Lecture 2


LTS
0.2-2 µl
LTS
2-20 µl
LTS
20-200 µl
LTS
100-1000 µl
LTS
30-300 µl
LTS
10-100 µl

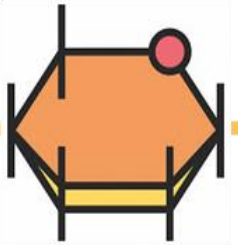
Table of content

LABORATORY PRACTICE

REPORT WRITING

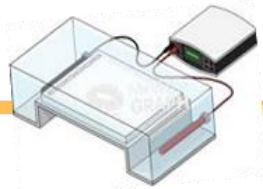
LAB 1: BIOSAFETY

LAB 2: THE MICROPIPETTE

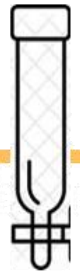


LAB 3: QUALITATIVE ANALYSIS OF CARBOHYDRATES

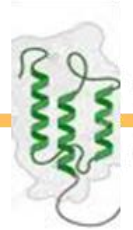
LAB 7: CAPSTONE PROJECT



LAB 6: ANALYSIS OF DNA BY AGAROSE GEL ELECTROPHORESIS

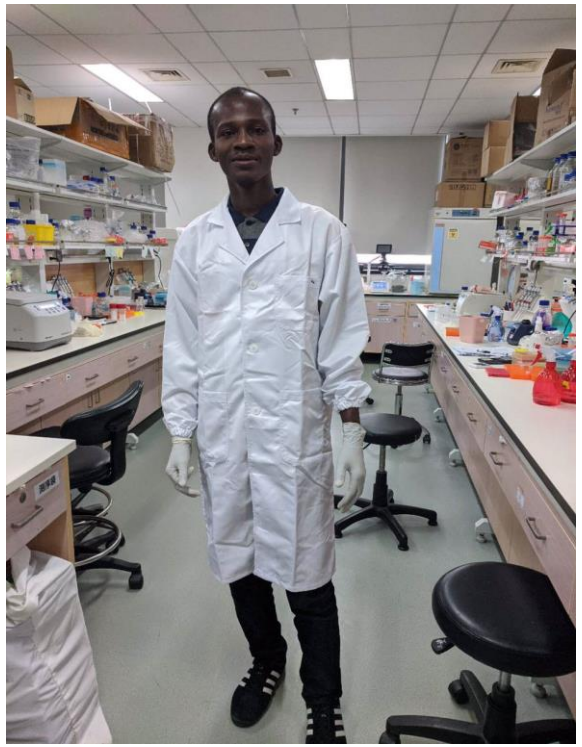


LAB 5: CHROMATOGRAPHIC SEPARATION OF PLANT



LAB 4: ANALYSIS OF PROTEINS - QUALITATIVE AND QUANTITATIVE METHODS

This week Lecture



Introduction & Fundamentals

Why this tool is the heart of the lab and how it works

The User's Guide

Choosing the right tool, setting it up, and avoiding common damage

The Core Skills

Mastering the two essential techniques: Forward and Reverse Pipetting

Quality Control

How we use science to prove our skill is accurate and precise

Lab Citizenship

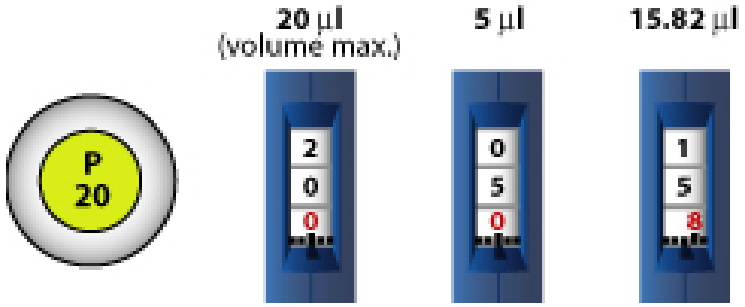
The rules of care, safety, and troubleshooting.

Micropipette: Precision and Accuracy in the Biochemistry Lab

The Importance of Micro pipetting

Why Master the Micropipette?

- The most **fundamental tool** in the modern biochemistry laboratory.
- Allows for the **accurate transfer** of **liquid volumes** in the **microliter (10^{-6} L) range**.
- The **validity** of your **data** depends on your pipetting skills.
- **Goal:** To master its operation as a prerequisite for all future practical work.



What is a Micropipette & Its Types?

What is a Micropipette?

A precision laboratory instrument for **accurately** transferring **small, adjustable liquid volumes**, typically from **1 to 1000 microliters (µL).**

The Micropipette Family: Key Variations

- **Variable Volume Micropipette:** This is the standard pipette we will use most often.
  - ✓ The volume can be adjusted within a specific range (e.g., 100-1000 µL).
  - ✓ Highly versatile for various experimental needs.

What is a Micropipette & Its Types?

❖ **Fixed Volume Micropipette:**

- ✓ Dispenses only one specific, non-changeable volume (e.g., 1000 µL).
- ✓ Offers excellent reproducibility for repetitive tasks.

❖ **Multichannel Micropipette:**

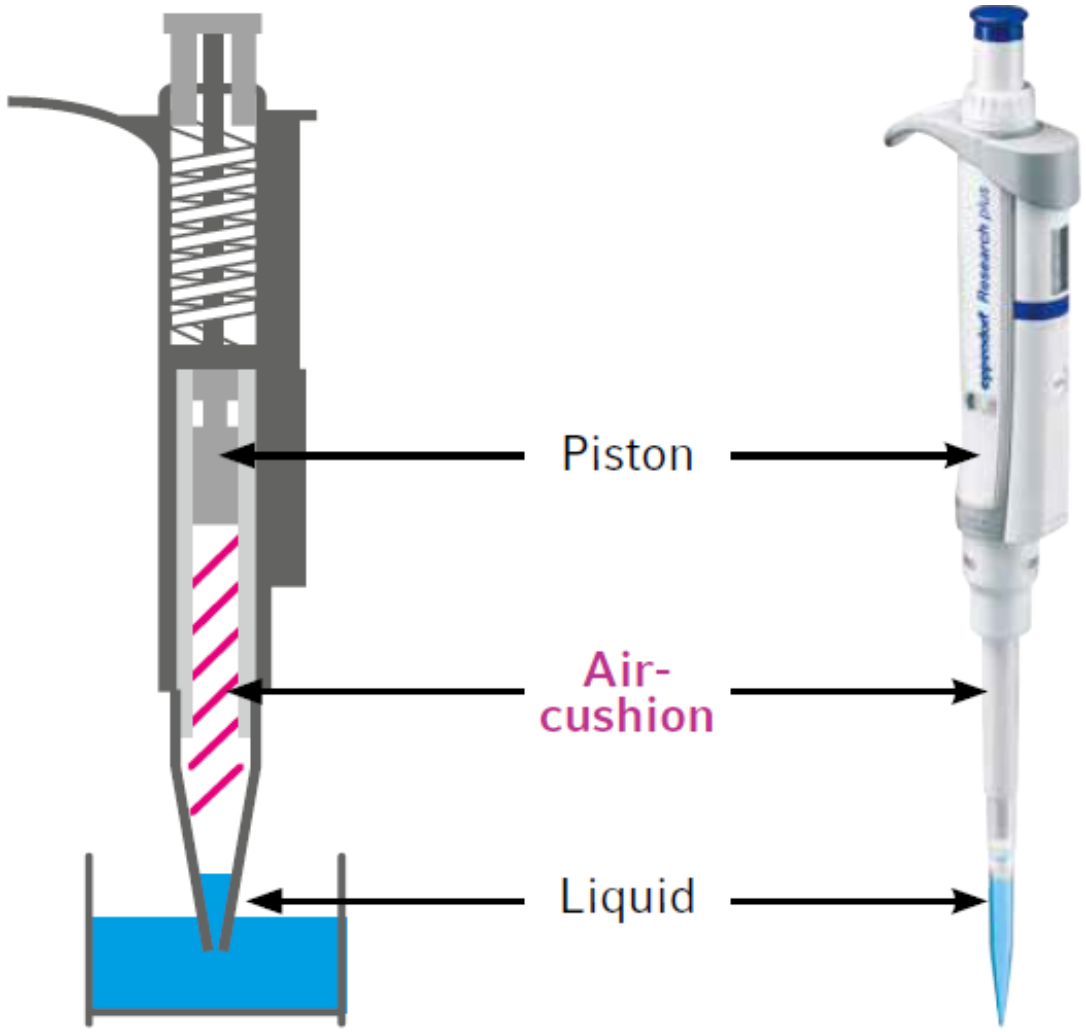
- ✓ Features multiple heads (typically 8 or 12) to handle samples in parallel.
- ✓ Essential for high-throughput work like filling 96-well plates.

Principle of Operation

How Does it Work? The Air Displacement Principle

A piston inside the micropipette moves, creating a cushion of air between the piston and your sample.

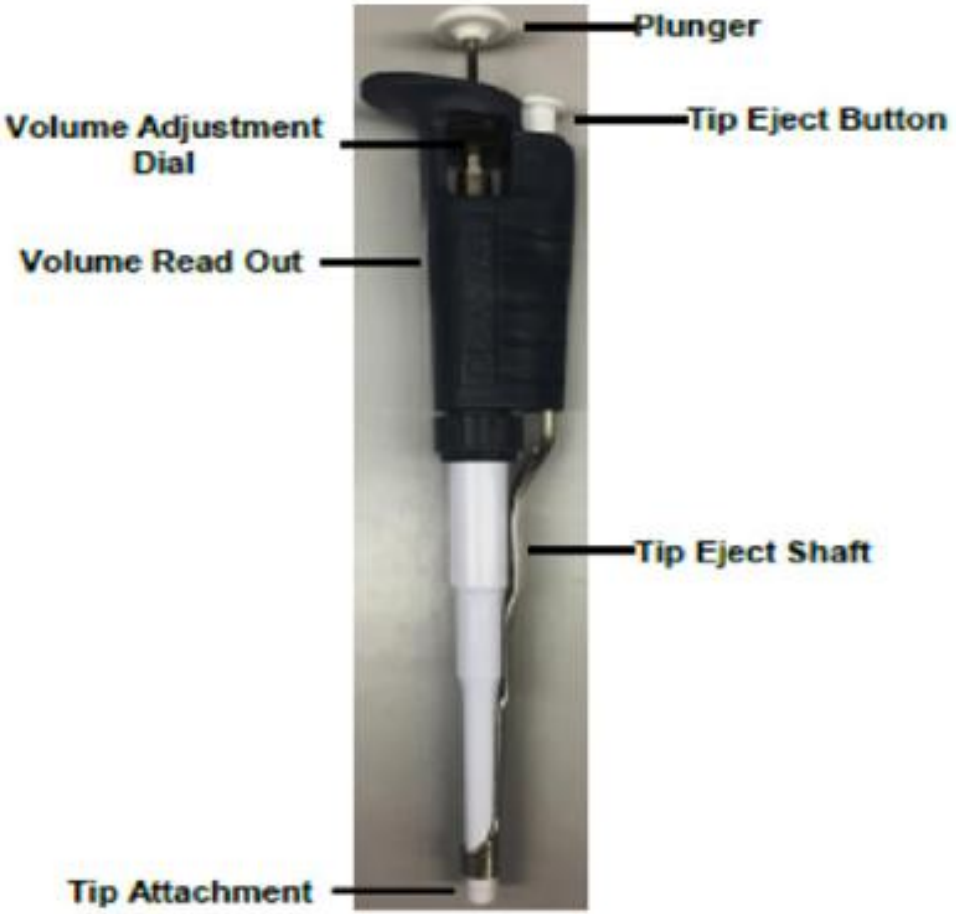
- **Depressing the plunger:** The piston expels a specific volume of air.
- **Releasing the plunger:** A partial vacuum is created. Atmospheric pressure forces liquid into the tip.
- **Dispensing:** The air column then forces the liquid out of the tip.



Anatomy of a Micropipette

Know Your Instrument

- **Plunger:** Sets the **piston** in motion. Has two "stops.
- **Volume Adjustment Dial:** Turn to set the desired volume.
- **Volume Read Out:** Displays the set volume.
- **Tip Eject Button:** Used to discard the tip after use.
- **Tip Attachment/Shaft:** Where the disposable tip is attached.



Getting Started - The Right Tools & Settings

Choosing the Right Pipette

- **The Golden Rule**: Always choose the smallest volume pipette that can handle the volume you need.
- Why? Accuracy decreases when using a pipette at the very bottom of its volume range.
- Example: To measure 35 µL:

P200 (20-200 µL): ✓ Correct Choice.

P1000 (100-1000 µL): ✗ Incorrect Choice (inaccurate).

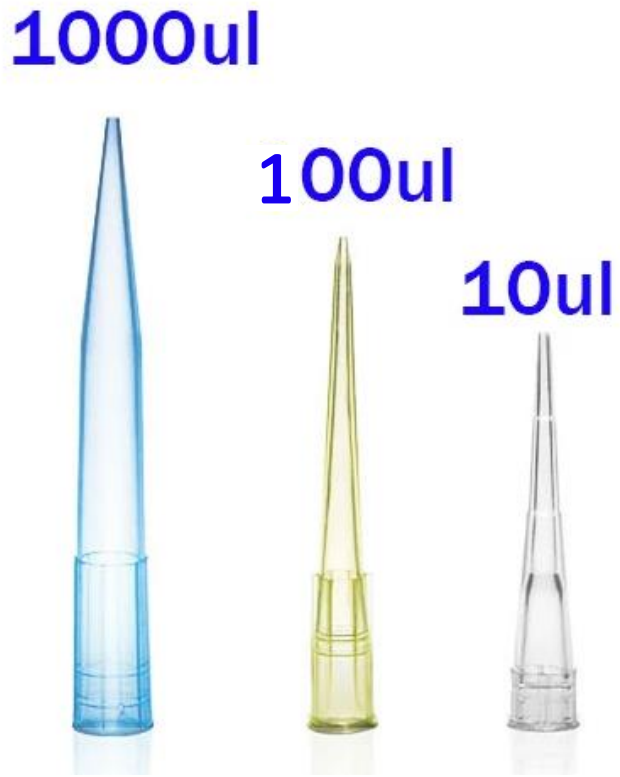
P20 (2-20 µL): ✗ Incorrect Choice (out of range).

All About Pipette Tips

Matching the Pipette to the Tip

Pipette tips are designed to create a perfect seal with a specific pipette model.

- **General Color Code (Brand Dependent):**
  - ✓ **P1000:** Blue or large clear tips.
  - ✓ **P200/P100:** Yellow or medium clear tips.
  - ✓ **P20/P10:** White or small clear tips.
- **Action:**
  - ✓ Ensure you have the correct tip box for your pipette.
  - ✓ When loading, press down firmly but gently to create a good seal.



Reading the Volume

How to Read the Volume Display

- **P1000 (100-1000 µL):** Top digit is thousands. 1-0-0 is 1000 µL.
- **P200 (20-200 µL):** Top digit is hundreds. 1-0-0 is 100 µL.
- **P20 (2-20 µL):** Bottom digit (often red) is tenths. 1-0-0 is 10.0 µL.
- **CRITICAL RULE:** NEVER turn the volume dial beyond the pipette's stated range. This will damage the instrument.

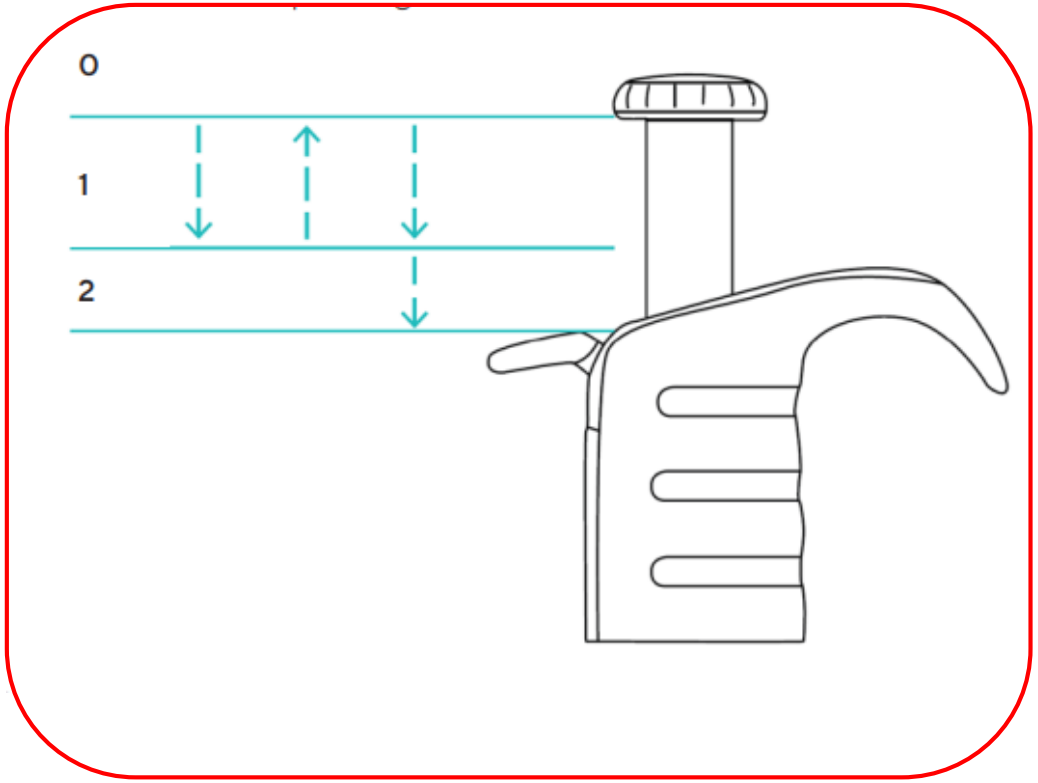
Pipette	P1000	P200	P20	P10
Range	100 – 1000 µl 200 – 1000 µl	20 – 200 µl 50 – 200 µl	2 – 20 µl	1 – 10 µl
Display	0 thousands 5 hundreds 8 tens ones	1 hundreds 4 tens 7 ones tenths	1 tens 3 ones 5 tenths hundredths	0 tens 2 ones 0 tenths hundredths
Volume	580µl	147 µl	13.5 µl	2 µl

Type of Pipetting

- **Forward pipetting**: The target volume is aspirated and dispensed, and a separate blowout step is used to completely empty the tip by pressing the plunger to the second stop.

Understanding the Plunger's Two Stops

- **First Stop:**
  - ✓ Achieved by depressing the plunger gently.
  - ✓ This is the stop you press to when **aspirating (drawing up)** your desired volume.
- **Second Stop:**
  - ✓ Achieved by pressing the plunger past the first stop.
  - ✓ Used only when **dispensing (expelling)** to blow out the last droplet.



Step 1: Aspirating Liquid (Forward Pipetting)

How to Aspirate Liquid Correctly

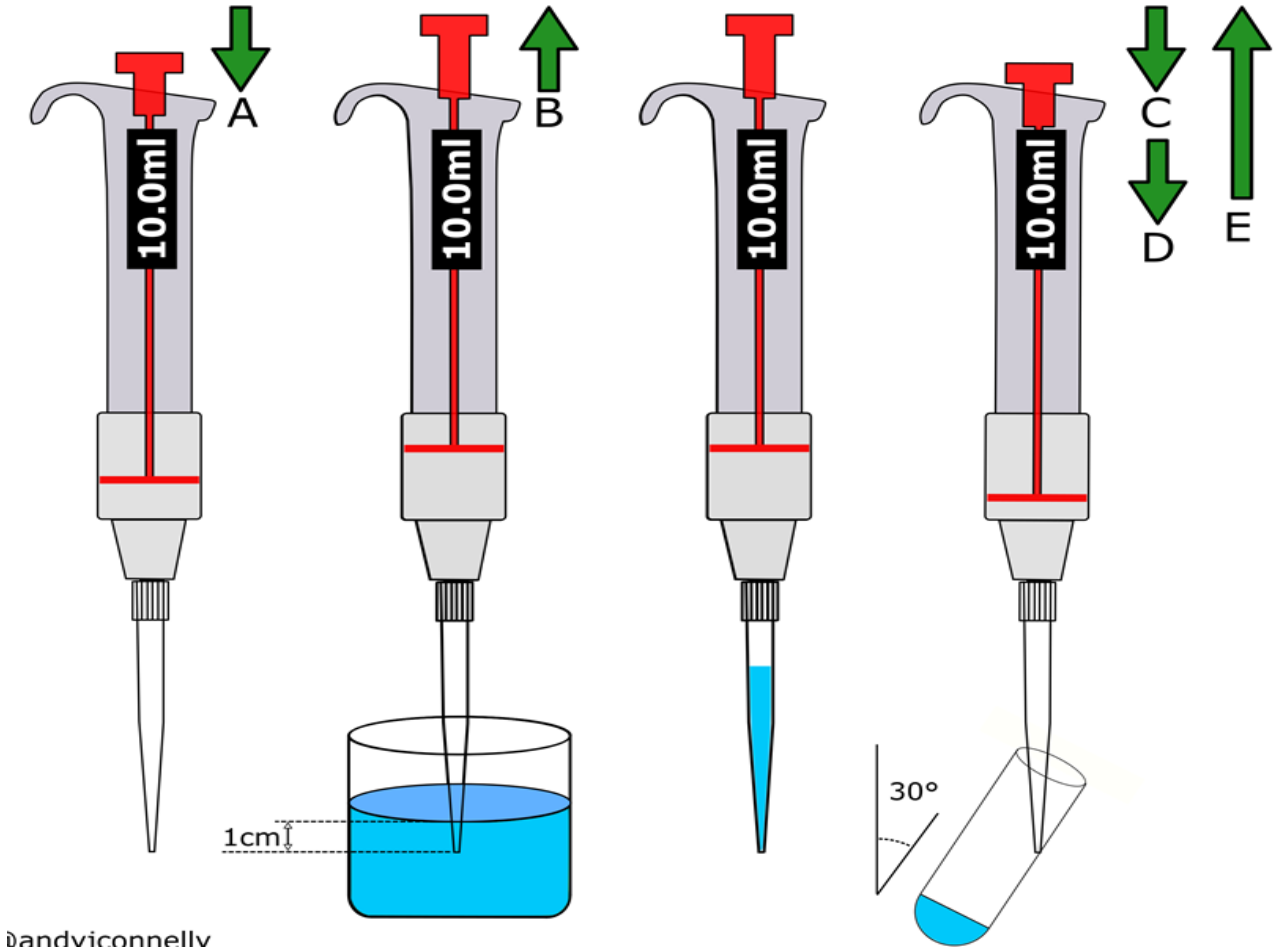
1.**Set the volume** and attach a fresh, sterile tip.

2.**Depress the plunger to the first stop.**

3.**Insert the tip** just a few millimeters below the liquid's surface.

4.To pull up the liquid, **slowly and smoothly lift your thumb off the plunger.**

5.Wait a second to ensure the full volume has entered the tip.



Step 2: Dispensing Liquid (Forward Pipetting)

How to Dispense Liquid Correctly

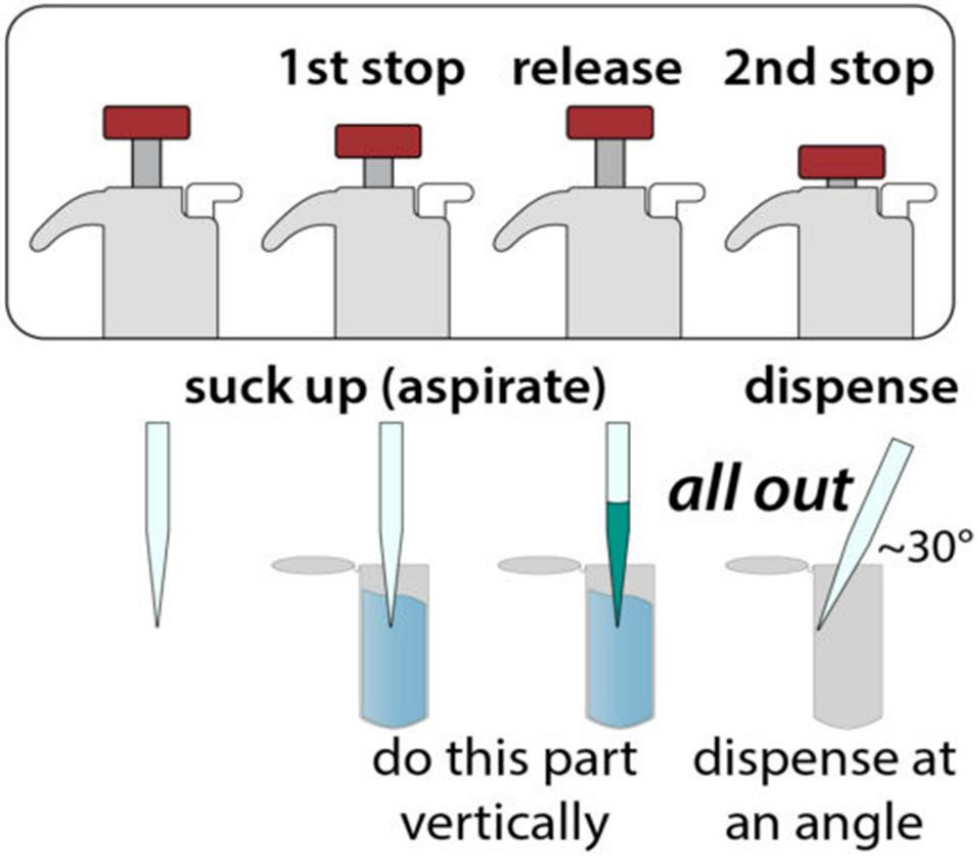
1.Place the tip against the inside wall of the receiving container.

2.Push the plunger smoothly back down to the *first stop*.

3.Pause, then **push the plunger down to the *second stop*** to expel the final droplet.

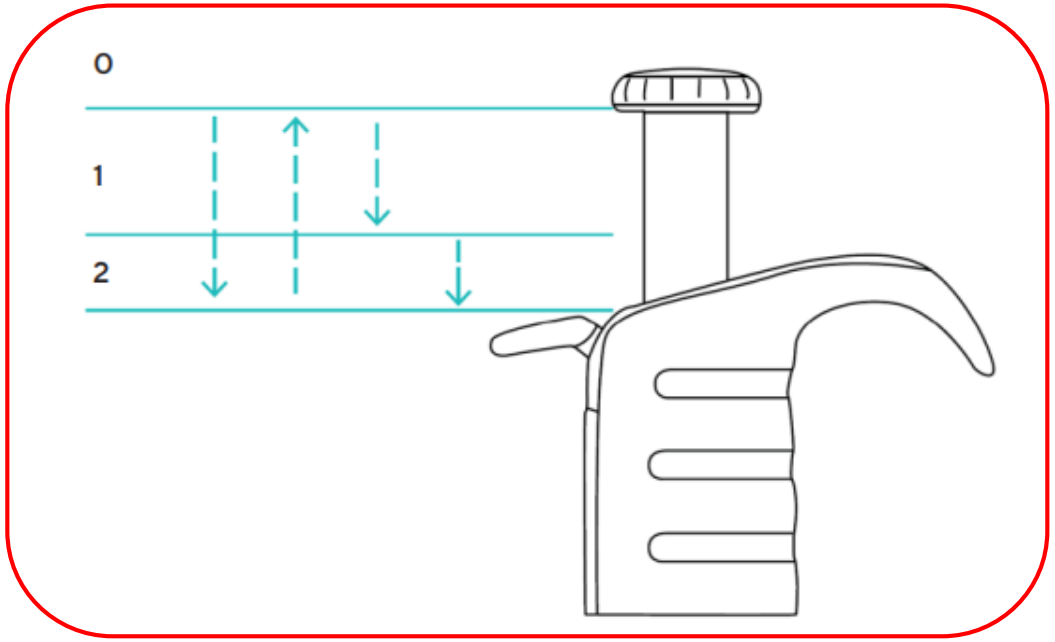
4.Keeping the plunger pressed down, remove the tip from the container.

5.Release your thumb and eject the tip into a waste bin.



Reverse Pipetting

Reverse pipetting is a specialized technique where you intentionally draw up more liquid than you need. You then dispense only the exact volume set on the pipette by pressing to the first stop, leaving the excess liquid behind in the tip to be discarded.



An Alternative Method: Reverse Pipetting

- The standard method is Forward Pipetting.
- Reverse Pipetting is a technique used to improve accuracy with difficult-to-manage liquids.
- Key Difference: You press to the second stop to aspirate and only to the first stop to dispense.
- A small amount of liquid will remain in the tip, which is normal.

When to Use Reverse Pipetting

When is Reverse Pipetting Useful?

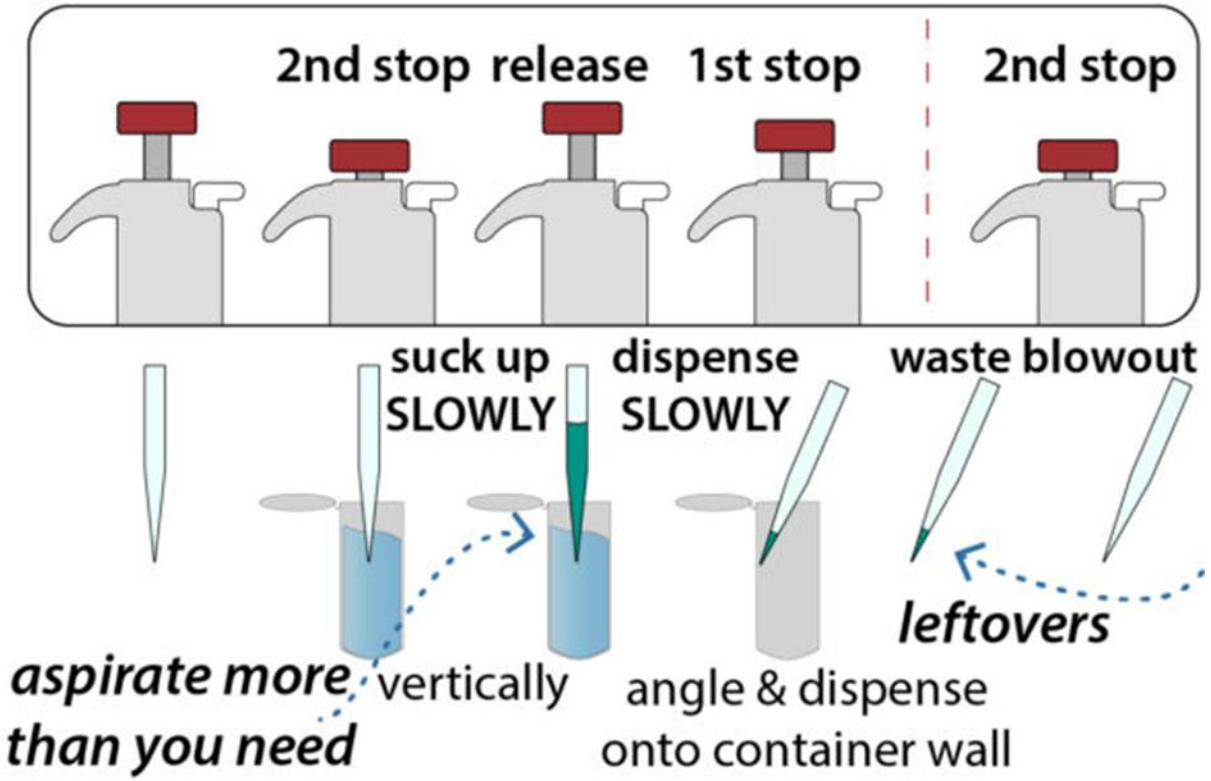
This technique improves accuracy when working with:

- **Viscous solutions** (e.g., glycerol, protein solutions).
- **Volatile solutions** (e.g., ethanol, organic solvents).
- **Solutions that tend to foam** (e.g., detergents).
- Dispensing very **small volumes** (<10 µL).

How to Perform Reverse Pipetting

Step-by-Step Guide to Reverse Pipetting

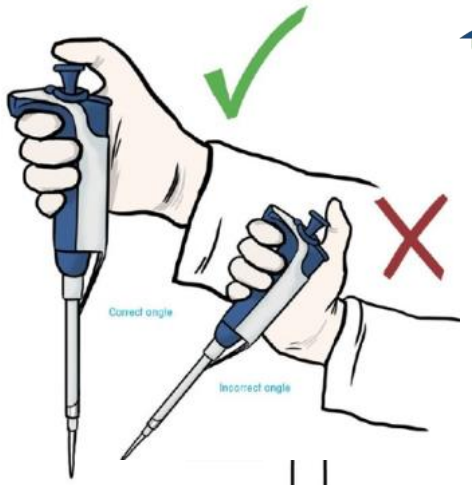
- Press plunger to the SECOND STOP.
- Immerse the tip and slowly release the plunger to aspirate.
- To dispense, press slowly and smoothly only to the FIRST STOP.
- A small volume of liquid will remain in the tip.
- Discard the remaining liquid along with the tip into a waste container.

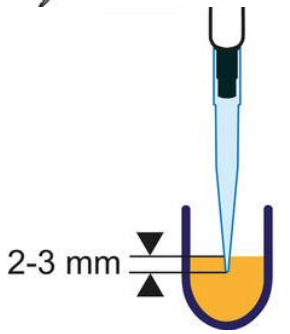


Best Practices for Accuracy

Tips for Optimal Pipetting

- Use a slow, smooth, and consistent rhythm for both aspiration and dispensing.
- Keep the pipette as vertical as possible (within 20 degrees) when aspirating.
- Only immerse the tip 2-3 mm below the surface.
- For viscous liquids, aspirate and dispense the liquid back into the source once or twice to coat the inside of the tip.
- Always use a fresh tip for each different reagent.





Common Pipetting Errors to Avoid

What Not To Do

- **Releasing the plunger too quickly:** Causes air bubbles, splashes inside the tip, and inaccurate volume.
- **Plunging too deep into the liquid:** Causes excess liquid to cling to the outside of the tip.
- **Using the wrong stops:** Aspirating from the second stop takes up too much liquid. Forgetting the second stop on dispense leaves liquid behind.

Quality Control - Proving Your Skill

Why Calibrate? Ensuring Your Data is Reliable

- A micropipette's performance can drift over time.
- **Calibration** quantitatively assesses your pipetting technique and the instrument's performance.
- We will use **gravimetric calibration**, a standard QC procedure that links mass to volume.

Theory of Gravimetric Calibration

The Principle Behind Calibration

- It uses the known **density** of a liquid (distilled water) to convert a measured **mass** into a **volume**.
- **Density of Water (ρ) ≈ 1.0 g/mL or 1.0 mg/µL**
- Therefore:
  - ✓ A volume of **1000 µL** should have a mass of **1.000 g.**
  - ✓ A volume of **200 µL** should have a mass of **0.200 g.**

Accuracy

Assessing Performance: Accuracy

- **Definition:** The closeness of a measured value to the true or target value (a measure of systematic error).
- **Calculation:** Percent Error (% Error)
- **Formula:** % Error = [(Average Measured Mass - Target Mass) / Target Mass] * 100%
- **Goal for Scientific Work: < 2%**

Precision

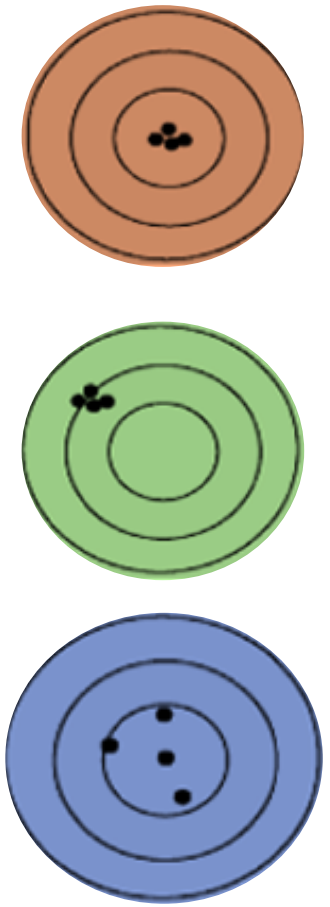
Assessing Performance: Precision

- **Definition:** The closeness of repeated measurements to each other (a measure of random error).
- **Calculation:** Coefficient of Variation (% CV)
- **Formula:** % CV = (Standard Deviation / Average Measured Mass) * 100%
- **Goal for Scientific Work: < 1%**

Visualizing Accuracy vs. Precision

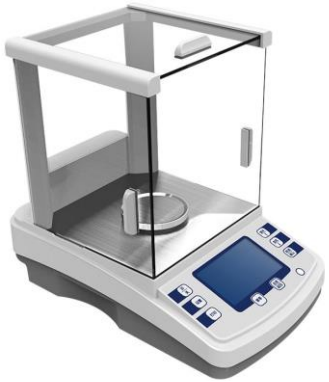
What's the Difference?

- **ACCURATE & PRECISE:** All shots are close to the center and to each other (The Goal!).
- **PRECISE, NOT ACCURATE:** All shots are close to each other, but not near the center.
- **ACCURATE, NOT PRECISE:** Shots are scattered, but their average is near the center.



Calibration Procedure: The "How-To"

How to Test Your Accuracy and Precision



1.Place a weigh boat on the analytical balance and press **"Tare."**

2.Set your pipette to the target volume (e.g., 1000 µL).

3.Dispense the water into the weigh boat and **record the mass.**

4.Press "Tare" again. **Eject the tip** and get a fresh one.

5.Repeat for a total of **five independent measurements.**

6.Repeat the entire process with the second pipette (e.g., P200 at 200 µL).

Data Collection & Analysis

Recording and Calculating Your Results

- **Data Table:** Record your five mass measurements for both the P1000 and P200.
- **Calculations:** For both data sets, calculate:
  - ✓ Average Mass
  - ✓ Standard Deviation (SD)
  - ✓ Percent Error (% Error)
  - ✓ Coefficient of Variation (% CV)

Data Table:

Measurement	Mass P1000 (g) (Target = 1.000 g)	Mass P200 (g) (Target = 0.200 g)
1		
2		
3		
4		
5		
Average:		
Std. Dev.:		

Sample Calculation & Analysis

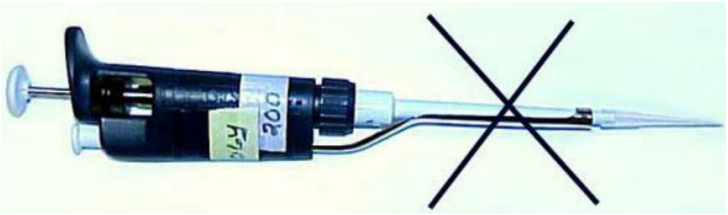
Let's Analyze an Example

- **Scenario:** A P200 is set to 150 µL. Analysis yields an average mass of 0.141 g. The manufacturer's tolerance is ±0.8%.
- **Target Mass:** 0.150 g
- **Calculation:** % Error = [(0.141 - 0.150) / 0.150] * 100% = -6.0%
- **Conclusion:** A -6.0% error is **much larger** than the ±0.8% tolerance. This pipette is out of calibration.

Lab Citizenship - Care & Troubleshooting

Pipette Safety & Handling Rules

- **Never** use a pipette without a tip.
- **Never** lay a pipette down on its side with liquid in the tip.
- **Never** let the plunger snap back after aspiration.
- **Never** pipette hazardous substances without specific training and safety precautions (e.g., in a fume hood).



Pipette Care & Storage

Taking Care of Your Most Important Tool

- **Storage:** Always store pipettes vertically on a pipette stand.
- **Volume Setting:** After use, set the pipette to the **highest volume setting** in its range. This releases tension on the internal spring.
- **Cleaning:** Wipe the outside of the pipette with 70% ethanol to decontaminate.
- **Report Damage:** If you drop a pipette or suspect it's not working, report it immediately.

Troubleshooting Common Problems

What to Do When Things Go Wrong

- **Problem**: Air bubbles in the tip.

  Solution: Re-pipette slowly. Ensure tip is properly immersed.

- **Problem**: Liquid dripping from the tip.

  Solution: Check for the correct tip. If it persists, the pipette may need service. Try reverse pipetting for volatile liquids.

- **Problem**: Inconsistent results during calibration.

  Solution: Focus on consistent technique. If precision remains low (%CV > 1%), alert the supervisor.

Application & Summary

Summary of Key Points

- Choose the **right pipette** for the volume.
- Master the **two-stop system** for forward pipetting.
- Know when and how to use **reverse pipetting.**
- **Accuracy** (% Error) is closeness to the true value.
- **Precision** (% CV) is reproducibility.
- **Proper care and handling** extends the life of the instrument.

Technique Check Quiz

Quick Quiz: Are You Ready?

You need to transfer 185 µL. Which pipette do you use? (Answer: P200)

You are pipetting glycerol. Which technique is best? (Answer: Reverse Pipetting)

To aspirate a sample, you press the plunger to which stop? (Answer: First Stop)

After finishing with your P200, what volume should you set it to for storage? (Answer: 200 µL)

Questions?