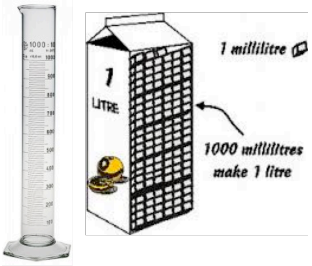
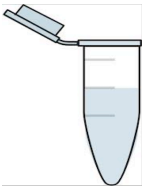
Pipetting exercise

The ability to safely and accurately transfer volumes of liquid from one container to another is one of the most important skills used in the biology laboratory. This exercise will familiarize you with the equipment used to transfer smaller amounts of liquid, and give you the skills to use these tools in future experiments. **Micropipettors** are used to transfer liquids less than 1 millilliter (ml) - an amount equal to 1000 microliters (µl).

Micropipettors



1 Liter (L) = 1000 milliliter (ml)



1ml

1µl

1 milliliter (ml) = 1000 microliter (µl)

Part 1:

Materials:
B stock fluid
R stock fluid
Y stock fluid
Micropipettors and tips
15 ml conical tube filled with water
microcentrifuge tubes (9)

Protocol:

1. Familiarize yourself with the micropipettors: set and eject tips a couple times (note different sizes), change volume settings (note range), note the different resistance of the thumb piston at different settings **(first stop – "to aspirate the set volume"; second stop – "expel all tip contents")**; gently pipette different volumes of water from the 15 ml conical tube of water and expel into the same 15 ml conical tube a couple times (try from 5 µl to 1000 µl).
2. Label 3 microcentrifuge tubes: "V Stock", "G Stock" and "O Stock".

3. Using P10 and P100 (with tip!), pipette 10 µl of “B Stock” and 100 µl of “R Stock” solutions into “V Stock” microcentrifuge tube (remember to change tips!); cap and mix gently.
4. Using P100 (with tip!), pipette 20 µl of “R Stock” and 100 µl of “Y Stock” solutions into “O Stock” microcentrifuge tube (remember to change tips!); cap and mix gently.
5. Using P100 (with tip!), pipette 20 µl of “B Stock” and 100 µl of “Y Stock” solutions into “G Stock” microcentrifuge tube (remember to change tips!); cap and mix gently.
6. You should now have six “stock solutions”.
7. Label remaining 6 microcentrifuge tubes: R, O, Y, G, B and V.
8. Using P1000, pipette 1 ml of water from the 15 ml conical tube into each of the 6 microcentrifuge tubes that you labeled R, O, Y, G, B and V. Each student pipettes 1ml into 3 tubes.
9. Using P100 (with tips and remember to change each time)….
    - pipette 100 µl of “R Stock” into the R tube containing 1 ml of water
    - pipette 100 µl of “O Stock” into the O tube containing 1 ml of water
    - pipette 100 µl of “Y Stock” into the Y tube containing 1 ml of water
    - pipette 100 µl of “G Stock” into the G tube containing 1 ml of water
    - pipette 100 µl of “B Stock” into the B tube containing 1 ml of water
    - pipette 100 µl of “V Stock” into the V tube containing 1 ml of water
    - close cap completely and mix gently by inversion.

Note your results.

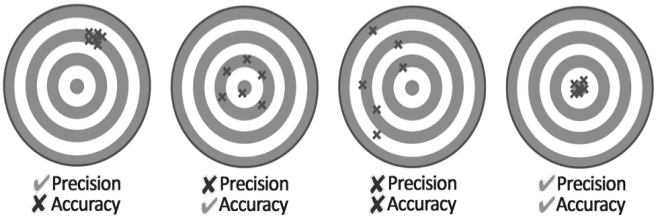
Part 2: Precision and Accuracy

Measurements are precise when multiple values are repeatable.

Measurements are accurate when the actual value achieved is the intended value.

When the pipettor is set at 100 µl, it should deliver 100 µl to be considered accurate, and do this consistently to be considered precise.

Error may be a result of the instrument (pipettor error), technique (pipetter error) or method (using a pipettor outside of its acceptable range). The first is avoided by regular maintenance and calibration. You can minimize the latter two yourself with consistent practice and attention to detail. When pipetting it is important to be both precise and accurate.



Materials:
Micropipettors and tips
15 ml conical tube filled with water
1 square of parafilm (4X4 inches)

Protocol:

1) Using a P10, pipette 5 µl of any colored water from the Part 1 onto the square of parafilm provided. Repeat 4 additional times. Do the amounts of the liquid dispensed appear to be the same? Are any larger or smaller? If they look to be different, keep trying until you get 5 consecutive drops that appear to be the same size. (Note: slow smooth pressure on the pipette plunger is key to consistency).

2) Again using a P10, pick up each "drop" of colored water and dispense in a different area of the parafilm. Was all of the liquid transferred or did any get left behind? Did any air bubbles get in your tip? Do all of the amounts still look the same? Practice this step until you and your partner are comfortable picking up and moving the drops in their entirety, without air bubbles.

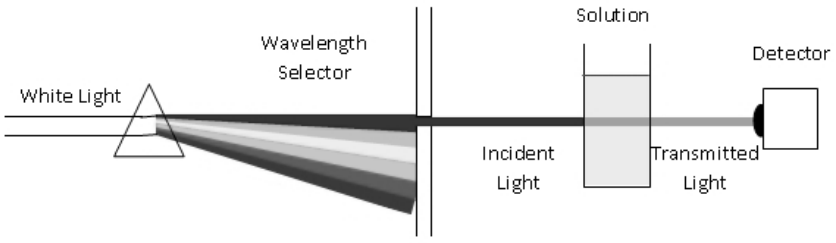
3) Using a P10, pipette 1 µl of any colored water onto the square of parafilm provided. Adjust the pipette to transfer 2 µl and pipette 2 µl of the same colored water onto the parafilm next to the 1 ul droplet. Repeat this process increasing 1 µl at a time until you pipetted 10 µl in side by side dots. When can you visualize differences in the different amounts?

Part 3: Serial Dilution

Serial dilutions are an essential skill for a variety of experiments as well as general stock preparation. You will be making a series of dilutions to test your new pipetting skills. Often the original concentration of a stock solution may not be known, or may be too concentrated to be measurable by whatever method is to be used. It is then necessary to dilute the stock solution to a concentration that is measurable. Why not just take a very small volume and dilute it into a very large volume (i.e. 1 µl into 1 L)? Because this method is much more likely to introduce error and yield flawed results. We avoid this error by making a series of dilutions referred to as a serial dilution. A serial dilution covers a range of concentrations that is more likely to result in at least one measurable sample. When the concentration is determined for the measurable diluted sample, the original concentration can be calculated by multiplying by the dilution factor.

You will be making two initial larger dilutions of the blue food coloring followed by a 2 fold serial dilution series.

The concentrations of solutions are often measured by determining the optical density (OD) at the appropriate wavelength with a spectrophotometer. The concentration of cells, microorganisms, DNA and RNA (quantity and quality), and in this case dye can be measured this way. Samples are placed in a specialized holder called a cuvette and placed in the spectrophotometer. Light at the appropriate wavelength is passed through the sample and the amount of light that is absorbed/optical length is determined. This is referred to as the OD.



The difference between the incident and transmitted light indicates the absorbance

Materials:
Original Blue Sample (labeled OBS)
Micropipettors and tips
15 ml conical tube filled with water
microcentrifuge tubes (7)
prelabeled cuvettes in rack

Protocol:

Dilution Steps:

Note: Remember to change tips between steps to prevent carryover from skewing your results.

1) Label 6 microcentrifuge tubes P,1,2,3,4, and 5.

2) Pipette 98 µl of water into Tube P, 950 µl of water into Tube 1, and 500 µl of water into Tubes 2-5.

3) Pipette 2 ul of blue dye (OBS) into Tube P and mix well by gently flicking. (50 fold dilution, total dilution 1/50)

4) Pipette 50 µl of Tube P into Tube 1 and mix well. (20 fold dilution, total dilution 1/1000)

5) Pipette 500 µl of Tube 1 into Tube 2 and mix well (2 fold dilution, total dilution 1/2000)

6) Pipette 500 µl of Tube 2 into Tube 3 and mix well (2 fold dilution, total dilution 1/4000)

7) Pipette 500 µl of Tube 3 into Tube 4 and mix well (2 fold dilution, total dilution 1/8,000)

8) Pipette 500 µl of Tube 4 into Tube 5 and mix well (2 fold dilution, total dilution 1/16,000)

Measurement Steps:

You will measure the OD of the colored solutions in Tube 1-5 on the GenSys spectrophotometer. Cuvettes are utilized to hold your samples in the instrument. After you have transferred your samples to the cuvettes, your instructor or teaching fellow will guide you on how to measure the OD. Tube 1-5 should be measurable at 595 nm. If you have pipetted correctly, each dilution should read ½ the OD of the previous diluted tube.

Note: When handling cuvettes only touch the upper most part, fingerprints may interfere with light penetration and skew the results. Gently tap the cuvette on the counter if necessary to remove bubbles or ensure all the liquid is at the bottom of the cuvette.

1) Pre-labeled cuvettes B and 1-5 are located in a cuvette holder

2) Into the cuvette labeled B, pipette 400 µl of water. This will be used to blank the instrument. Blanking is necessary to tell the instrument what "0" is. In this case, water alone.
3) Into the remaining cuvettes: pipette 400 µl of Tube 1 into the cuvette labeled 1. Continue this process by pipetting 400 µl of Tube 2 into cuvette 2, 400 µl of Tube 3 into cuvette 3 etc.
4) Read the OD of each solution with the help of your instructor or teaching fellow. Record the results on the sheet provided.