)
- \( \bar{x} \) = the mean of your dataset
- \( x_i \) = an individual measurement

The Standard deviation tells you how much uncertainty you have in the mean generated from a set of data. In this case, the measurements in the first row have a mean of 12. The mean is then subtracted from each measurement to give the values in the second row (e.g. 15 - 12 = 3). The differences are then squared, so that positive and negative differences contribute to a standard deviation that is always a positive number. The squares of the differences are entered in the third row. They add to 26. The next step is to divide the sum of the squared differences by (n - 1). Since we have four measurements, n = 4, and n - 1 = 3. You should divide 26 by 3 to give 8.7. The square root of this number is the standard deviation, 2.9. That means that we have estimated the mean to be 12 +/- 2.9.