Use and Maintenance of Micro-pipettes
Introduction

• Automatic pipettes are used to accurately transfer small liquid volumes.

• Glass pipettes are not highly accurate for volumes less than 1 milliliter (1 ml), but the automatic pipettes are both accurate and precise.

• These are continuously adjustable digital pipettes.

• Each pipette can be set to transfer any volume within its own volume range.
The Automatic Pipette

After successful completion of this module the participant will be able to perform following:

• Select the proper automatic pipette to transfer a specified volume of sample
• Set a specified volume on the pipette volume indicator using the volume adjustment knob
• Read a digital volume setting in both micro liter (µl) and milliliter (ml) units
• Demonstrate the correct technique to accurately transfer a sample of a stock solution to another vessel
• Correctly answer questions based on the material for the Automatic Pipette
Parts of the Automatic Pipettor

PLUNGER BUTTON
TIP EJECTOR BUTTON
VOLUME ADJUSTMENT KNOB
DIGITAL VOLUME INDICATOR
PLASTIC SHAFT
TIP EJECTOR ARM
DISPOSABLE TIPS

Parts of the Pipette

Pipette tips
Operating the Micropipette

Step 1: Set the Volume

Pipetters – 3 Volumes:

Volume Adjustment Knob:

Always open the volume control above the final setting you need and then go down to that setting. This helps ensure the accuracy of the internal mechanism.

Digital Volume Indicator:
### Operating the Micropipette

#### Step 1: (Continued) Read the Volume

**How to Read the Volume Indicator:**

<table>
<thead>
<tr>
<th>Model</th>
<th>Volume (µl)</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a): P-20</td>
<td>6.86</td>
<td>0.00686</td>
</tr>
<tr>
<td></td>
<td>6.86 x 10^{-3} ml</td>
<td></td>
</tr>
<tr>
<td>(b): P-200</td>
<td>132.4</td>
<td>0.1324</td>
</tr>
<tr>
<td></td>
<td>1.324 x 10^{-1} ml</td>
<td></td>
</tr>
<tr>
<td>(c): P-1000</td>
<td>262</td>
<td>0.262</td>
</tr>
<tr>
<td></td>
<td>2.62 x 10^{-1} ml</td>
<td></td>
</tr>
</tbody>
</table>
Operating the Micropipette

Step 2: Attach the Disposable Tip

Example of tip sizes:

Attaching the disposable tip
Operating the Micropipette

Step 3: Depress the Plunger to the First Stop

Step 4: Immerse Tip in Sample

Step 5: Draw up the sample
To aspirate the sample into the tip, 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.

Step 6: Pause
Wait a few seconds to ensure that the full volume of sample is drawn into the plastic tip. WAIT LONGER FOR LARGER VOLUMES. WAIT LONGER FOR MORE VISCOUS ("SYRUP-LIKE") SUBSTANCES.
Operating the Micropipette

Step 7: Withdraw the Tip
Remove the tip from the sample liquid. No liquid should remain on the OUTSIDE of the tip. 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.

Proper Droplet Removal

WRONG Droplet Removal
Operating the Micropipette

Step 8: 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.

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).
Step 9: Withdraw the Pipette
With the plunger fully depressed, withdraw the pipet from the receiving vessel carefully, sliding the tip along the wall of the vessel. Holding the tip against the side of vessel is especially important when transferring small volumes of liquid.

Step 10: Release the Plunger
Gently allow the plunger to return to the UP position. DO NOT allow it to SPRING BACK!

Step 11: Discard the Tip
Discard the tip by depressing the tip ejector button, as shown below. A fresh tip should be used for each sample to prevent sample carryover.
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 larger errors
Pipetting 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