Course
Advanced Biotechnology

Unit
Lab Overview

Essential
How do scientists deliver small amounts of liquid with accuracy and precision?

TEKS
130.364 1G, 1H, 1K, 2E, 2G

TAKS
Science 1A, 2B

Prior Student Learning
Metric Conversions

Estimated Time
50 minutes

Rationale

This lab instrument is commonly used in biotechnology experiments. It is essential for students to master this equipment with accuracy and precision.

Objectives

Students will:

- Use proper technique when working with a micropipette.
- Show competency of equipment.

Engage

- Students complete **Working with Micropipette Pre-Lab.**

Key Points

- Refer to Micropippetor Use Video and Notes.

Activity

1. Students complete **Working with Micropipettes Lab.**
2. Student will turn in 6 tubes to their instructor. Check volumes and color for a quick lab grade to ensure proper equipment use.
3. Students complete **Working with Micropipettes Post Lab.**
4. Students complete **Skill Standard: Using the Micropipette.**

Assessment

- Sample tubes are correct volume and color.
- Completion of post lab questions.
- Completion of Skill Standard practical. Students should deliver volume to an electric balance with 100ths place accuracy.

Materials

- Working with Micropipettes Pre Lab
- Working with Micropipettes Lab
- Working with Micropipettes Post Lab
- Skill Standard: Using the Micropipette

Accommodations for Learning Differences

- Visit the **Special Populations** section of the CTE Career and Technology Education Website: http://cte.unt.edu/special-pops.

National and State Education Standards

Texas College and Career Readiness Standards
Science Standards
I. C3, D1, D2, D3
II. F1, F2

Working with Micropipettes   **Name:**_____

Pre-Lab

Laboratory work in molecular biology and biotechnology is usually done in minute quantities. The unit of measure used for setting up reactions is the microliter (µl). One microliter is one millionth (10^{-6}) of a liter.

$$\text{So: } 1 \text{ L} = 1,000,000 \text{ µl, and } 1 \text{ ml} = 1,000 \text{ µl}$$

Use the following resource to answer the questions below
- http://www.austincc.edu/biocr/1406/labm/ex1/prelab_1_7.htm

1. Convert the following:

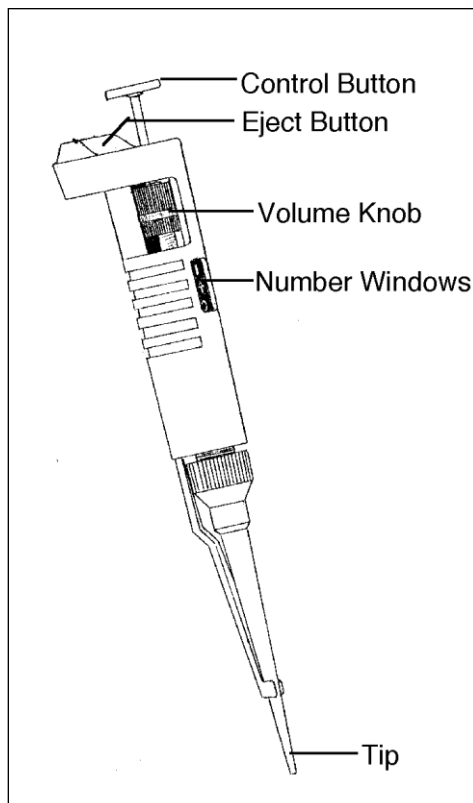100 µl   =_____ml   5 ml   =_____ µl

500 µl   =_____ml   0.5ml   =_____ µl

3,000 µl =_____ml   0.004ml   = _____ µl

10 µl   =_____ml   0.000001ml   = _____ µl

Control Button
Eject Button

Volume Knob

Number Windows

Tip

2. The micropipette is an instrument that allows us to accurately measure µl volumes of reagents. Micropipettes are delicate, very expensive, and the cornerstone of our work with DNA. A micropipette uses suction to draw up specific amounts of liquid. Its parts allow you to control how much liquid to suck up and dispense. On the left is a diagram of a micropipette and its specific parts. Fill in the blanks below using this diagram.

a. The _____ allows the user to suck up and dispense liquid.

b. The _____ allows ejection of micropipette tips after use.

c. The _____ allows the user to dial the amount of liquid to be measured.

d. The _____ shows the amount dialed.

e. The _____ of the micropipette is where the micropipette tips are placed. The entire white part is called the *barrel*.

3. Damaging the micropipette can be avoided by following a few simple rules:

- Never rotate the _____ knob beyond the upper or lower range of the micropipette.

- Never use a micropipette without a _____ in place.

- Never lay down a micropipette with a _____ tip.

- Never allow _____ to snap back after ejecting fluid.

- Never immerse barrel of micropipette in _____.

- Never flame micropipette _____.

4. Micropipettes are designed to deliver a specified volume within a certain range, with the appropriate tip in place. Fill in the table below with the appropriate ranges.

Name of micropipette	Range of Volumes	Tip To Use
P1000	µl	Blue
P200	20-200µl	
P20	µl	Yellow

5. Perhaps the most difficult part of using micropipettes is setting them properly. On each of the micropipettes, you will find 3 or 4 numbers places in the number windows depending on the type of micropipette you are using. However, the numbers represent different volumes for P1000, P200, and P20:

Number	P100	P200	P20
1st	X,000µl	X00µl	X0µl
2nd	X00µl	X0µl	Xµl
3rd	X0µl	Xµl	0.Xµl

   a. Red lines represent decimals. Indicate any red lines that appear in the windows of your micropipettes by circling its location using red ink in the table above.
   b. If you micropipettes have 4 windows, add the 4th window values to the table above using blue ink.

6. Practice setting the volumes below using the appropriate micropipette. Check your settings with a lab partner. **Then sketch the numbers displayed in the windows of the micropipette.**

  a. P1000: 324, 1000, 546µl

  b. P200: 24, 156, 87µl

  c. P20: 2.4, 18.9, 6.0µl

d. The window of a P20 micropipette displays 0100. How many microliters does this represent?

e. The window of a P200 micropipette displays 0100. How many microliters does this represent?

f. The window of a P1000 micropipette displays 0100. How many microliters does this represent?

Working with Micropipettes Lab

Name:_____

Part A: Checking Your Technique:
1. Observe the instructor's demonstration on the proper use of the micropipette before beginning this exercise.
2. Obtain two 1.5 ml microfuge tubes and fill one with distilled water. You will practice transferring liquid from one tube to the other.
3. Choose a micropipette and set the dial to deliver 330ul. Which micropipette should you use? What should the window read? Check your answer before moving on.
4. Place your thumb at the top of the plunger with your fingers wrapped around the body.
5. Place a tip onto the micropipette by pressing the tip of the micropipette barrel firmly into a tip of the appropriate type (blue or yellow).
6. Depress the plunger to the **first stop**.
7. **While holding the plunger down**, place the tip into microfuge tube and into the liquid.
8. Slowly withdraw your thumb to suck liquid into tip. Watch that it goes up without air bubbles. **Do not snap back plunger!**
9. Place the tip into the bottom of the receiving microfuge tube.
10. Press plunger to **first stop** to dispense liquid. Continue to press beyond to first stop to the **second stop** to get out all of the remaining liquid in the tip. *Do not let go of the plunger yet!*
11. Pull tip out of liquid before relaxing the plunger back to original position.
12. Eject tip into waste container by pressing the ejector button.
13. Compare your liquid volumes with your lab partners. They should be equal.
14. Once you feel comfortable using the micropipette, check your technique by delivering the 330ul into a weigh boat on an electric balance. If your technique is perfect the scale will read 0.330 grams. You will be graded on this technique. Continue to complete this task until the tenths and hundreds place are perfect. A perfect thousandths place will receive bonus points.

Part B: Small Volume Pipetting Exercise

1. Obtain a P20 micropipette, yellow tips and 2 microfuge tubes.
2. Label the tubes A and B. Use the matrix below as a guide for adding appropriate volumes of the colored solutions to each tube.

Tube	Red	Blue	Yellow	Water	Color Observed
A	-	2 µl	2 µl	11 µl	
B	3 µl	2 µl	-	10 µl	

3. First add the appropriate amount of water to the bottom of each tube. Be sure all of the liquid comes out and forms a small bubble of liquid at the bottom of the tube.
4. Next add the colored solutions one at a time. Dispense the solution directly into the small bubble of liquid at the bottom of the tube.
5. After you have added all of your solutions into each tube, practice mixing the contents with a micropipette. Set your micropipette to 15 µl and slowly pipet the mixture up and down until well mixed.
6. Place all the tubes in the microfuge (see instructions on how to use at the equipment station-WRITE THIS DOWN) and apply a short (1-2 seconds) pulse. Make sure the tubes are placed in a balanced configuration!
7. Record the final color of the solution in each tube in the table above.
8. A total of 15 µl was pipetted into each tube. Check that your measurements were accurate by setting the P20 to 15 µl and withdrawing the contents of each tube.
9. Save your tubes. You will turn them into your instructor for a grade at the end of this exercise.

Part C: Large Volume Pipetting Exercise

1. Obtain a P200 and P1000 micropipette, yellow tips and blue tips and 4 microfuge tubes.
2. Label the tubes C, D, E, & F
3. Use the matrix below as a guide for adding appropriate volumes of the colored solutions to each tube.

Tube	Red	Blue	Yellow	Water	Color Observed
C	40 µl	-	40 µl	920 µl	
D	-	20 µl	260 µl	720 µl	
E		120 µl	20 µl	860 µl	
F	60 µl	30 µl	30 µl	880 µl	

4. Mix well (by vortexing or micropipetting) and place all the tubes in the microfuge and apply a short (1-2 seconds) pulse. Make sure the tubes are placed in a balanced configuration!
5. Record the final color of the solution in each tube in the table above.
6. A total of 1,000 µl (1ml) was pipetted into each tube. Check that your measurements were accurate by setting the P1000 to 1,000 µl and withdrawing the contents of each tube.
7. Gather all of your tubes labeled A-F and put in order. Place a single piece of tape on top of the tubes to hold them together in one strand. Write your name and class on the tape and turn into your instructor for a grade.

Working with Micropipettes   **Name:**_____
Post Lab

1. Why should you avoid touching the micropipette tips?

2. Why should you avoid submerging the micropipette tip too deep in the liquid?

3. Predict what would happen if you push the plunger to the second stop before drawing up the liquid? Try it out for yourself if you don't know.

4. What does the phrase -pipetting up and down- mean and how is this technique used?

5. What is the purpose of vortexing?

6. Explain how you checked your technique using the electric balance.

7. Record your colors observed for tubes A-F below.

8. What is the purpose of the microfuge?

9. Why must the microfuge be balanced?

10. Explain the purpose of the first stop and second stop on the micropipette.

11. Complete your **Skill Check** with your instructor for a lab grade.

Use of Microfuges

*** Improper use will result in a reduction of lab grade***
So pay attention and READ directions

1. Observe the instructor demonstrate the proper use of the microfuge and how to insert tubes in a balanced configuration. This is extremely important, because spinning tubes in an unbalanced position will damage the microfuge!

2. Be sure tubes you are spinning are in pairs and have approximately the same weight/volume in them. To create a balance if you do not have two equal volume tubes, create a dummy tube using distilled water.

3. Open lid and remove rotor cover. Place tubes in pairs arrange so that they are at opposite ends in the rotor.

4. **Replace rotor cover** and close lid.

5. Select appropriate time and push start. For short pulses, hold the pulse button for the desired time.

6. Wait for rotor to stop completely before opening lid and removing your tubes.

Skill Check: Using the Micropipette

1. Your teacher will ask you to deliver a particular amount using the micropipette.
2. You will be asked to
   a. Convert from liters to milliliters to microliters.
   b. Identify micropipette parts and their purposes.
   c. Choose and set the correct micropipette for the job.
   d. Properly use the micropipette. Accuracy will be checked using an electric balance.
   e. Properly use a microfuge.

Criteria	Needs Improvement 1pt	Adequate 2pt	Adequate 3pt
Convert from liters to milliliters to microliters.	Rarely / Never	Sometimes	Usually / Always
Identify micropipette parts and their purposes.	Rarely / Never	Sometimes	Usually / Always
Choose and set the correct micropipette for the job.	Rarely / Never	Sometimes	Usually / Always
Properly use the micropipette.	Rarely / Never	Sometimes	Usually / Always
Properly use a microfuge.	Rarely / Never	Sometimes	Usually / Always

Name:_____ **Class:**_____ **Total Points: ___/15**