UNCC Biotechnology and Bioinformatics Camp

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Lab Intro

- Using Micropipettes

Micropipettes

- How do you control volume in the microliter range?
- How do you verify that the volume delivered by such a device is accurate?

Use of Micro-pipettes

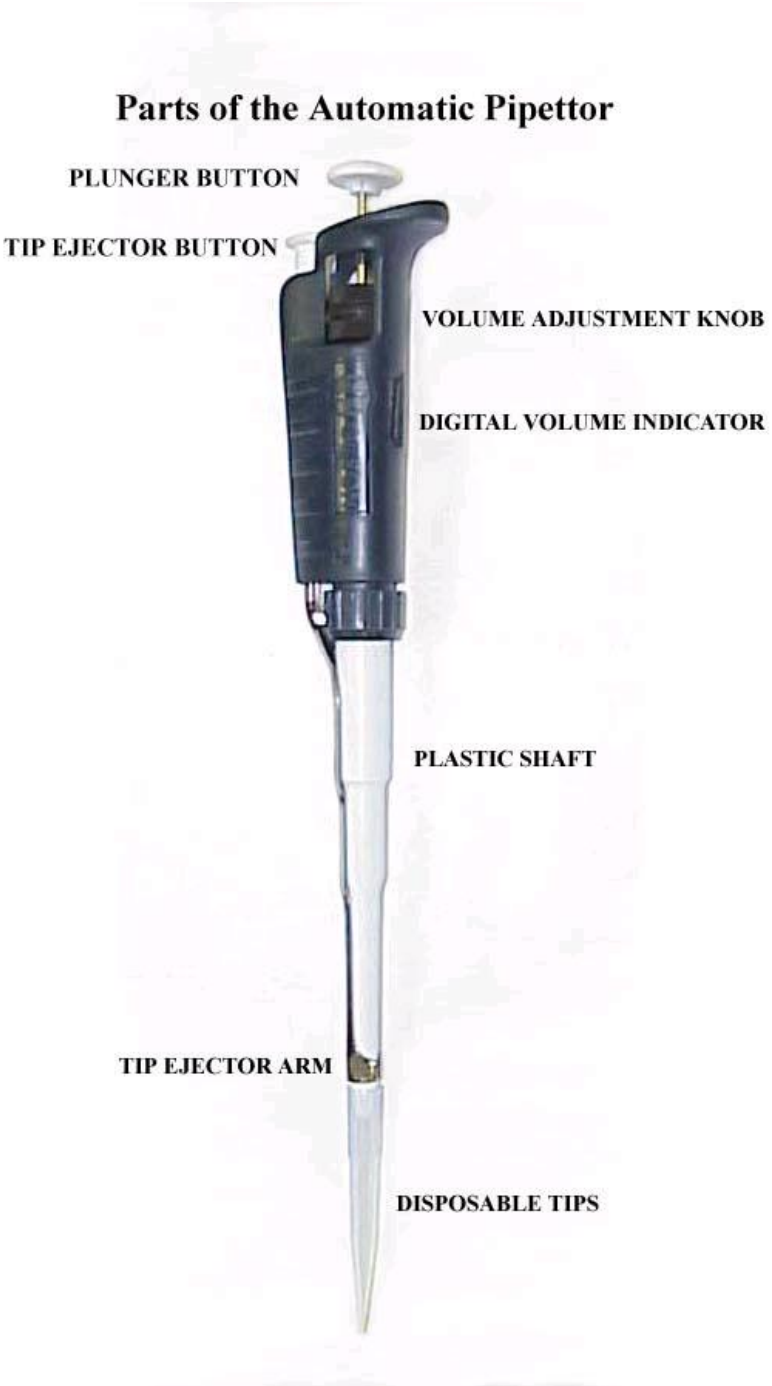
Introduction

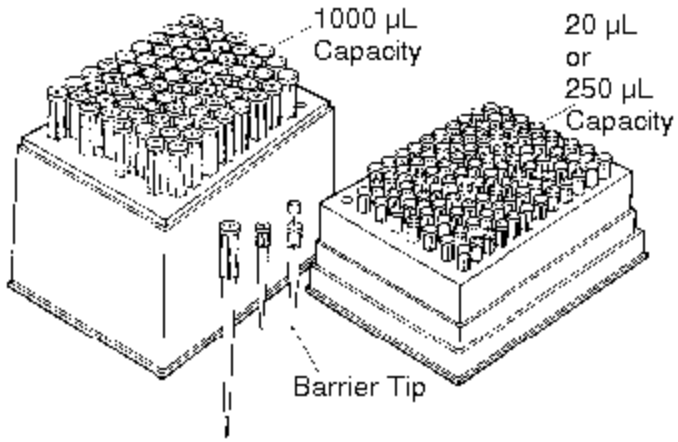
- Micropipettes or ‘Pipetteman’
  - Hand-held devices used to transfer small volumes
- Glass or plastic 1.0 ml pipettes are not highly accurate for volumes less than 0.1 milliliter, but the automatic pipettes are both accurate and precise.
- There are analog and digital models for setting the delivery volume
- Each pipette can be set to transfer any volume *within its own volume range*

Using Automatic Micropipettes

- Select the proper pipette to transfer the specified volume of sample
- Set the specified volume on the micropipette
- Read the digital volume setting correctly
  - convert micro liter (µl) to milliliter (ml) units when necessary
- Demonstrate the correct technique to accurately transfer a sample of a stock solution to another vessel
- Verify the delivery volume by pipetting water onto a piece of film on a fine-scale balance

Parts of the Pipette and tips





Pipette tips

Operating the Micropipette

Step 1: select the correct pipetter

Step 2: Set the Volume

Operating the Micropipette

Read the Volume

		
(a): P-20 Model **6.86 μ l = 0.00686** **or 6.86 x 10^{-3} ml**	**(b): P-200 Model** **132.4 μ l = 0.1324** **or 1.324 x 10^{-1} ml**	**c): P-1000 Model** **262 μ l= 0.262** **or 2.62 x 10^{-1} ml**

Operating the Micropipette

Step 2: Attach the Disposable Tip

Operating the Micropipette

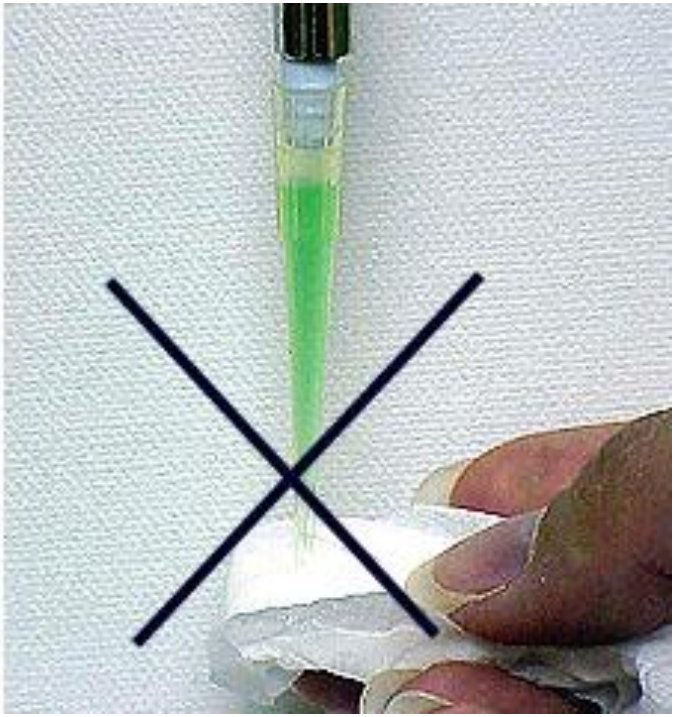
To pull the sample into the tip
A.. Allow the pushbutton to return slowly and smoothly to the fully extended UP POSITION.

NEVER LET THE PLUNGER SNAP UP! This draws the exact calibrated volume into the tip if the tip remains below the liquid surface during withdrawal.

Operating the Micropipette

Remove the tip from the sample liquid. No liquid should remain on the OUTSIDE of the tip.
Depending on the experiment you may wipe away any droplets on the outside of the tip with a lint-free tissue, such as KIMWIPES, but only wipe droplets from the side of the tip.

NEVER TOUCH THE TIP OPENING or you may absorb part of your sample.



Operating the Micropipette

Dispense the Sample

To dispense the sample from the pipette:
a) Touch the tip end to the side wall of the receiving vessel and
b) Depress the plunger to the FIRST STOP.
c) Pause for at least one second-- 1-2 seconds for P-1000, 2-3 seconds for P-5000, or longer for viscous liquids.
d) Press the plunger to the SECOND STOP (the second point, of greater resistance, at the bottom of the stroke) to expel any residual liquid in the tip (like "blowing out" a glass pipette).

(a) Start Dispensing	(b) 1st Stop = Dispense	(c) 2nd Stop = Expel

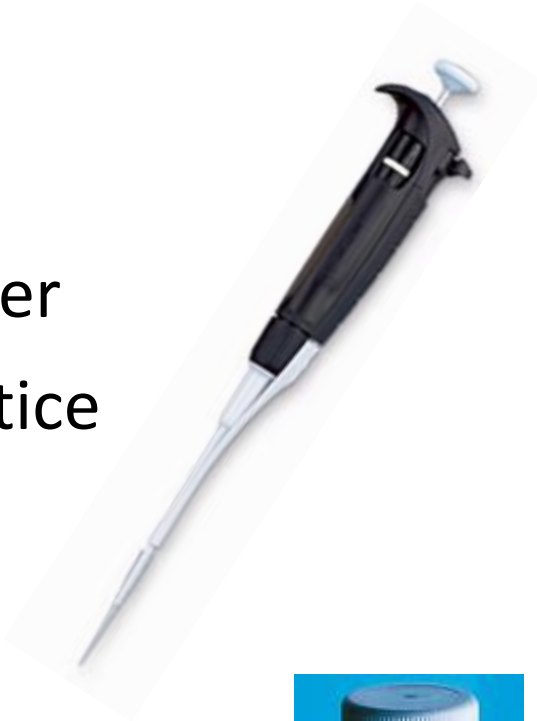
Operating the Micropipette

With the plunger fully depressed, withdraw the pipet from the receiving vessel, sliding the tip along the wall of the vessel.
Holding the tip against the side of vessel is <u>especially important when transferring small volumes of liquid</u>.

Gently allow the plunger to return to the UP position. DO NOT allow it to SPRING BACK!

Equipment and Supplies

- 4 automatic pipettes and matching tips
- A blue-capped tube of water
- A piece of Parafilm to practice pipetting onto
- Gloves, Kimwipes



Step-wise Operation of the Automatic Pipette

(1) Set the volume
(2) Attach disposable tip
(3) Depress the plunger to the first stop
(4) Immerse tip in sample
(5) Draw up the sample
(6) Pause
(7) Withdraw the tip
(8) Dispense the sample
(9) Withdraw the pipette
(10) Release plunger
(11) Discard the tip

Accuracy and Precision

- Accuracy means the closeness with which the dispensed volume approximates the volume set on the pipette
- Accuracy is specified as mean error, the average deviation of replicate measurements from the expected set volume
- Precision is the "scatter" or reproducibility of individual measurements of the same volume
- Precision can also be expressed as standard deviation

Accuracy and Precision (Continued)

- Relative accuracies are generally about 1% or less
- Precision is less than 0.5 % except when transferring the smallest recommended volume for a given pipette model
- Using the pipettes to transfer volumes which are below the recommended range will introduce

Pippetting Guidelines and Precautions

For optimal reproducibility, use the following pipetting procedures:

(1) Consistent SPEED and SMOOTHNESS when you press and release the PLUNGER

(2) Consistent pressure on the PLUNGER at the FIRST STOP

(3) Consistent and sufficient IMMERSION DEPTH

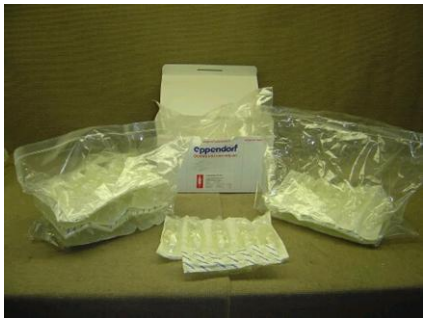
(4) Nearly VERTICAL POSITIONING of pipette

(5) AVOID ALL AIR BUBBLES: Since the plastic pipette shaft can be damaged if liquids are drawn beyond the tip into the shaft

(6) NEVER lay the pipette on its SIDE nor INVERT the pipette if liquid is in the tip

Practice with Pipettes

- Practice using the pipette
- Practice setting a few volumes
- Practice reading the digits of set volumes
- Practice drawing up and dispensing samples
- Get the "feel" of the 1st and 2nd stops
- Practice "blowing out" the pipette



Verifying your pipetting skill

- Note: these micropipettes were recently calibrated
- Note: you do not have to change pipette tips unless you touch one to a surface.
- Put a piece of parafilm on the weighing tray of the Mettler Balance
- Tare the parafilm
- Pipette a series of volumes and record the mass. Record the model of pipetter also.
- 5ul, 7.5 ul, 14.5, 20ul,42.5ul and 75.8ul, 162.5ul.