



MICROPIPETTE TECHNIQUES

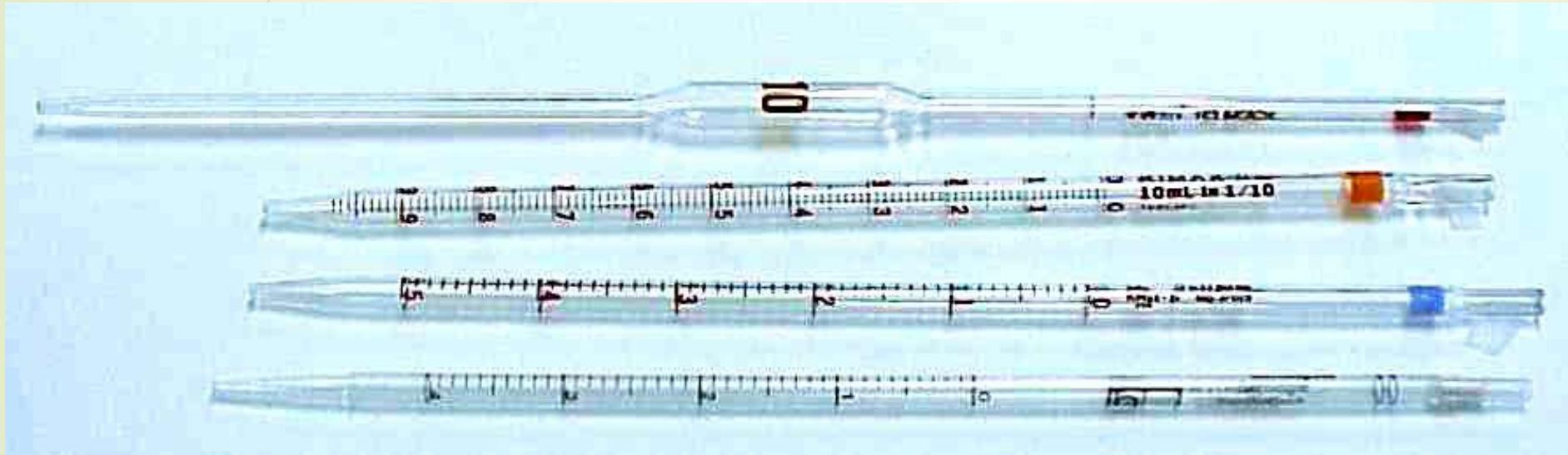
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2nd Term 2015



WHAT ARE PIPETTES?

- Pipettes are glass or plastic tubes, usually open at both ends, which are used to transfer specific amounts of liquid from one container to another.
- They are usually used for volumes between 1 and 100 milliliters.

TYPES OF PIPETTES



- Volumetric
- Measuring
 - Mohr
 - Serological

USE OF MICROPIPETTES

- Automatic micropipettes are usually used for volumes between 1 microliter and 1 milliliter.
- Refer to the SLC Module D11, Automatic Micropipette, for instructions on how to use these instruments.

Specific Reasons for Pipetting

- ➔ Dilutions of patient samples when the concentration of a substance is too high to be accurately measured
- ➔ Preparation of stock standards to yield various target concentrations for working standards
- ➔ Reconstitution of controls used to assess instrument performance and validity of test results.
- ➔ Dilution or preparation of reagents used in the testing method.

Uses of Micropipettes

- Glass pipettes are not highly accurate at dispensing < 1 milliliter (1 mL)
- Automatic pipettes are both accurate and precise for small volumes
- These may be adjustable digital pipettes

Role of Pipettes

- Accurate pipetting is a critical skill required of all clinical laboratory scientists
- Good pipetting skills are required to prepare accurate and precise dilutions of:
 - Reagents
 - Patient specimens
 - For quantitative measurements in the clinical laboratory

Good laboratory practice requires accurate and precise pipetting. It is part of Quality Assurance.

Micropipettes: Small Volumes Dispensed



A micro-volume pipette is used when pipetting ≤ 1.0 mL

Multi-Channel and Adjustable Micropipettes



Mechanical Micropipettes

- There are 2 basic types of mechanical or manual micropipettes
 - Piston (air displacement)
 - The piston style may have 2 stops, in which the lowest position is to ensure that air blows out the last drop
 - Note: a one-stop piston micropipette is easiest to use but prone to having too much of the sample retained behind in the pipette
 - Syringe (positive displacement)

Quality Assurance of Micropipette Use

- ➔ Select the appropriate pipette for the volume required.
- ➔ Select the appropriate pipette tip.
- ➔ Ensure that there are enough tips for the procedure.
- ➔ Ensure that the pipettor, tips, and specimen are at the same temperature.

Getting to Know your micropipet

- There are 4 parts to a micropipet
 - Plunger button
 - Ejector button
 - Volume display (setter)
 - Dispensing Tip



Getting to Know your micropipette

- Plunger button
 - Typically there are 2 stops
 - The first evacuates the air in the micropipette
 - The second stop evacuates the volume plus another 50% or so.
 - Practice to feel the difference

Operating the Micropipette

Step 1: Set the Volume

Pipettors – 3 Volumes:



Digital Volume Indicator:



Volume Adjustment Knob:



Picking the proper size micropipet

- Look at your plunger
 - It will give you the min. and max. that it can deliver
- 3 numbers will be displayed
 - The top # is the digit for the maximum
 - I.e. 2 is the top # on the P200

Operating the Micropipette

Step 1: Read the Volume

How to Read the Volume Indicator



(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

Interpret the following settings

How many microliters is this

on a m10?

3	5	0
---	---	---

3.50 μL
or 0.00350 mL

on a m100

3	5	0
---	---	---

35.0 μL
or 0.0350 mL

on a m1000

3	5	0
---	---	---

350.0 μL
or 0.350 mL

Setting Adjustable Volume



- ▶ Course and fine setting of the pipette line. Because the course and fine settings are separate, it is not necessary to "wind" the pipette as would be done on a screw-type adjustment

Operating the Micropipette

Step 2: Attach the Disposable Tip

Attaching the disposable tip:



Example of tip sizes:



Operating the Micropipette Steps 3-6

- Step 3: Depress the Plunger to the First Stop



- Step 4: Immerse Tip in Sample
- Step 5: Draw up the sample
- Step 6: Pause before withdrawing tip
- *Wait a few seconds*



Mechanical Micropipette Procedures

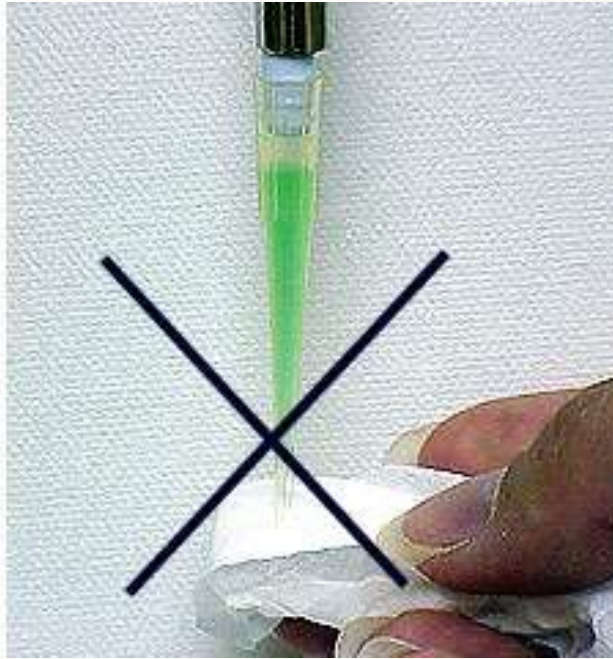
- ▶ Particularly important is removing excess fluid from the outside of the pipette tip
 - ▶ **For syringe pipettes**
 - ▶ The filled syringe should be dipped in distilled water to remove excess fluid clinging to the outside
 - ▶ **For piston pipettes**
 - ▶ The outside of the pipette should be carefully wiped with a disposable tissue, taking care not to touch the bottom of the pipette tip

Operating the Micropipette

Step 7: Wiping the Tip



Proper Droplet Removal



WRONG Droplet Removal

Operating the Micropipette

Step 8: Dispense the Sample



**(a) Start
Dispensing**

**(b) 1st Stop =
Dispense**

**(c) 2nd Stop =
Expel**

Operating the Micropipette Steps 9-11



➤ Step 9: Withdraw the Pipette

➤ Step 10: Release the Plunger

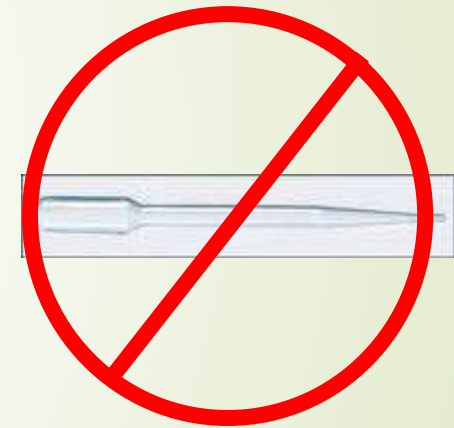


➤ Step 11: Discard the Tip

Press ejector button to discard tip

Pipette: Use and Care

- Discard contaminated tips in appropriate container after completion of task
- Handle carefully
- Store in an appropriate rack or stand
- **DO NOT RE-USE**
 - Pipette tips
 - Graduated plastic bulb transfer pipettes



Routine Maintenance

- Check at the beginning of each workday for dust and dirt on the outside surfaces. Pay particular attention to the tip cone
- Clean with 70% isopropyl alcohol
- In addition to routine calibration, pipettes should be cleaned and lubricated as recommended by the manufacturer

Routine Maintenance

- Inspect the pipette for scratches or other damage to the nose cone
- Check for leaks by aspirating the maximum listed volume of distilled water
- If the pipette is dropped or you suspect any type of damage, do calibration check

Mechanical Pipette Maintenance

- Maintain *complete records* of pipette calibration function check
 - Include serial and other identifying numbers
- Annual check of calibration with gravimetric or spectrophotometric method

Mechanical Pipette Maintenance

- ▶ The accuracy and precision should be checked ***the first time of use and periodically*** thereafter
- ▶ Accuracy and precision should be checked ***at least once every year***
 - ▶ If either fails, it is important to follow the manufacturer's instructions

See job aid for complete maintenance procedures