

LAB #2: Microquantity Measurement

Objectives:

- Convert mL to μL and μL to mL.
- Identify techniques for proper handling of pipettes, micropipettes and a microcentrifuge.

Introduction:

This part of the lab introduces micropipeting and sterile pipeting techniques used throughout DNA laboratories. Mastery of these techniques will be important for good results. Most microchemical protocols involve very small volumes of DNA and other reagents. These require you to use an adjustable micropipet that measures as little as one microliter (μL) a millionth of a liter, compared to millileters (mL) which are only one thousandth of a liter.

SAFETY

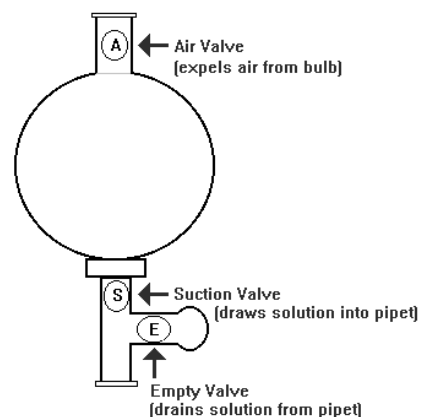
THE SOLUTIONS FOR THIS PART OF THE LAB ARE COLORED WATER. THE SAFETY PRECAUTIONS LISTED BELOW ARE TO PREVENT DAMAGE TO THE MICROPIPET.

- NEVER SET THE MICROPIPETTE TO A VOLUME BEYOND ITS RANGE.
- NEVER ATTEMPT TO USE THE PIPET WITHOUT A TIP IN PLACE.
- NEVER LAY DOWN A PIPET THAT HAS A FILLED TIP.
- NEVER LET THE PLUNGER SNAP BACK AFTER WITHDRAWING OR EJECTING FLUID.

Procedure:

USING A PIPET WITH A SAFETY BULB

1. Take a 10 mL glass (or nalgene) pipet. Carefully place the attachment of the three-way bulb over the mouth of the pipet. Squeeze the air valve (A) and the bulb simultaneously to empty the bulb of air. Place the tip of the pipet below the solution's surface in the beaker. Gradually squeeze the suction valve (S) to draw liquid into the pipet. When the liquid is above the specified volume, stop squeezing the suction valve (S). Do not remove the bulb from the pipet.
2. DO NOT ALLOW LIQUID TO ENTER THE PIPET BULB. If the level of the solution is not high enough, squeeze the air valve (A) and the bulb again to expel the air from the bulb. Draw up more liquid by squeezing the suction valve (S).
3. Touch the tip of the pipet to the inside of the beaker to remove the drop hanging from the tip. If this drop is not eliminated, the volume transferred will be slightly higher than the volume desired.
4. Once you have drawn up the desired volume of solution and removed the drop hanging from the tip, record the initial volume in the pipet.
5. To transfer the solution into the desired vessel, press the empty valve (E) until the meniscus is at the mark corresponding to the appropriate volume. Touch the tip of the pipet to the wall of the receiving vessel to remove any liquid from the outside of the tip. Record the final volume in the pipet. The volume transferred is equal to the final pipet reading minus the initial pipet reading.

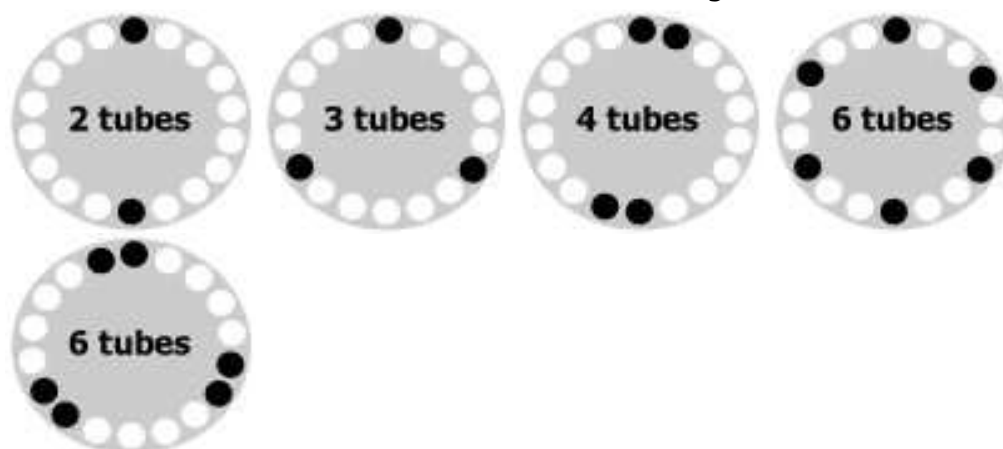


USING A MICROPIPETTE

6. Rotate the control button to the desired volume. Notice the change in the plunger length as the volume is changed.
7. Push the pipet end firmly in the proper size tip.
8. While withdrawing or expelling fluid, always hold the vessel at nearly eye-level. It is important that you watch while you pipet.
9. Hold the pipet in a vertical position when filling.
10. To draw fluid, depress the button to the first stop, and hold in this position. Then, dip the tip into the solution to be pipeted, and draw the fluid into the tip by gradually releasing the plunger.
11. Slide the tip out along the inside wall of the reagent tube to dislodge any excess fluid adhering to the outside of the tip.
12. To withdraw the sample, touch the pipet tip to the inside wall of the reaction tube into which you wish to empty the sample. This creates a capillary effect which helps draw fluid out of the tip.
13. Slowly depress the button to the first stop. Then press on to the second stop to blow out the last bit of fluid. Hold the button down in the second position.
14. Slide the pipet out of the reagent tube with the button depressed to the second stop to avoid sucking any liquid back into the tip.
15. To eject the tip, depress the separate thumb button to 'launch' tip into a waste jar.
16. To prevent contamination of your reagents:
 - Always add appropriate amounts of a single reagent sequentially to all reaction tubes.
 - Release each reagent drop onto anew location on the inside wall of the reaction tube. In this way you can use the same tip to pipet reagent into each reaction tube.
 - Use a fresh tip for each new reagent you pipet.
17. Obtain a 0.5—10 μL micropipet.
18. Label three (3), 1.5 mL reaction tubes and label them A, B, and C.
19. Use the matrix below to add each solution sequentially to each of the three (3) tubes. Be sure to use a fresh pipet tip for each change in solution.

Tube	Solution I	Solution II	Solution III	Solution IV
A	4 μL	5 μL	1 μL	----
B	4 μL	5 μL	----	1 μL
C	4 μL	4 μL	1 μL	1 μL

20. Close the tops, and place the reaction tubes in a balanced configuration in the microfuge rotor. Spinning tubes in an unbalanced position will damage the microfuge rotor. See the next page for balanced rotor positions. If you have an odd number of tubes, you can put in blanks that will balance out the arrangement.

Balanced Rotor Configurations

21. Spin tubes for a 1-2 second pulse in the microfuge. This will mix and pool reactants into a droplet in the bottom of each tube.
22. You added a total of 10 μL of reactants into each test tube. Now, set your pipet to 10 μL , and very carefully withdraw the solution from each tube. Discard into the waste jar.
 - Are you able to just fill the tip?
 - Did you find that a small volume of fluid is left behind?
 - Did you find that after extracting all the fluid you are left with a small air space in the tip?
23. Obtain the mid-range (10 μL –100 μL) micropipet.
24. Label two (2) 1.5 mL tubes and label them E and F.
25. Using the matrix designed for your micropipet, fill each tube to the desire volume.

Tube	Solution V	Solution V	Solution V	Solution V
E	15 μL	25 μL	32 μL	28 μL
F	11 μL	44 μL	18 μL	27 μL

26. Close the tops, and place the reaction tubes in a balanced configuration in the microfuge rotor. Spinning tubes in an unbalanced position will damage the microfuge rotor.
27. Spin tubes for a 1-2 second pulse in the microfuge. This will mix and pool reactants into a droplet in the bottom of each tube.
28. You added a total of 100 μL of reactants into each test tube. Now, set your pipet to 100 μL , and very carefully withdraw the solution from each tube. Discard into the waste jar.
29. Obtain the large-range (100 μL –1000 μL) micropipet.
30. Label two (2) 1.5 mL tubes and label them G and H.
31. Using the matrix designed for your micropipet, fill each tube to the desire volume.

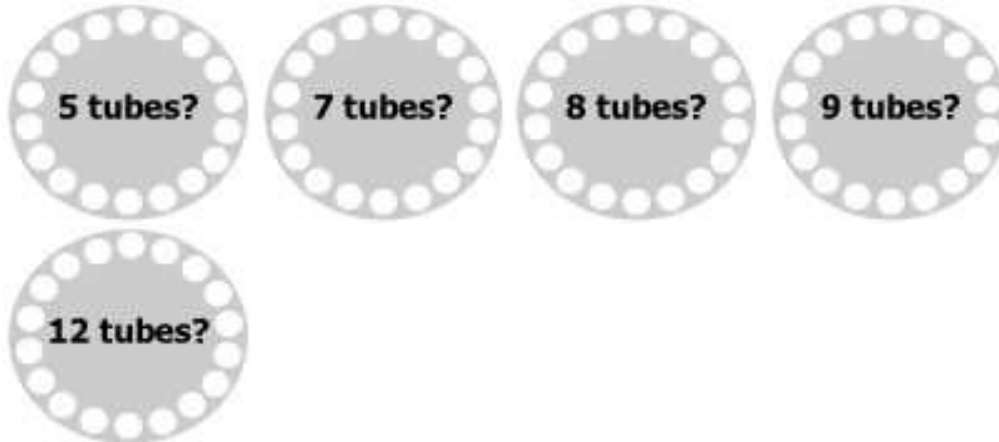
Tube	Solution V	Solution V	Solution V	Solution V
G	100 μL	200 μL	150 μL	550 μL
H	150 μL	250 μL	350 μL	250 μL

32. Close the tops, and place the reaction tubes in a balanced configuration in the microfuge rotor. Spinning tubes in an unbalanced position will damage the microfuge rotor.
33. Spin tubes for a 1-2 second pulse in the microfuge. This will mix and pool reactants into a droplet in the bottom of each tube.
34. You added a total of 1000 μL (1 mL) of reactants into each test tube. Now, set your pipet to 1000 μL (1 mL), and very carefully withdraw the solution from each tube. Discard into the waste jar.
35. Complete review questions on the back page of this handout.

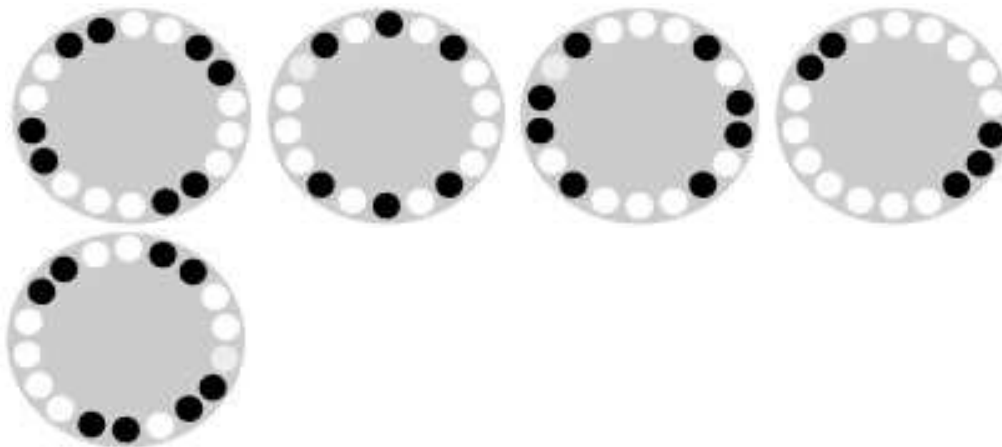
NAME: _____

Lab Questions:

1. Using the diagram below, draw balanced rotor configurations for 5, 7, 8, 9, and 10 tubes.
HINT: You must use at least that number of tubes...



2. Which of the following shows an unbalanced rotor? (put an **X** through the diagram)



3. The small range digital micropipet measures volumes between 0.5 μL and 10.0 μL . If you wish to dispense seven and five-tenths microliters of a fluid with the instrument, what sequence of numerals would you see on the digital dial?
- 75/00
 - 75/10
 - 00/75
 - 07/50
4. A student presses the button on the micropipet to the first position, places it in a liquid and slowly releases the button. What will most likely occur?

- a) ejection of the tip
 - b) fluid will be drawn up into the tip
 - c) the last drop of fluid will be pushed out of the tip
 - d) most, but not all fluid will be expelled from the tip
5. A student presses the button on the micropipet to the second position. What will most likely occur?
- a) ejection of the tip
 - b) most of the fluid will be removed from the tip
 - c) fluid will be drawn up into tip
 - d) fluid will be ejected and then redrawn into the tip
6. On a large-range Eppendorf digital micropipet, what volume of liquid is indicated by these numbers **0 5 0 0**
- a) 5 μL
 - b) 50 μL
 - c) 500 μL
 - d) 5000 μL
7. Complete the following conversions:
- a) 0.167 mL to μL _____
 - b) 0.05 mL to μL _____
 - c) 42 μL to mL _____
 - d) 182 μL to mL _____
 - e) 0.9 μL to mL _____
8. Identify four (4) important precautions in micropipet use:
