



LECTURE 3: Pipetting Techniques

MT 103: PMLS

2ND SEMESTER | AY 2025-2026 | PROF. SIR TYRONNE
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TOPIC OUTLINE

★ Pipetting Techniques

WHAT IS PIPETTE?

- Utensil to transfer liquid
- Eumycotic Mycetomas
- Subcutaneous Phaeohyphomycosis
- Sporotrichosis

Classification

1. Manual Pipets
2. Mechanical devices

Manual Pipets are

1. Transfer pipet (volumetric)
2. Measuring pipet
3. Micropipet

subclassification ■ TC TD TD/Blow out

The volume of TD pipette = Volume of TC pipette + drainage holdback error

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• Drainage holdback error- The amount of liquid required to wet the inner surface of the pipette and the remaining liquid left at the tip of the pipette after it has been used

• ex- "adjusted to deliver"

• in- "adjusted to contain"

TD vs TC

- ★ TD pipettes are much more common than TC pipettes.
- ★ Most typical graduated pipettes or bulb pipettes are usually calibrated to deliver (TD), whereas capillary pipettes are adjusted to contain (TC).
- ★ TD pipettes are commonly used in quantitative analysis when precise volumes of liquid are required, as they ensure the accurate delivery of a specific volume.
- ★ TC pipettes are useful when it is important to dispense the exact same volume each time, such as in serial dilutions or when preparing solutions with specific concentrations.

TC

- ★ Contain exact amount
- ★ Accurate measurement
- ★ Must be completely drain
- ★ Rinsed with solvent
- ★ E.g.:
 - Sahli-Hellige
 - Volumetric Pipette
 - Lang-Leavy

BLOW-OUT

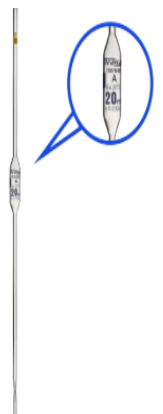
- ★ Deliver exact volume after blowing out
- ★ Allowed to drain
- ★ Then blow out
- ★ Not rinsed out
- ★ double band / frosted ends
- ★ E.g. :
 - Ostwald-folin
 - Serological

TD

- ★ Deliver exact volume
- ★ Allow to drain by gravity
- ★ No blow out
- ★ Eg:
 - Automatic micropipette
 - Volumetric transfer
 - Serologic pipette

Volumetric Pipet

- Long glass tube
- Open ended bulb
- Max line limit
- Single volume
- Tapered delivery tip
- For dilute aqueous Solution
- Self draining
- Reliability decrease as size



Mohr pipet

- Uniform diameter
- Tapered delivery tip
- Graduations incised on stem only
- Self draining
- Long tips



Serological pipet

- Long glass or plastic tube
- Uniform diameter
- Tapered tips
- Graduated till tip
- Blow out
- Variable openings
- Large opening for viscous fluids



Bacteriologic pipet

- Glass or polypropylene
- Use for transport of bacterial specimen esp. pus
- Sealed by flame and cotton plug
- Provide maximum sterility



Ostwald-Folin pipet

- Bulb closer to delivery tip
- Viscous fluids
- Blow out
- Opaque fluid read top of meniscus



Pasteur pipet

- Don't have calibration marks
- Transfer fluids
- Without consideration of volume
- One time use
- Plastic or glass
- Beral pipet
- flexible soft plastic, has a built-in bulb on the end.



Pipetting technique

- Pipetting bulb
- Held vertical
- Read at eye level
- Lowest part of meniscus at line level
- Unrestricted flow
- Inclined container and touch tip
- Etched ring or frosted end
- Blow out after drain

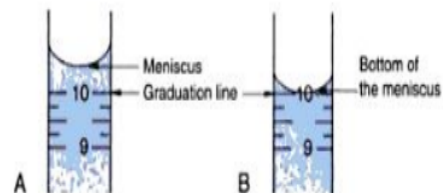


FIGURE Pipetting technique. (A) Meniscus is brought above the desired graduation line. (B) Liquid is allowed to drain until the bottom of the meniscus touches the desired calibration mark.

SUBCUTANEOUS MYCOSES

- ★ Semiautomatic devices
- ★ ≤ 1ml
- ★ Mostly are TC (rinsed out)
- ★ 1-1000µl
- ★ 0.5µl-20ml
- ★ Fixed or adjustable



Pipet terms

- **Adjustment** - altering the pipet so that the dispensed volume is within the specifications.
- **Aspirate** - to draw up the sample.
- **Dispense/distribution** - to deliver the sample.
- **Calibration check** - checking the difference between the dispensed volume and the selected volume.
- **Accuracy** - is the degree of closeness of volume indicated and dispensed.
- **Precision** - reproducibility the degree to which further measurements show the same or similar results.
- **Purge** - blow out

Advantages

- ★ Adjustable
- ★ Ergonomic design
- ★ Multiple wells
- ★ Piston driven
- ★ Stability
- ★ Safety
- ★ Ease of use
- ★ Disposable tips
- ★ No washing or drying
- ★ Save time
- ★ Avoid cross contamination
- ★ Improve precision

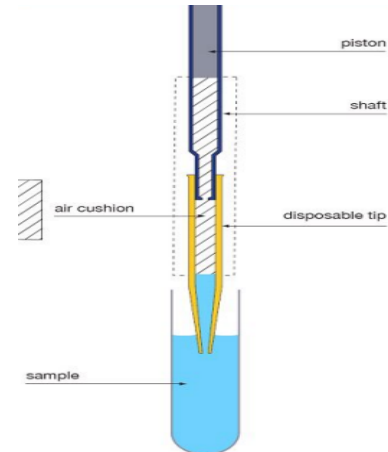


Micropipette types

- ★ Air displacement
- ★ Positive displacement
- ★ Dilutor or dispenser

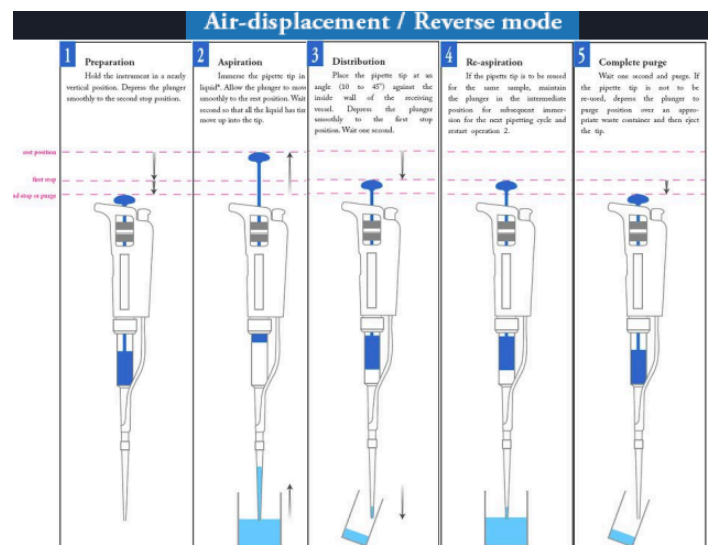
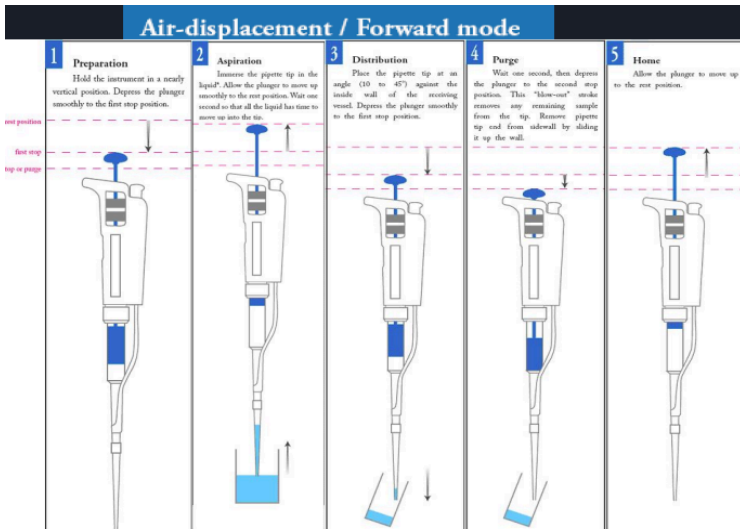
Air displacement pipet

- ★ Accurate & precise
- ★ Relies on piston for suction creation
- ★ Air cushion between piston and disposable tip
- ★ No contact of piston and sample
- ★ Can be used as
- ★ Forward Pipetting
- ★ Reverse Pipetting



EUMYCOTIC MYCETOMA: Treatment

- ★ Preparation
 - Hold in vertical position.
 - Depress the plunger to first stop position.
 Prolonged antifungal therapy
- ★ Aspiration
 - Immerse tip in the liquid.
 - move plunger smoothly to the rest position.
- ★ Distribution
 - Place tip at an angle (10 to 45°) against the inside wall of the receiving vessel.
 - Depress plunger to the first stop position.
- ★ Purge
 - depress the plunger to the second stop position ("blow-out")
 - Remove pipette tip end from sidewall by sliding it up the wall.
- ★ Home
 - Allow the plunger to move up to the rest position

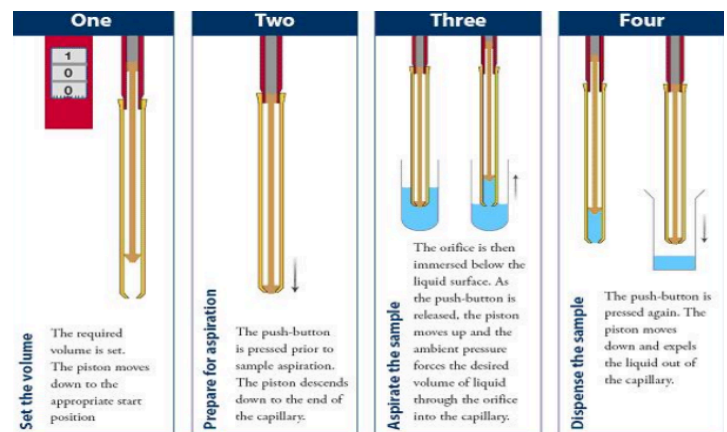
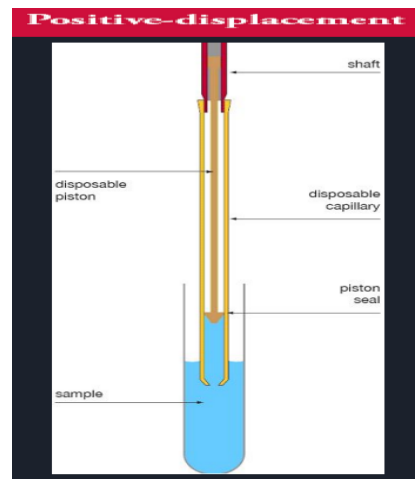


Reverse Pipetting

- ★ Preparation
 - Hold vertical position.
 - Depress the plunger to the second stop position.
- ★ Aspiration
 - Immerse the pipette tip in the liquid.
 - Allow the plunger to move up smoothly to the rest position.
- ★ Distribution
 - Place tip at an angle (10 to 45°) against the inside wall of the receiving vessel.
 - Depress the plunger to the first stop position. Wait one second
- ★ Re-Aspiration
 - pipette tip is to be reused for the same sample.
 - maintain the plunger in the intermediate position for subsequent immersion for the next pipetting cycle and restart operation 2.
- ★ Complete purge
 - Wait one second and purge.
 - If the pipette tip is not to be re-used, depress the plunger to purge position over an appropriate waste container and then eject the tip.

Positive displacement pipet

- Moving piston in pipet tip
- Carry over concern
- Rinsing and blotting b/w samples



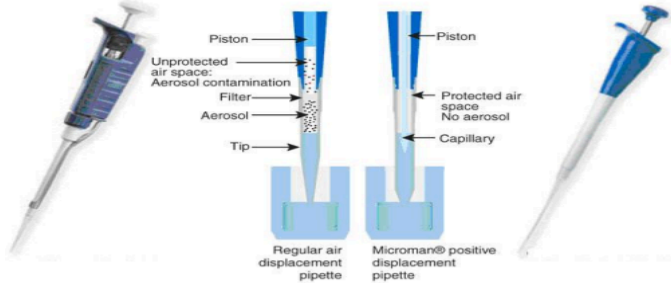


Figure | Two pipetting concepts: air displacement using standard filter tips, and positive displacement as applied in the Microman pipette.

General guidelines

- Check at the beginning of work, wipe with 70% ethanol.
- Set the volume.
- Fluid and pipet tip on same temp.
- Recommended tip for pipet.
- High-quality tips of contamination-free polypropylene.
- Tip for single use.
- Avoid inverting pipette when liquid in the tip.
- Avoid fingers contamination by using the tip ejector and gloves.
- Use Pipet stands
- Check calibration regularly.
- Follow the instructions for recalibration by the manufacturer.

Risk factors

- Repetitive motion and excessive force of the thumb.
- Awkward postures of the hand, wrist, arms and shoulders.
- Bending and twisting of the wrist.
- Forceful gripping.
- Holding the hand in an elevated position and distant from the body.
- Sustained postures.

Preventing cross-contamination

- **Pipet-to-sample**
 - contaminated pipette or tips can cause contamination of samples.
- **Prevention:**
 - Use sterilized tips
 - if possible autoclave the pipet.
 - Change the tip after pipetting of each sample.
 - Store the pipet vertically.
- **Sample-to-pipet**
 - Two ways
 - Samples can enter the cone of the pipet.
 - aerosols of sample
- **Prevention:**
 - vertical pipette
 - Release the push-button slowly.
 - To avoid aerosol contamination, use filter tips or use a positive displacement
- **Sample-to-sample (carry-over)**
 - The remains of sample A can mix with next sample B.
- **Prevention:**

Bipolaris species

- To dispense repeatedly specified volume
- Attach to reagent bottle directly
- Depression of plunger dispense specified volume
- Error rate 1%
- Precision rate 0.1%
- Useful for serial dispensing



Mechanical devices

- Single well or multiple wells
- Use disposable tips or washing out
- Programmable
- 96 or 384 wells



- Change the tip after each sample.
- autoclave or cleaning of pipette

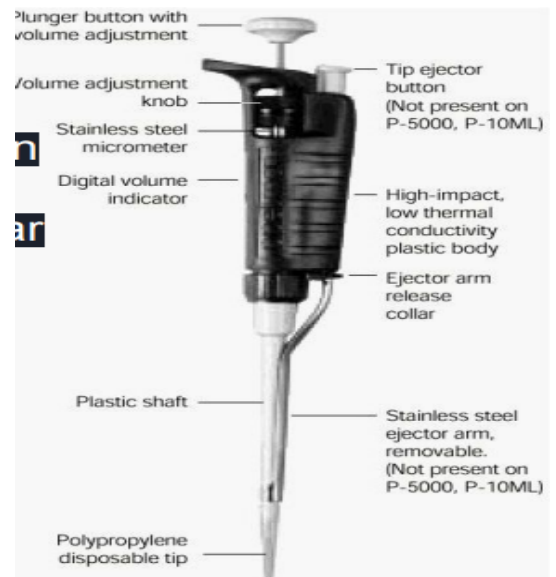
Tip Selection

- Use recommended tips only
- High quality pure polypropylene
- Free from dust or particles
- Uniform in size and shape
- Accurately centered for tip orifice
- Free from metals, like cadmium
- Securely sealed: There should be good tip cone match for a perfect seal and tip eject
- Free from blemishes, flashing or any protrusions
- Highly resistant to chemicals
- Highly stable thermally



Quality control

- **General**
 - Check accuracy and precision
- Depend amount of use
- At least once or twice per year
- Routine maintenance
- Air displacement
 - Stroke length
 - Air seal
- Positive displacement
 - Spring check
 - Replacement of Teflon tip



Criteria for selection of pipet

- Level of accuracy and precision required
- How well the device perform
- Autoclaved or not
- Ease of use
- Work load
- Maintenance and calibration
- Cost, consumable and replacement parts
- availability