1 MB experiment 1: Lab measurements

Purpose: This is up to you to write down.

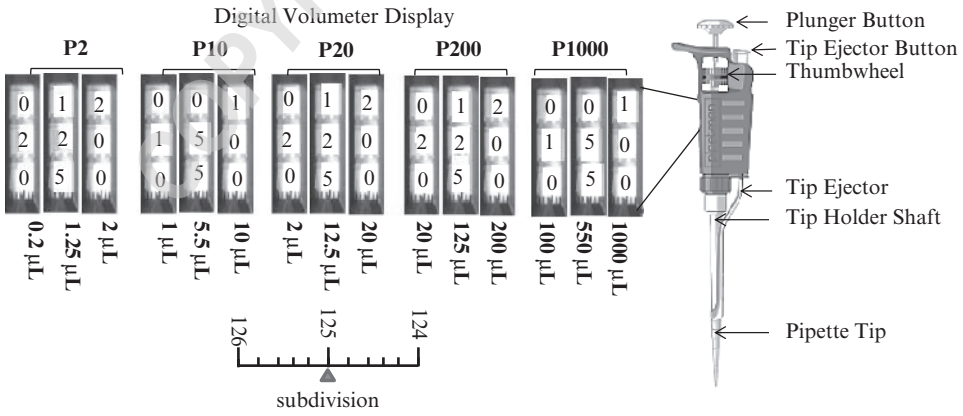
Introduction

Experiments frequently require measurement of volume and mass. Accurate and precise measurements are necessary to produce the reliable and reproducible data. In a biotechnology lab, volume is often measured in microliters (μL) and mass is calculated in terms of microgram (μg), nanogram (ng), or picogram (pg) in order to set up reactions in microcentrifuge tubes. You will be using micropipettors throughout the experiments in order to draw up small volumes ranging from 0.1 to 1000 μL. Depending on the volume to be withdrawn, you have to choose the correct model of micropipettor (P2, P10, P20, P200, or P1000). You must familiarize yourself with setting the volume and the effective range of volume that can be taken by each micropipettor. The maximum microliter volume that can be withdrawn is indicated by each pipettor model number, and the minimum volume is typically one-tenth of the model number. Most of the digital volume indicator consists of three numbers reading from top to bottom, as shown below.

In P1000, the top, middle and bottom numbers are for 1000s, 100s, and 10s of μL, respectively. In P200, the top, middle, and bottom numbers denote 100s, 10s, and 1s of μL, respectively. In P20 and P10, the top, middle, and bottom numbers indicate 10s, 1s, and 0.1s of μL, respectively. In P2, the top, middle, and bottom numbers refer to 1s, 0.1s, and 0.01s of μL, respectively.

There are five subdivision line marks between the last digits in all the micropipettors. For example, five subdivisional increments between 125 and 126 or between 124 and 125 exist for P2, P20, and P200. The same subdivisional increments between 055 and 056 or between 054 and 055 exist for P10 and P1000. Accordingly, each subdivision mark equals 2 μL for P1000, 0.2 μL for P200, 0.02 μL for P20 and P10, and 0.002 μL for the P2 micropipettor.

The volume is set by either turning the plunger or thumbwheel to read the numerical settings displayed. To use the plunger button makes it easier and faster to set volumes, especially when you wear gloves. However, you must not rotate the plunger or thumbwheel beyond the upper or lower range limit of the pipettor, though the 0.1 μL setting for P2 and 0.5 μL setting for P10 can be used when a sample is taken carefully according to the manufacturer's instruction. If you want to draw up the volume that goes beyond the upper limit of a pipettor, you may first set the lower volume within the range to draw a sample and then set the additive volume to draw the sample using the same pipettor and tip instead of changing to a larger volume pipettor. Of course, you need to use a lower volume pipettor when the minimum volume of a pipettor is higher than the volume you want to take. Most micropipettors have two stop points on the plunger to draw and expel a liquid, as shown below.



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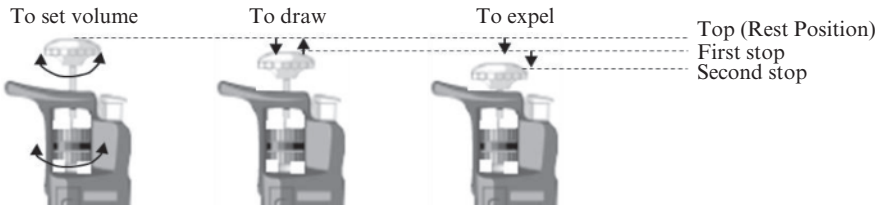


First, fit the tip to the end of the shaft and press down to ensure an airtight seal. Make sure that the pipet is securely placed on the micropipettor. If the tip is loose on the pipettor, the volume will not be correct and the tip may fall down. However, do not impose an excessive pressure pushing down on the pipette tip as this may cause breakage of the shaft or pipette tip box. Second, press the plunger to the first stop point, immerse the pipet tip about 2 mm below the surface of the solution (not too deeply into the solution!) and slowly release the plunger to fill the tip. If you release the plunger too quickly, the liquid may splash up into the micropipettor and contaminate it. If you draw up viscous (thick) liquids, such as dense glycerol, sucrose, and detergent solutions, too quickly, the liquid will not enter the tip fast enough and your measurement will be inaccurate. Sometimes this happens with thin liquids as well, so you should pipette slowly and wait a few seconds to ensure that the tip is filled with the full volume. Lastly, insert and touch the tip end to the bottom of tube, and press the plunger down to the second final stop point to expel the fluid. Neither drop the liquid nor shake the tip to expel the last drop of tiny liquid adhering to the tip point.

If you are working with a power-assisted pipette-aid to deal with larger volumes (>5 mL), the upper button fills the pipet and the lower button expels the fluid. Do not draw up the liquid beyond the upper marking of the pipet because this will wet the cotton in the neck of the pipet and the pipet will no longer work.

When working under sterile condition, the following rules apply:

– Anything that your hand (even glove-worn hand) touches is no longer sterile. If your hand passes over an open sterile plate or bottle, it is likely that you have contaminated these contained items.

– If the pipet tip touches anything other than a sterile surface, it is no longer sterile.

– Keep your workspace clean and in good order. A messy workspace could cause contamination of your experiment.

– When transferring sterile liquids from a stock bottle, wipe out the micropipettor plastic shaft with 70% ethanol paper towel, remove the cap with one hand, and insert the pipet at a slight angle into the bottle without touching the neck of the bottle with the micropipettor shaft. While transferring the fluid to the plate or tube, replace the lid on the bottle. If multiple transfers are needed from one bottle to several plates or tubes, the lid is placed on the bottle loosely and replaced after all transfers. Do not pass your hand over the open bottle.

It is recommended to watch the following YouTube videos as to how to use a micropipettor and Spectronic 20 prior to coming to the lab:

- https://www.youtube.com/watch?v=tL0acTneiNY (Auto pipet technique)
- https://www.youtube.com/watch?v=y-OHnnhWCdo (How to pipette: lab survival skills)
- http://www.youtube.com/watch?v=jmZomizSPxw&NR=1 (Using the Spec-20)

Pre-lab assignment

(Typing and submission must be completed before lab work begins.)

1. You have P2, P10, P20, P200, and P1000 micropipettors, as shown below. Fill out the table to indicate the µL volume for each of the five pipettors according to the volume setting, as shown below. Indicate "NO" if the volume setting cannot be used for the pipettor chosen.

1	0	0	2
3	3	8	2
2	2	2	0

Pipettor / Volume setting	P2	P10	P20	P200	P1000
132 (top to bottom)					
032 (top to bottom)					
082 (top to bottom)					
220 (top to bottom)					

2. How would you draw 205 µL using a P200 pipettor? How would you draw 1010 µL using a P1000 pipettor?

3. What micropipettor would it be best to take all of the individual volumes in each of Part I, C1 and C2 exercises?

4. How would you prepare 1.5 mL of 1 mg/mL blue dextran solution using a 10 mg/mL blue dextran stock solution (at Part I, step D8)? Show your work.

5. How much 3 M NaCl is needed to conduct the Part II experiment? Show your calculations.

Materials and equipment

- Four different dye solutions, dH_2O
- 50% glycerol
- Solutions A, B, C, D (different color solutions)
- Blue dextran (10 mg/mL in dH_2O)
- 3 M NaCl, unknown NaCl solutions I and II
- Parafilm
- P2, P10, P20, P200, P1000 micropipettors, micropipette tips
- 0.5-mL and 1.5-mL microcentrifuge tubes (non-sterile)
- Borosilicate glass test tubes (13 mm × 100 mm)
- Test tube racks and microcentrifuge tube racks
- 70% ethanol wash bottle
- Spectronic 20, cuvette
- Analytical balance

Procedure

Part I. Pipetting skill

A. Volume setting practice

Pipetting error is a major contributor to poor laboratory results. Two important sources of pipetting error are the use of an uncalibrated micropipettor causing systematic error and the practice of inaccurate pipetting introducing random error.

1. Practice setting the volume on the micropipettors; each person in your group should set at least one and have it checked by other group members and/or your instructor. Look at the plunger button top of the micropipettor to identify its measuring range. Remember that the value listed on the top is the largest volume you can measure on that pipettor and that the lowest volume is 1/10 of the largest volume. On a P1000 micropipettor, the largest and lowest measurable volumes are 1000 and 100 µL, respectively; 200 and 20 µL on a P200 micropipettor; 20 and 2 µL on a P20 micropipettor. Set the P1000 pipettor to 0.45 mL (450 µL), the P200 micropipettor to 0.15 mL (150 µL), and the P20 micropipettor to 0.015 mL (15 µL). You should practice doing that kind of conversion in your head.

2. Insert a tip into the end of the pipettor by pushing the end of the micropipettor firmly into the tip in the box. Do not touch the tip with your hands. The smaller tips fit both the P20 and the P200 micropipettors. They are often yellow or clear. The larger tips are for the P1000 micropipettor and are often blue.

3. Take up the water volume (microliter) you want, empty into a waste beaker, and discard the tip in a waste beaker by pressing the eject button. You may want to practice this technique a few times as it is a very important skill to master.

B. Checking pipettor calibration

- 1 mL H_2O = 1.0 (0.995–0.998) g at 25 °C
- Check the mass of each volume using an electronic analytical balance:

– P1000: 500 µL = 0.5 mL = 0.5 g
– P200: 100 µL = 0.1 mL = 0.1 g
– P20: 10 µL = 0.01 mL = 0.01 g

1. Place a strip of parafilm on the balance pan.
2. Set the balance to zero.
3. Dispense the water on to the balance and read the reading.

C. Pipetting practice (accuracy and precision)

Accuracy is how close a measurement value is to the true value or accepted value, referring to a mean value of measurements. Precision is the consistency of a series of measurements or tests, referring to standard deviation. Familiarize yourself with the amount of liquid filled in a tip. Try to develop a sense for the volume of liquid you are pipetting by looking at the amount of liquid in the tip. It is easy to grab the wrong pipettor or to set it incorrectly; however, you should be able to tell the difference between 1 µL in a tip and 10 µL in a tip just by looking at the amount of liquid in the tip.

1. Add the following solutions to two separate 0.5-mL microcentrifuge tubes:

Solution A	6.5 µL
50% glycerol	9.0 µL
Solution B	1.5 µL
Solution C	1.0 µL
Solution D	2.0 µL

The total volume should be 20 µL. Check the accuracy of your measurements by setting a micropipettor to the total volume in the tube and slowly withdrawing all of the solution. Your pipetting is accurate if you leave no solution behind and have no air bubble trapped in the liquid solution in your tip.

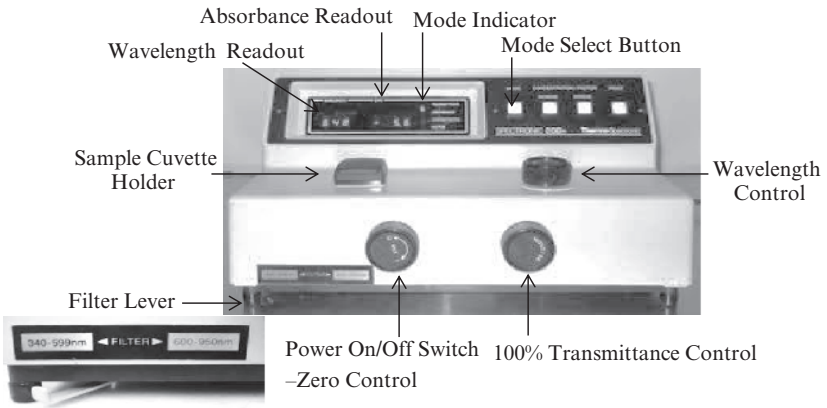
2. Add the following solutions to two separate 1.5-mL microcentrifuge tubes:

Solution A	75 µL
50% glycerol	50 µL
Solution B	125 µL
Solution C	150 µL
Solution D	250 µL

The total volume should be 650 µL. Check the accuracy. Is the tip just filled? If so, is any solution left in the tube? Is the tip underfilled with air space?

D. Spectrophotometric test for pipetting

1. Turn on the Spectronic 20 instrument to warm up.
2. Set the wavelength control knob to 630 nm. Make sure that the filter lever on the bottom left is positioned at 600 to 950 nm.
3. Using the zero control knob on the left side, set the absorbance to read 0% transmittance (% T) on the top of the meter. Nothing should be in the sample cuvette holder.



4. Prepare the following triplicate solutions (A1, A2, A3) in test tubes # 1 to 5.

Tube #	Volume of blue dextran (10 mg/mL)*	Volume of water needed† (µL)	Total volume (µL)	µg of blue dextran
0	0 µL (A1, A2, A3)	3000	3000	
1	2 µL (A1, A2, A3)		3000	
2	4 µL (A1, A2, A3)		3000	
3	6 µL (A1, A2, A3)		3000	
4	10 µL (A1, A2, A3)		3000	
5	20 µL (A1, A2, A3)		3000	

*Remove each dextran volume of water, add each volume of dextran starting from tube #1 to tube #5, and rinse pipette tip by pipetting up and down. Do not change the pipette tip.
†Add 3 mL of water first to all tubes using a 5-mL pipette.

5. Fill one cuvette with H_2O as a blank and insert it in the sample cuvette holder with the line of the cuvette facing the front. Close the top.

6. Adjust % transmittance to read 100 using the transmittance control knob, and push the "mode" button to get 0.000 absorbance. Do not touch the wavelength control, zero control, and transmittance control knobs once the instrument is calibrated.

**If you accidently touch them during sample measurements, re-set following steps 5 and 6.*

7. Take out the cuvette, pour off the blank water, and add each sample to the empty cuvette, and read the absorbance of each sample at 630 nm.

Tube #	µg of blue dextran	A_{630} sample A1	A_{630} sample A2	A_{630} sample A3	Mean value	Standard deviation
0						
1						
2						
3						
4						
5						

8. Prepare 1.5-mL of a 1-mg/mL blue dextran from a 10-mg/mL stock solution, and then make the following triplicate solutions (B1, B2, B3) in glass test tubes # 1 to 5.

Tube #	Volume of blue dextran (1 mg/mL)	Volume of water needed (µL)	Total volume (µL)	µg of blue dextran
0	0 µL (B1, B2, B3)	3000	3000	
1	20 µL (B1, B2, B3)		3000	
2	40 µL (B1, B2, B3)		3000	
3	60 µL (B1, B2, B3)		3000	
4	100 µL (B1, B2, B3)		3000	
5	200 µL (B1, B2, B3)		3000	

9. Repeat steps 5 to 7 to read the absorbance of each sample at 630 nm.

Tube #	µg of blue dextran	A_{630} sample B1	A_{630} sample B2	A_{630} sample B3	Mean value	Standard deviation
0						
1						
2						
3						
4						
5						

10. Using the table data obtained from step 7 and 9, plot a graph indicating µg of blue dextran on the *x* axis and A_{630} on the *y* axis. Use the mean A_{630} values of three samples to display data points. Connect the data points using different colors or symbols to distinguish between the two best-fit lines on the same graph. Put a standard deviation bar (|) on to each data point. Compare the two graphs.

Part II. Density and specific gravity

Density (ρ) is defined as mass (m) per unit volume (v): $\rho = m/v$ with units of kg/m^3, g/cm^3, or g/mL. The specific gravity for a liquid has the same numerical value as the density of that liquid if the unit of the density is g/mL. The specific gravity is used to find out the purity of a drug compound since each chemical has a distinct specific gravity, as well as to calculate volume of a fluid using the specific gravity of the fluid. Conversely, the weight can be calculated if the volume is known.

In this experiment, you will determine the densities of different concentrations of NaCl solutions by measuring the weight of a known volume of liquid. You will then determine the concentration of unknown NaCl solutions from the standard curve.

Procedure

1. Label each duplicate tube 1 and 2 for six NaCl molarity solutions (see the table below showing the effect of NaCl concentration on solution density), measure the individual weights of 8 empty 1.5-mL microcentrifuge tubes, and record the weights.

NaCl molarity	Mass of tube		Volume of solution		Mass of tube plus solution		Mass of solution		Mean value of solution mass	Density of solution
	1	2	1	2	1	2	1	2		
3.0										
1.5										
0.75										
0.375										
Unknown 1										
Unknown 2										

2. Prepare 1 mL of 3.0 M, 1.5 M, 0.75 M, and 0.375 M NaCl solutions in the above-measured microcentrifuge tubes. (*Show calculations of how to prepare them using 3 M stock solution in the pre-lab notebook*.) Add unknown NaCl solutions 1 and 2 to the measured microcentrifuge tubes.
3. Measure the individual weights of the microcentrifuge tubes containing solutions and determine the weight of the solution by subtracting the weight of the microcentrifuge tube from the weight of the microcentrifuge tubes containing solution.
4. Calculate the density of the solution.
5. Plot the density and solution concentration (M NaCl) data on the graph with the density on the *y* axis and the M NaCl on the *x* axis.

**Adjust the axis scales so that the data points are spread out and occupy as much of the graph space as possible with a 45 degree slope and the major and minor tick marks on both axes*.

6. When the data points have been plotted, draw a best-fit line through them. Do not connect the dots. Use this graph to determine the concentrations of NaCl in unknown samples. You may use a Microsoft Excel program to plot a calibration curve. Regarding how to use, visit the online website (http://www.youtube.com/watch?v=NJYAMNlBGb4).

Discussion

(Do not copy the number and discussion point. Write a paragraph in your own words.)

1. If your micropipetting technique was good, the graph you produced should be a nearly straight line from start to end. The more points that deviate from the line, the more inaccurate is the pipetting skill.
2. The standard deviation for the three values for triplicate solutions provides a measure of the reproducibility of your pipetting skills. The smaller the standard deviation, the more precise is your pipetting skill.
3. Compare the two best-fit lines on the same graph. Do the two lines overlap? If not, explain why.
4. Suggest something you could do to ensure that your micropipettor is measuring correctly.
5. Describe the relationship between the concentration and the density of solution. How do they differ from each other?

Post-lab assignment

1. Why are there no units for specific gravity?
2. How do you determine the density of water-insoluble solids like gold and the density of water-soluble solids like sugars and salts?
3. Convert the molar NaCl solutions used in Part II into g/mL and compare with their respective densities of NaCl solution measured in Part II. How does solution density differ from solution concentration in g/mL?
4. If the density of propylene glycol (antifreeze) is 1.04 g/mL, what is the volume in mL of 4.92 lb of antifreeze (1 lb = 454 grams)?
5. An aqueous vinegar sample contains 4.4% acetic acid (v/v) and has a density of 1.006 g/mL. Calculate the amount of acetic acid in grams in 750 mL of this vinegar.
6. What instrument other than analytical balance is used to determine the concentrations of salt solutions and purity of water?
7. Draw the position lines of 10, 20, 50, and 100 µL volumes when you pipetted a solution into 0.5-mL and 1.5-mL microcentrifuge tubes. Sketch the lines as accurately as possible on the basis of *actual size* of the microcentrifuge tubes. (Hand drawings to the actual scale of both the volumes and centrifuge tubes are required.)
8. Draw the position lines of 2, 5, and 10 µL volumes in P20 pipette tip. Sketch the lines as accurately as possible on the basis of *actual size* of a P20 pipette tip. (Hand drawings to the actual scale of both the volumes and P20 pipette tip are required.)

Further reading

Miller, J.S., Sass, M.E., Wong, S.J., and Nienhuis, J. (2004). Micropipetting: an important laboratory skill for molecular biology. *The American Biology Teacher*, **66** (4): 291–296.

Seiman, L.A. and Moore, C.J. (2000). The measurement of volume. In *Basic Laboratory Methods for Biotechnology*, pp. 301–339. Prentice Hall, Inc. ISBN 0-13-795535-9.