TITLE: Pipetting by Design

KEY QUESTION(S):

- What is a micropipette?
- How do you properly use a micropipette?
- Why is a micropipette necessary in biotechnology laboratories?

OVERALL TIME ESTIMATE:

- Advanced Preparation: 10 Minutes
- Student Procedure: 30 Minutes

LESSON SUMMARY: In this lesson, students learn and practice the proper technique for measuring small volumes of liquid using a micropipette. The students will a follow a micropipetting protocol to create an image by pipetting the proper color and volume of water into their well plate.

STUDENT LEARNING OBJECTIVES:

The student will be able to:

1. Properly operate a micropipette.
2. Determine the appropriate micropipette to use according the volume of liquid being measured.
3. Correctly read the volume indicator on the micropipette.
4. Measure volume in microliters (µl) using a micropipette.
5. Convert volume into mass.
6. Use a balance to determine accuracy of pipetting.

MATERIALS:

- (1) Multicolor Food Coloring Package (Red, Blue, Yellow, Green)
- 15 mL conical tubes, 4 per group

Needed For Each Student Group:

- 10mL aliquots of Colored Water stock solutions (Red, Blue, Yellow)
  - 10 mL of water plus 5 drops
  - One tube of water only
- (1) 96 Well Plate
- (1) P20 Micropipette
- (1) P200 Micropipette
- (1) 2-200ul Tip Box

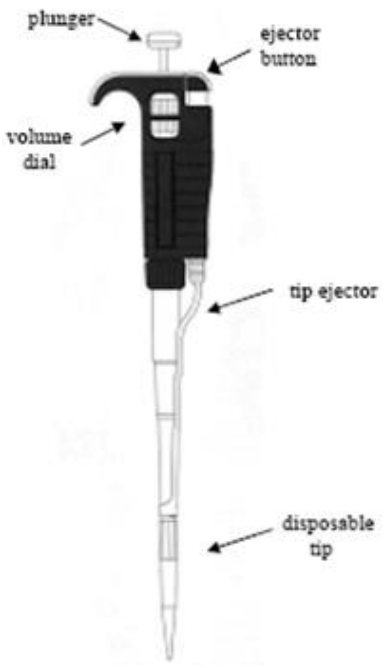


Figure 1

BACKGROUND INFORMATION:

Micropipettes are precise instruments used to accurately measure very small quantities of liquids. Figure 1 shows a micropipette and the main components of the instrument. They are available in a variety of sizes to best match your measurement needs. The size of the micropipette is indicated directly on the instrument. The most commonly used micropipettes are the P10, P20, P200, and P1000. The number following the "P" refers to the maximum volume in microliters (µl) that can be measured using the instrument.

In this activity, P20 and P200 micropipettes will be used. The proper method for reading the volume indicator and directions on how to use the P20 and P200 micropipettes are listed below (for FisherBrand Micropipetters):

Reading the volume on the micropipette:

- **P20 Micropipettes:** The volume indicator consists of three number dials and is read from left to right. A P20 is used to measure volumes up to 20µl. **NOTE: Do not dial past below 2.0 µl or above 20.0 µl.**

0	7	. 3

7.3 µl

2	0	.0

20.0 µl

- **P200 Micropipettes:** The volume indicator consists of three number dials and is read from left to right. Some pipets have the numbers arranged vertically, so they are read from top to bottom. A P200 is used to measure volumes between 20µl and 200µl. **NOTE: Do not dial past below 20 µl or above 200 µl.**

0	7	3

73 µl

2	0	0

200 µl

Directions on how to use a micropipette:

- Be gentle.
- Hold micropipette in one hand. With the other hand turn the volume adjustment dial (the plunger on this model) slightly above the desired setting then slowly down until the required volume shows on the indicator. *This prevents mechanical backlash from affecting accuracy*.
- Press disposable tips firmly onto the shaft to ensure an airtight seal. Do this by pushing the micropipette into the tip while the tip is still seated in the tip box.
- Depress plunger to the **first stop**. Holding the micropipette vertically, immerse the tip approximately two mm into the sample liquid. Allow the pushbutton to return **slowly** to the up position! **[NOTE: If you release the plunger too quickly you may not withdraw the correct volume. It could also cause the liquid to "jump" and get onto the pipette. This can result in cross contamination of solutions and damage to the pipette.]**
- Withdraw the tip from the liquid. Touch the tip end against the side wall of the receiving vessel and depress the plunger slowly to the first stop.
- Wait one second then press the plunger to the second stop, expelling any residual liquid in the tip.
- With the plunger fully depressed, withdraw micropipette and allow the plunger to slowly return to the up position.
- Discard the tip by depressing the ejector button. **Use a fresh tip for the next sample to avoid contamination.**

Important information to note:

- NEVER use the micropipette without a disposable tip in place. Moisture can damage the piston and reduce accuracy.
- NEVER lay a liquid loaded micropipette down. Moisture can run back inside causing damage to the micropipette.
- Do not allow the button to snap back after pushing the plunger. Allow it to return gradually.

ADVANCE PREPARATION:

1. (10 Minutes)Prepare colored water solution.
   - Each group of students needs one tube of each colored water, red, yellow, and blue, any one tube of water only.
     - For color solutions, use 10 mL of water and 5 drops of food coloring
2. (5 Minutes) Prepare student protocols
   - Copy protocol for each student pair or group. For prolonged use, consider laminating.

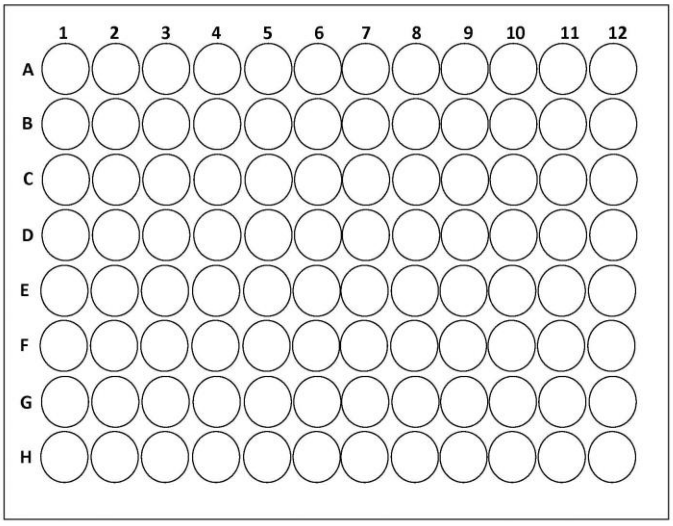
PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

A. Introduction (10 Minutes)

1. Teach students the parts and proper method for handling the micropipette.
2. Model using the micropipette to measure the volume of several samples of water.
   - Explicitly instruct students on how to set the volume using the adjustment knob, properly read the volume indicator, how to eject the tip and when each micropipette should be used.
3. Separate students into pairs.
4. Distribute a micropipette protocol sheet and all the necessary materials to each pair of students or have student collect items from a common workstation.

B. Micropipetting Activity (20 Minutes)

1. Students work in small groups or pairs to complete the micropipette protocol activity.
   - Activity results are dependent upon which protocol the students followed. Use colored pencils to draw your results below:



C. Check for Accuracy (20 minutes)

1. As an extension, students can check for accuracy by determining the mass of their design. To do so, the students should do the following (you may wish to have your students come up with this protocol on their own):
   - Determine the total volume of colored water added to their plate
   - Convert volume into mass (1000µl = 1ml; 1ml = 1g)
   - Determine the mass of an empty 96-well plate
   - Measure mass of plate with colored water
   - Subtract the mass of the empty plate from the pas of the plate with the colored water
   - Calculate percent error: [(actual-predicted)/predicted] * 100%

Protocol 1:
Micropipette the indicated volumes into designated wells on the 96 well plate.

Using the **RED** dye:

10 µl: C9, D9, E9
20 µl: B4, C1, D1, E1, F4
120 µl: B6, B7, D6, D7, F6, F7

Using the **Yellow** dye:

115 µl: A10, A11, B9, D11, D12, E12, F9, G10, G11
150 µl: A2, A3, B1, B4, C1, D1, E1, F1, F4, G2, G3

Using the **Blue** dye:

3 µl: C9, D9, E9
19 µl: A10, A11, B9, D11, D12, E12, F9, G10, G11
170 µl: B12, F12

Using the **water:**

15 µl: B12, F12
50 µl: A2, A3, B1, F1, G2, G3
70 µl: B6, B7, D6, D7, F6, F7
175 µl: C9, D9, E9

Protocol 2:
Micropipette the indicated volumes into designated wells on the 96 well plate.

Using the **RED** dye:

6 µl: D1, E1, F1, G1, H1, D5, E5, F5, G5, H5
10 µl: A3
20 µl: D7, D8, F7, F8
120 µl: B2, B4, C1, C5

Using the **Yellow** dye:

100 µl: G10,H10, A8. A12
115 µl: A9, A11, D10, E10, F10
130 µl: A10, B10, C10
150 µl: D7, D8, F7, F8

Using the **Blue** dye:

3 µl: A3
4 µl: A10, B10, C10
6 µl: E2, E4
19 µl: A9, A11, D10, E10, F10
30 µl: E3
50 µl: G10, H10, A8, A12

Using the **water:**

15 µl: E3
70 µl: B2, B4, C1, C5
175 µl: A3
195 µl: D1, E1, F1, G1, H1, D5, E5, F5, G5, H5, E2, E4

Protocol 3:
Micropipette the indicated volumes into designated wells on the 96 well plate.

Using the **RED** dye:

6 µl: B3, C3, D3, E3, F3
10 µl: C1, D1, E1, C5, D5, E5
120 µl: B2, C2, D2, E2, F2, B4, C4, D4, E4, F4

Using the **Blue** dye:

3 µl: C1, D1, E1, C5, D5, E5, A11, G11
6 µl: C9, E9
30 µl: B10, F10
170 µl: D6, D7, D8, D9

Using the **water:**

15 µl: D6, D7, D8, D9
70 µl: B2, C2, D2, E2, F2, B4, C4, D4, E4, F4
160 µl: B10, F10
175 µl: C1, D1, E1, C5, D5, E5, A11, G11
195 µl: B3, C3, D3, E3, F3, C9, E9

Protocol 4:
Micropipette the indicated volumes into designated wells on the 96 well plate.
Using the **RED** dye:

8 µl: D2, E2, D11, E11
10 µl: A4, A5, A6, A7 A8, A9, B3, B10, G3, G10, H4, H5, H6, H7, H8, H9
Using the **Blue** dye:

3 µl: A4, A5, A6, A7 A8, A9, B3, B10, G3, G10, H4, H5, H6, H7, H8, H9
30 µl: D4, E4, D9, E9
170 µl: C5, C6, C7, C8, F5, F6, F7, F8

Using the **water:**

15 µl: C5, C6, C7, C8, F5, F6, F7, F8
160 µl: D4, E4, D9, E9
175 µl: A4, A5, A6, A7 A8, A9, B3, B10, G3, G10, H4, H5, H6, H7, H8, H9
195 µl: D2, E2, D11, E11

Protocol 5:
Micropipette the indicated volumes into designated wells on the 96 well plate.

Using the **RED** dye:

8 µl: B7, G7
10 µl: A6, F6
20 µl: B2, E2, C9, G9
120 µl: A1, F1, B8, G8

Using the **Yellow** dye:

115 µl: B2, C4, C9, D4, E2, E11, F11, G9
150 µl: C3, D3, D10, F10

Using the **Blue** dye:

3 µl: A6, F6
19 µl: C4, D4, E11, F11
170 µl: B5, E5, D12, G12

Using the **water:**

15 µl: B5, E5, D12, G12
50 µl: C3, D3, D10, F10
70 µl: A1, F1, B8, G8
175 µl: A6, F6
195 µl: B7, G7

Protocol 6:
Micropipette the indicated volumes into designated wells on the 96 well plate.

Using the **RED** dye:

6 µl: F5, G6, G7
20 µl: B4, B5, B6, B7, B8, B9, C3, C4, C6, C7, C9, C10, D4, D5, D6, D7, D8, D9, D10
120 µl: C5, C8

Using the **Yellow** dye:

115 µl: E7
150 µl: A5, A6, A7, A8, B4, B5, B6, B7, B8, B9, C3, C4, C6, C7, C9, C10, D4, D5, D6, D7, D8, D9, D10, E4, E5, E6, E8, E9, F4, F6, F7, F8, F9, G5, G8, H6, H7

Using the **Blue** dye:

19 µl: E7

Using the **water:**

50 µl: A5, A6, A7, A8, E4, E5, E6, E8, E9, F4, F6, F7, F8, F9, G5, G8, H6, H7
70 µl: C8
195 µl: F5, G6, G7

Protocol 7:
Micropipette the indicated volumes into designated wells on the 96 well plate.

Using the **RED** dye:

6 µl: B3, C3, D3, E3
10 µl: A1, B1, C1, D1, E1, F1
20 µl: C10, C11, F10, F11
120 µl: A2, F2, D12, E12, F12, G12, H12

Using the **Yellow** dye:

150 µl: D9, E9, F9, G9, H9, C10, C11, F10, F11

Using the **Blue** dye:

3 µl: A1, B1, C1, D1, E1, F1
6 µl: A5, B5, C5, D5, E5, F5
30 µl: B6, C7, D7
170 µl: A8, B8, C8, D8, E8, F8

Using the **water:**

15 µl: A8, B8, C8, D8, E8, F8
50 µl: D9, E9, F9, G9, H9
70 µl: A2, F2, D12, E12, F12, G12, H12
160 µl: B6, C7, D7
175 µl: A1, B1, C1, D1, E1, F1
195 µl: B3, C3, D3, E3, A5, B5, C5, D5, E5, F5

Protocol 8:
Micropipette the indicated volumes into designated wells on the 96 well plate.

Using the **RED** dye:

10 µl: E8, E9
20 µl: D2. D3

Using the **Yellow** dye:

115 µl: A2, A3, B4, C4
130 µl: A1, B1, C1, D1, E1, F1
150 µl: D2, D3, E4, F4

Using the **Blue** dye:

3 µl: E8, E9
4 µl: A1, B1, C1, D1, E1, F1
19 µl: A2, A3, B4, C4
170 µl: B7, C7, D7, E7, F7, G7, H7, B8, B9, B10

Using the **water:**

15 µl: B7, C7, D7, E7, F7, G7, H7, B8, B9, B10
50 µl: E4, F4
175 µl: E8, E9