Module 4: Micropipettes

Objectives Covered

Pipetting Concepts, Micropipettes:
# 2h
# 5b
# 6ci, 6cii
# 8, 9

Automatic Micropipettes

- **Micropipettes** are used to deliver small volumes of liquid
- Range: 1 uL to 5000 uL (5 mL)
  - Most often <1.0 mL up to 1.0 mL
- **Automatic** implies the parts of the pipette used to aspirate and dispense volumes is an integral part of the pipette

Be Careful

I need to deliver 5.0 mL of liquid but I only have a 1.0 micropipette.

What happens if I use a 1.0 mL micropipette 5 times?

Error is Cumulative

Each pipetting stroke (step) has a potential of delivering an inherent amount of error

By delivering the 1.0 volume 5 times, you are introducing 5X the amount of error.

Best to use a 5.0 serologic pipette or automatic micropipette capable of delivering 5.0 mL

Types of Micropipettes

- Single Stop
- Double Stop
- Fixed Volume
- Variable Volume
**Single Stop Micropipette**

**Single Stop:** there is only ONE plunger stop to fill pipette tip and dispense liquid into receiving vessel.

<table>
<thead>
<tr>
<th>Ready position</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>First stop</td>
<td></td>
<td></td>
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</table>

Begin with plunger at ready position
Depress plunger to the first stop (1)
Immerse tip into liquid
Aspirate liquid into tip by allowing plunger to move up smoothly to the ready position (2)
Remove tip from liquid
Dispense liquid by depressing plunger to the first stop (1)
Return to ready position (2)

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**Double Stop Micropipette**

**Double Stop:** there are TWO plunger stops to fill pipette tip and dispense liquid into receiving vessel.

<table>
<thead>
<tr>
<th>Ready position</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>First stop</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Second stop</td>
<td></td>
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</tbody>
</table>

Begin with plunger at ready position
Depress plunger to first stop (1)
Immerse tip into liquid
Aspirate liquid into tip by allowing plunger to move up smoothly to the ready position (2)
Remove tip from liquid
Depress plunger to first stop, wait one second, then depress plunger to second stop (3) to expel all liquid (this is blow out step)
Return to ready position (4)

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**Fixed Volume Micropipette**

This pipette delivers one specific volume

Used in laboratories with limited variety of testing procedures

Limitation: fixed volume

Examples: Oxford, MLA

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**Oxford micropipette Double stop**
Variable Volume Micropipette

This pipette is able to deliver various volumes by changing the indicator dial to the desired volume setting.

Used in larger laboratories having a larger testing menu.

Limitation: cost; must check calibration at each volume used for testing.
Forward Pipetting Technique

Routinely used when pipetting aqueous solutions

Clinical Laboratory: serum, plasma, urine, body fluids

Reverse Pipetting Technique

Not routinely used when pipetting aqueous solutions.

Error: Increases delivery volume

Clinical Laboratory: used when pipetting viscous, dense, volatile solutions such as whole blood, solvents, alcohols

Refer to ‘Forward and Reverse Micropipetting Technique’ supplemental document
Pipetting Technique - 1
Forward Pipetting Technique: Double Stop

Before you begin:
- Choose correct pipette size for task
- Check the pipette for external dust and dirt; clean if needed
- Apply plastic tip straight and snug
- Hold pipette vertical

Pipetting Technique - 2
Forward Pipetting Technique: Double Stop

1. Depress plunger to first stop (1)
2. Immerse tip into liquid at proper depth
3. Aspirate liquid into tip by allowing plunger to return to ready position (2). Wait one second
4. Remove tip from liquid

Pipetting Technique - 3
Forward Pipetting Technique: Double Stop

5. Depress plunger to first stop, wait one second, then depress plunger to second stop to expel all liquid (3)
6. Return to ready position (4)

At this point, you have pre-wet the tip once
7. Repeat steps 1 through 6 to pre-wet tip a second time

Pipetting Technique - 4
Forward Pipetting Technique: Double Stop

To deliver specified volume of sample:
8. Depress plunger to first stop (1)
9. Immerse tip into liquid at proper depth
10. Aspirate liquid into tip by allowing plunger to return to ready position (2). Wait one second

Pipetting Technique - 5
Forward Pipetting Technique: Double Stop

11. Remove tip from liquid and position pipette over appropriate receiving vessel to deliver the selected volume
12. Depress plunger to first stop, wait one second, then depress plunger to second stop to expel all liquid to appropriate receiving vessel (3)

Pipetting Technique - 6
Forward Pipetting Technique: Double Stop

14. Touch tip to sidewall of receiving vessel
15. Remove pipette from receiving vessel. Do not drag tip along sidewall of receiving vessel
16. Return to ready position (4)

Specified volume delivered in Steps 9 through 16
Tip Immersion Depth
‘Don’t Drown the Tip’

Depth and Position Errors

Proper depth and vertical

Too deep

Too deep and not vertical

Proper Ergonomic Grip

Push this ‘lever’ with thumb to eject tip

Do not pull tip off

Things to Remember

1.Always change tips between specimens
   *Cross contamination error

2. If delivering the same sample and same sample volume in multiple (repeated) tubes, use the same tip.
   *Improves precision (reproducibility/precision)

Eliminate Sources of Error

Increase Accuracy and Precision: Improve Test Results

Automatic Micropipettes

- Choose correct pipette size
- Use correct pipetting technique
- Always pre-wet plastic tips
- All samples/equipment at room temperature
- Insert tip 2-3 mm (small), 4-6 mm (large)
- Hold pipette vertical
- Pipette at steady speed
- Wait one second for sample to fill tip
- No bubbles

Wait, No Wait, Wait Too Long

http://www.artel_usa.com How Timing is Critical for Good Pipetting Technique
Safety: Ergonomics

- Posture
- Feet on floor
- Supplies in close reach
- Relax arms and hands
- Relaxed grip
- Hold pipettes using proper technique
- Elbows close to your side
- Wrist in neutral position
- Avoid static position: 20-30 minutes