Pipetting 101
Developed by BSU CityLab

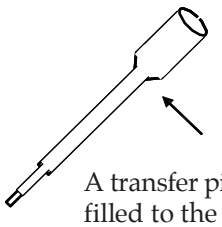
Color Comparisons
Pipetting Exercise #1

STUDENT OBJECTIVES
Students will be able to:

- Choose the correct size micropipette for the volume of liquid to be moved
- Demonstrate how to correctly set the volume on different sizes of micropipettes
- Demonstrate the sequence of steps for using a micropipette to draw up liquid from one container and transfer it to another
- Demonstrate the proper use (and care for) these finely-tuned instruments

BACKGROUND INFORMATION
Micropipettes are used to accurately measure microliter volumes of liquid. The prefix "micro" means millionth, and one microliter is one millionth of a Liter. In decimal notation, one microliter is 0.000001 L. In scientific notation, one microliter is 1×10^{-6}. A microliter is abbreviated with the symbol μL, which comes from the Greek letter μ (pronounced "mew").

When thinking about a microliter, it may be helpful to first consider a milliliter (mL). A typical transfer pipette, when filled to the base of the bulb as in the diagram, will hold about 1.0 mL of liquid. One thousand microliters will occupy the same one milliliter space! Small plastic tubes with attached caps, called microcentrifuge tubes (or microfuge tubes) are used in this activity, and they hold only about 1.5 mL (or 1,500 microliters). In fact, the largest micropipette holds 1,000 μL of liquid – a single milliliter!



A transfer pipette, filled to the base of the bulb (as indicated by the arrow), holds about one milliliter of liquid (1.0 mL).

When you use a measuring instrument, you are comparing an unknown quantity of something with a known quantity, called a standard. In the case of micropipettes, the standard is the microliter. If you draw up 350 μL of liquid in a micropipette, the instrument will contain a precise amount indicated with a number (350) and a unit (μL). Measurements with numbers are described as quantitative measurements.

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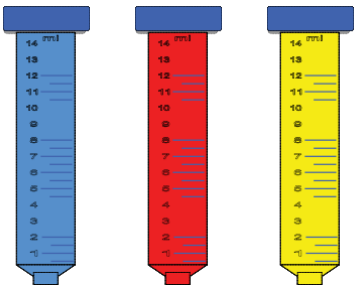
Some standards are less specific and cannot be assigned a number value. For example, if you have four identical containers of water, and each contains a different number of drops of blue food coloring, it might not be obvious which blue is darkest in color, which is the lightest, and where the other two fit in between them. Measurements like these are more subjective (in the eye of the observer) and are called qualitative measurements.

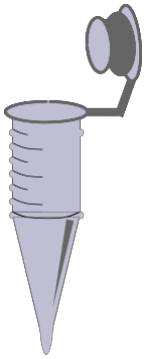
You have been introduced to the basic operation of the adjustable micropipettes, and now it is time to become comfortable using them. Refer often to the diagrams and details in the handouts provided by the teacher. In this activity, you will use micropipettes to measured food-colored water.

The first step is to make three new solutions according to the concentrations given in Table #1 of the protocol. The lab protocol is simply the step-by-step instructions you will follow (the written protocol has small boxes to be checked off as you complete each step). The three new solutions are the color standards you will use for comparison in the second step. Following the concentrations provided in Table #2, you will pipette different amounts of liquids from the original containers to make five more color combinations and then compare them to the three original standards.



ColorStandards

As you work, keep in mind any sources of error you encounter along the way. When carrying out experiments, scientists always strive to perform each step in exactly the same way every time they do that step. Any change in the protocol, or a change in technique, is considered a variable – something that could change the outcome of the experiment. Whenever you use a measuring instrument, some degree of variability is assumed. For example, if you are asked to measure out a cup of water, and then a classmate is asked to use the same measuring cup to do the same task, the chances are good that you will NOT measure out the EXACT same amount of water as your classmate! Variability describes the range of possible values or amounts of something, and all measuring instruments have variability (although the variability range in a micropipette is quite small).



Microcentrifuge Tube

Pipetting 101

Color Comparisons Protocol

Name:_____ Date: _____

MATERIALS

- 200 µL Micropipette
- 1000 µL Micropipette
- Box of Micropipette tips for 200 µL Micropipette
- Box of Micropipette tips for 1000 µL Micropipette
- Used tip disposal container
- Waste liquid disposal container
- Microcentrifuge tube rack containing:
  Eight, empty 1.5 mL microcentrifuge tubes with attached caps
- Test Tube or Centrifuge rack with:
  One capped test tube or conical centrifuge tube containing 15 mL blue dye
  One capped test tube or conical centrifuge tube containing 15 mL red dye
  One capped test tube or conical centrifuge tube containing 15 mL yellow dye
- Permanent marker
- Student Handouts, optional
- Safety goggles for each student
- Gloves for each student

PROCEDURE

Step #1

- Take three of the microcentrifuge tubes (with attached caps), and label them #1, #2, and #3 with the permanent marker. Set them aside in the microcentrifuge tube rack. Do the same with your other five microcentrifuge tubes, but label them A, B, C, D and E.

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Read the next four items in the protocol before doing anything else!

- Create your reference tubes, or standards, by combining the liquids according to the amounts given in Table #1 below. For example, to make standard #1, you will move 100 µL of blue dye into microcentrifuge tube #1. Then change the micropipette tip and move 100 µL of yellow dye into microcentrifuge tube #1. Snap the cap shut and lightly tap tube #1 on the table to mix the two colors. Return the microtube to the rack.

- Write your results in the third column of Table #1.

- Make sure that all members of your group take turns completing the exercises.

- Things to remember each time you use the micropipette:

First, determine which style and size micropipette to use!
Second, check or change the volume setting!
Third, use a new tip for each new color!

Table #1, Color Comparisons, Making Standards

STANDARD	COMBINE	RESULTING COLOR
#1	100 μL blue dye 100 μL yellow dye	
#2	100 μL red dye 100 μL yellow dye	
#3	100 μL red dye 100 μL blue dye	

Step #2

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Read these instructions before beginning:

- Combine liquids according to the amounts given in Table #2 to make five (A through E) new color combinations. Use the same protocols you followed to create standards #1 through #3 in Step #1. Make sure that all members of the group take turns completing the exercises.

- Write your observations in the two columns on the right side of the table. The last column asks you to compare the color of each new liquid to the corresponding standard from Step #1. For example, if you create a green liquid in Tube A, compare its intensity with the green in Tube #1, and make notes about whether it is lighter, darker, or the same shade as the standard.

Table #2, Color Comparisons, New Combinations

TUBE	COMBINE	RESULTING COLOR	COMPARE TO STANDARD (For example: lighter than, darker than, same as…)
A	50 µL blue dye	50 µL yellow dye	#1,
B	37 µL red dye	37 µL yellow dye	#2,
C	658 µL red dye	658 µL blue dye	#3,
D	100 µL yellow dye	25 ul red dye (4 times)	#2,
E	25 µL red dye (4 times)	25 µL yellow dye (4 times)	#2,

Notes:

OBSERVATIONS

1. In Table #1, what was the total volume of each standard you made?

2. What were the volume proportions of the two colors (what was their ratio) in each standard?

3. In Table #2, what volume proportions of each color were used to create each new solution (A-E)?

4. Can you suggest three or more variables (sources of error) that may account for your observations in the last column?

REVIEW QUESTIONS

1. What fraction of a Liter is a microliter?

2. If the window on the 20 μL micropipette shows the numbers to the right, what volume of sample will be delivered?

3. Why did you change micropipette tips each time you changed sample color?

Pipetting 101

A Solution for the Unknown Solutions: Starch and Iodine

Assay Protocol

Name:_____ Date: _____

MATERIALS

per Group

- 200 µL Micropipette
- Box of Micropipette tips for 200 µL Micropipette
- Used tip disposal container
- Waste liquid disposal container
- Microcentrifuge tube rack containing:
  - Six to eight empty, unlabeled 1.5 mL microcentrifuge tubes with attached caps
  - One 1.5 mL capped microcentrifuge tube, labeled "starch"
  - One 1.5 mL capped microcentrifuge tube, labeled "iodine"
  - One 1.5 mL capped microcentrifuge tube, labeled "W"
  - One 1.5 mL capped microcentrifuge tube, labeled "X"
  - One 1.5 mL capped microcentrifuge tube, labeled "Y"
  - One 1.5 mL capped microcentrifuge tube, labeled "Z"
- Permanent marker
- Student Handouts, optional
- Safety goggles for each student
- Gloves for each student

PROCEDURE

- Observe the microcentrifuge tubes (in the rack) that contain liquids. One tube contains starch. The other "known" tube contains iodine. The tubes are labeled, so you know what is in them.

- The other four tubes containing liquids are labeled W, X, Y and Z. They are considered the "unknowns," because you do not know exactly what is in them. You do know this, however: they contain starch or iodine or colored water or plain water.

- Using the marker, label one empty microcentrifuge tube as the "Standard"

- Observe the data table called A Solution for the Unknown Solutions. The first row of the table suggests that you will use a micropipette to combine 50 µL of starch and 50 µL of iodine in the tube you labeled as the "Standard."

- Select the appropriate size micropipette, set the volume and affix a clean tip. Then go ahead and combine 50 µL of starch and 50 µL iodine into the "Standard" tube.

- Observe the resulting color and write it in the table.

Read the following items before continuing:

As you can see, starch and iodine give a distinct color change when mixed. This color is now the standard, or indicator. It tells you that the liquid in the tube contains both starch and iodine.

Your task is to perform a series of trials to figure out what liquids are in the "unknown tubes" W, X, Y and Z. They may be starch or iodine or distilled water or colored water.

To solve the mystery, you will combine 50 µL samples from each of two different tubes into clean, empty tubes. Be sure to label each new tube and write on the data table which two samples you mixed together. Record the color of each new mixture and compare your results with the standard.

Test as many different combinations of two liquids as you need to in order to figure out the four unknowns. There is no set number of trials you must run because it depends on your procedure. There are at least two possible ways to perform the assay, so don't be alarmed if another group is doing something different. You may not need to use all the empty microcentrifuge tubes you have been given nor all the blank rows on the data table. You may even discover that you need more than the number of tubes and rows provided (in this case, just ask your teacher for assistance).

Remember, all micropipetting will be done in 50 µL volumes.

Use your best micropipetting technique, and be sure to use a fresh tip each time you pipette from a different solution.

When you have figured out what solutions are in tubes W, X, Y and Z, write their names in the conclusions section below the data table.

A Solution for the Unknown Solutions Data Table

TUBE LABEL	COMBINE		RESULTING COLOR
Standard	50 µL Starch,	50 µL Iodine,	
Trial #1			
Trial #2			
Trial #3			
Trial #4			
Trial #5			
Trial #6			
Trial #7			
Trial #8			
Trial #9			
Trial #10			
Trial #11			
Trial #12			
Trial #13			
Trial #14			

CONCLUSIONS

W = _____

X = _____

Y = _____

Z = _____

THOUGHT QUESTIONS

1. Did the mixing of starch and iodine cause a physical or chemical reaction? How do you know?

2. Another word for a test is an assay. The "starch-iodine assay" is commonly used in chemistry and biology. What might be your definition of it?

3. Describe in detail how you carried out your assay. What strategies did you use to determine the unknowns? How did the standard help you?

4. How positive do you feel about your conclusions? Describe any possible errors or variables in your assay.