

## PIPETTING TECHNIQUES AND VOLUMETRIC MEASUREMENTS

### Introduction:

The ability to accurately pipette is a critical skill required of all clinical laboratory scientists. Accurate and precise pipetting skills are required to prepare accurate and precise dilutions of various reagents and patient specimens when performing quantitative measurements in the clinical laboratory. This may include preparing

1. Dilutions of patient samples when the concentration of a substance is too high to be accurately measured
2. Stock standards to yield various target concentrations for working standards
3. Controls used to assess instrument performance and validity of test results
4. Reagents

Most clinical laboratory procedures require accurate volumetric measurements to ensure accurate results. Such measurements may be made by manual pipettes (serologic, volumetric and micro), dispensing devices, automated pipettes, burets, volumetric flasks and graduated cylinders. The reliability of these devices can vary considerably, and not all of these devices are suitable for the most accurate work. The maximum allowable error (or tolerance limits) for these devices are set by the National Institute of Standards and Technology (NIST). For exact work, only Class A glassware should be used, as this class of glassware meets NIST specifications.

### Calibration of pipettes

Pipettes are checked by the laboratory to ensure accurate delivery of a specified volume. When the laboratory does this 'check', the CLS refers to it as 'checking or verifying pipette calibration'. In other words, we are checking to make sure the pipette is still 'calibrated' to deliver a specified volume.

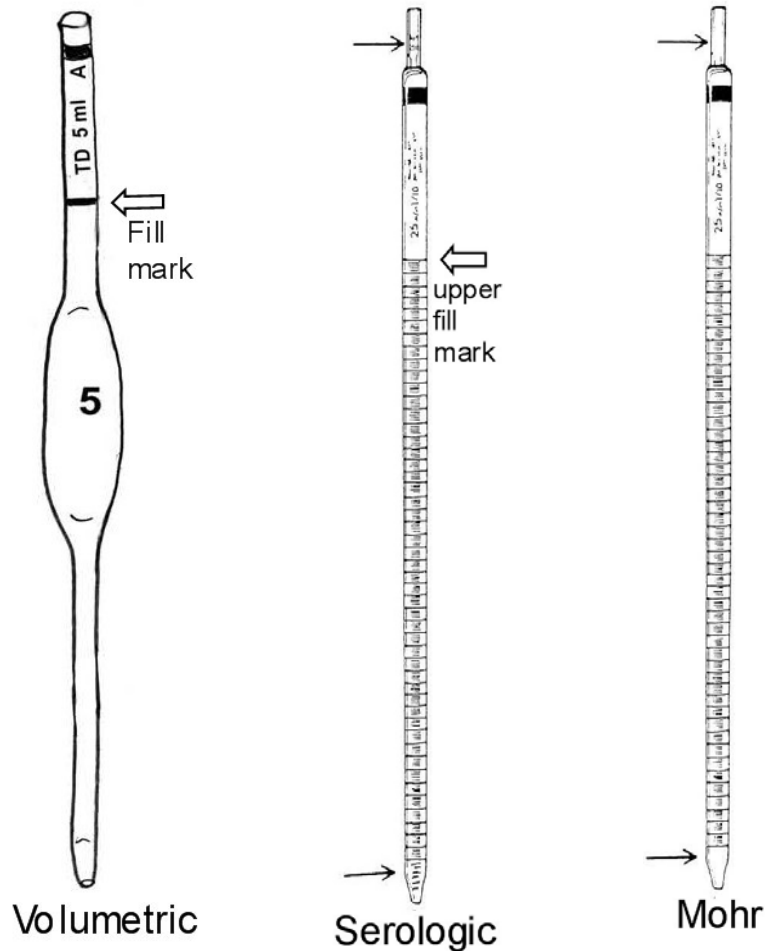
Macro-volume pipettes that are Class A do not need periodic calibration checks by the laboratory. These pipettes are calibrated to deliver a specified volume by the manufacturer. As long as the pipette is properly used this is true. Most laboratories will use only class A macro-pipettes and glassware so that in-house calibration checks are not needed. This saves time and money.

Calibration of micro-volume pipettes is required by the laboratory at periodic intervals (usually quarterly) to verify accurate delivery of a specified volume of liquid. Micro-pipette calibration may be verified several ways. The most commonly used methods in the clinical laboratory are:

1. Measuring the weight of a specific volume of distilled water at room temperature:  
**One milliliter (ml) of dH<sub>2</sub>O weighs 1 gram at room temperature**
2. The absorbance (or %T) of a specific volume of a colored solution is measured spectrophotometrically and the absorbance (or %T) is compared to established tolerance limits (most often done)
3. Acid/base titration

Types of MACRO-VOLUME PIPETS commonly used:

Most laboratories use volumetric and serologic pipettes. Mohr pipettes are seldom used in the clinical laboratory due to potential misuse of this pipette which causes invalid test results.



1. VOLUMETRIC: Also see video: Techniques: Volumetric
  - a. This type of pipette delivers a single, specified volume of fluid. Pipettes differ in size and thus volume to be delivered: 1ml, 2ml, 3ml, 5ml, 10 ml, etc
  - b. Designed with an open-ended bulb in the center of the pipette which holds the bulk of the fluid, a long glass tube at the upper end which has a mark indicating the proper filling level and a tapering lower end to deliver the liquid
  - c. When delivering the fluid to a receiving vessel,
    - 1) The contents of the pipette are allowed to **drain by gravity** without touching the inside walls of the receiving vessel
    - 2) After the fluid stops flowing into the receiving container, the bottom tip of the pipette is touched to the inside surface of the receiving container to allow fluid to be delivered to the receiving vessel by capillary draw. The fluid remaining in the tip after capillary draw is **allowed to remain** in the pipette tip (ie: pipette is not completely drained; pipette is not blown out)
    - 3) The tip of the pipette **SHOULD NEVER** come in contact with the accumulating fluid in the receiving vessel during drainage
  - d. This is the **most accurate pipette** and should be used when diluting or preparing standards and controls

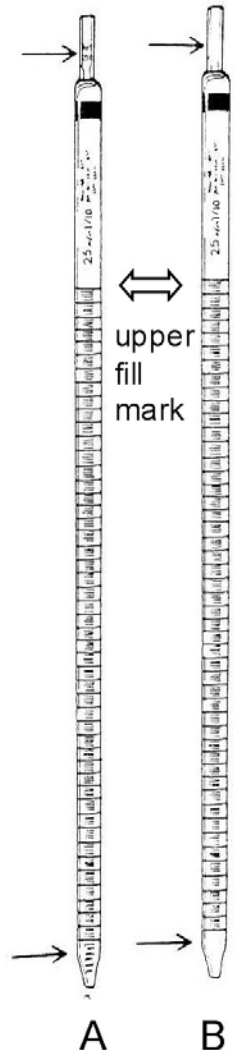
2. SEROLOGIC: (figure A) Also see 3 videos: Techniques: Serologic;  
Pipettes: Blow-out Pipet; Pipettes: Volumetric vs Serologic

- a. This type of pipette is capable of delivering multiple volumes of fluid. Pipettes differ in size and thus volumes to be delivered: 1ml, 5ml, etc
- b. Designed as long glass tubes of uniform diameter with volume graduations (usually at 0.1 ml intervals) that extend all the way to the very end of the pipette delivery tip. Volume graduations make this type of pipette capable of delivering intermediate volumes of liquid (3.9 ml, 4.5 ml, 5.0 ml, 9.8 ml, 10.0 ml, etc)

- 1) Measurement from the upper fill mark to a lower mark ensures accurate delivery of the pipette's specified volume
- 2) Partial measurements using the last few milliliters of the pipette in or near the delivery tip are inaccurate (and thus discouraged) due to changes in the diameter of the pipette

Serologic pipettes are also identified by the frosted rings (or pair of rings or a solid ring) near the upper end. The frosted/solid rings indicate the pipette is a **blow-out** pipette

- c. When delivering fluid to a receiving vessel,
  - 1) The contents are allowed to **drain by gravity** without touching the inside walls of the receiving vessel
  - 2) After the fluid stops flowing into the receiving container, the fluid remaining in the tip is completely expelled into the receiving container using one 'puff' of air from a safety bulb (blow-out step). The bottom tip of the pipette is touched to the inside surface of the receiving container to collect the last bit of fluid adhering to the pipette's tip.
  - 3) The tip of the pipette **SHOULD NEVER** come in contact with the accumulating fluid in the receiving vessel during drainage
- d. Used most often in the laboratory for routine work; ideal for titrations



3. MOHR: (figure B)

- a. These pipettes are similar to serologic pipettes in that they have multiple volume graduations, but they **do not have graduated volume markings to the bottom of the pipette tip,** nor do they have etched rings at the top of the pipette.
- b. These pipettes require a controlled delivery of the solution between the upper fill mark and the intended lower mark
- c. The tip of the pipette **SHOULD NEVER** come in contact with the accumulating fluid in the receiving vessel during drainage or the inside walls of the receiving vessel when dispensing of sample
- d. Most laboratories discourage use of the Mohr pipette because it is often mistaken as a serologic pipette and is used improperly:
  - 1) Error: fluid remaining in the tip after drainage is 'blown' into the receiving vessel, resulting in extra volume of liquid being delivered
  - 2) Error: when delivering sample to receiving vessel, the Mohr pipette tip is allowed to touch the inside of the receiving vessel, resulting in an inaccurate fluid volume delivery

### PROPER PIPETTING TECHNIQUE using macro-volume pipettes:

1. Choose the pipette which approximates the volume you wish to measure and the accuracy of measurement required:
  - a. If you want to measure 3.2 ml of reagent, you would choose a 5 ml serologic pipette rather than a 10 ml serologic pipette.
  - b. If you want to dilute a control using 10 ml of d-H<sub>2</sub>O, you would choose a 10 ml volumetric pipette
2. Inspect the pipette carefully. The pipette should be clean and the tip not chipped. Dirty pipettes will drain unevenly and inaccurately; chipped tips alter the volume delivered.
3. **NEVER MOUTH PIPET:** a rubber bulb or other device is used to fill the pipette with fluid above the mark. Careful aspiration of the fluid is required so that fluid is not aspirated beyond the pipette into the bulb. If this should happen, **alert your instructor**
4. Hold the pipette in a vertical position to increase accuracy and precision.
5. Pipette solutions that are at room temperature to avoid errors caused by contraction and/or expansion of fluids
6. Any air bubble, regardless of its size, will invalidate the volume of liquid you are trying to pipette. Should an air bubble appear in the pipette, re-sampling is required.
7. Refer to video demo (blackboard on-line course):
  - a. Grasp the pipette using the thumb and middle finger
  - b. Immerse the tip of the pipette below the surface of the liquid
  - c. Using a safety bulb, exert gentle and continuous suction drawing the liquid into the pipette until **slightly above the fill (calibration) mark**.
  - d. Quickly remove the safety bulb and place your index finger over the open end of the pipette
  - e. Withdraw the pipette from the fluid **and wipe the excess fluid from the outside** of the pipette with a clean cloth or tissue.
  - f. **Adjust the meniscus to the calibration mark** by gently releasing the pressure exerted by the index finger. When the meniscus of the fluid is at the correct mark, exert pressure using the index finger to stop and hold the fluid in the pipette so no drops of fluid are lost
  - g. Transfer the pipette to the desired receiving vessel and allow the liquid to drain freely, holding the pipette in a **vertical position**, taking care not to touch the wall of the receiving vessel nor the fluid in the vessel
    - 1) If using a volumetric pipette: After the pipette has drained, touch the tip of the pipette against the inside of the vessel allowing fluid to transfer into the receiving vessel by capillary draw. The fluid remaining in the pipette tip should remain in the pipette tip
    - 2) If using a serologic pipette: After the pipette has drained, ‘blow out’ the fluid remaining in the pipette tip into the receiving vessel using a ‘puff’ of air from the safety bulb. Touch the tip of the pipette against the inside of the vessel to deliver the last drop of fluid hanging on the pipette tip.

8. ALWAYS wipe off the outside of the pipette **before** adjusting the meniscus to the desired mark (refer to step 7e and 7f).

If the meniscus is adjusted before the outside of the pipette is wiped off, fluid will be pulled out of the pipette onto the wiping cloth by capillary action, thus decreasing the desired volume to be delivered.

9. The pipette tip **SHOULD NEVER** come in contact with the fluid in the receiving vessel when delivering the sample (refer to step 7g)

Types of MICRO-VOLUME PIPETS commonly used:

1. Generally, when pipetting volumes  $\leq 1.0$  ml, a micro-volume pipette is used
2. Several types are available for use; choice depends upon laboratory and intended use
  - a. Plunger with double stop
  - b. Plunger with single stop
  - c. Single fixed-volume delivery
  - d. Multiple volume delivery
  - e. Automatic or manual tip rejection
  - f. Automatic aspiration and delivery

Student Laboratory Rotation (UNMC site): you will learn proper pipetting technique using the 'oxford' pipette. This is a double stop pipette.

Clinical Laboratory Rotation: you will have the opportunity to use other types of micropipettes such as:

- a. 'MLA' and 'Eppendorf' pipettes: single stop pipettes
- b. 'Focus' pipette: double stop pipette

### PROPER PIPETTING TECHNIQUE using micro-volume pipettes:

1. Never drop a micro pipette as this may alter the calibration.
2. Verify all pipette parts are firmly assembled
3. Initially before use, depress the plunger knob several times. This acts to redistribute the lubricant within the pipette. It should feel smooth when the plunger knob is depressed and released. **Alert your instructor** if sticking is encountered
4. The plastic pipette tip must be properly fitted onto the pipette: it should be straight and held firmly in place.
5. When using a plastic tip, pre-rinsing is always required (unless otherwise indicated in the procedure)
6. Pipette solutions that are at room temperature to avoid errors caused by contraction and/or expansion of fluids
7. Hold the pipette in a vertical position to increase accuracy and precision
8. Depress and release the plunger with a controlled and smooth action. Try to maintain the same speed of intake and delivery for all samples (consistency is the key)
9. Never allow the plunger knob to snap back as this will cause aspiration of the liquid past the pipette tip into the pipette contaminating its interior assembly. If this should happen, **alert your instructor** as cleaning of the pipette is required
10. Depress the plunger knob BEFORE inserting the pipette tip into the fluid. Depression of the plunger knob while it is in the solution will cause bubbles to form in the tip, resulting in an inaccurate measurement
11. Insert the tip of the pipette into the sample at approximately the same depth each time. Try not to 'drown' the tip into the fluid (never deeper than 1/4")
12. Any air bubble, regardless of its size, will invalidate the volume of liquid you are trying to pipette. Should an air bubble appear in the pipette, re-sampling is required.
13. When delivering sample to receiving vessel, place plastic pipette tip inside the receiving vessel without touching the inside wall of the receiving vessel. Touching the wall with the tip of the pipette while delivering sample alters the 'flow' of sample delivery and also the volume being delivered.
14. Position the sample tip so that the sample does not 'dribble' down the entire length of the inside wall of receiving vessel. This may cause a loss of sample and invalid test results.
15. The plastic pipette tip **SHOULD NEVER** come in contact with the fluid in the receiving vessel while delivering the sample (unless otherwise instructed by the procedure)

'Oxford' pipette: Also see video: Techniques, Micropipette (Oxford)

This is a **TD** (to deliver) pipette, and has 2 plunger stop positions: primary and secondary

1. As the plunger knob is depressed, the first stop is encountered. This first depression of the plunger knob is the primary or calibrated movement that 'aspirates' the specified volume
2. The plunger knob may then be depressed to its lowest position. This is the secondary or blow out movement (similar to the 'blow-out' step when using a serologic pipette)

TO OPERATE the Oxford pipette:

1. Apply a clean plastic tip firmly to the pipette; verify the tip is on straight

This is the pre-wetting step that is required when using plastic pipette tips

2. Depress the plunger knob to the **FIRST** (upper-most) stop position
3. Immerse the tip approx 1/8" into the solution
4. Return the plunger knob to the release position, aspirating the sample into the plastic tip
5. Slightly remove the plastic tip from solution and return the sample to the original container: depress the plunger knob to the first (upper-most) stop, pause slightly, and then depress the plunger knob to the second (lowest) position
6. Return the plunger knob to its release position
7. Depress the plunger knob to the **FIRST** stop (upper-most stop)
8. Immerse the tip approx 1/8" into the solution
9. Return the plunger knob to the release position, aspirating the sample into the plastic tip
10. Remove the plastic tip from solution, and while removing the plastic tip from the solution, gently 'swipe' the tip against the side of the tube to avoid delivering any fluid adhering to the outside of the plastic tip into the receiving vessel
11. Place plastic tip inside the receiving vessel without touching the inside wall of receiving vessel. Position sample tip so that delivery of sample does not result in the sample dribbling down the entire length of the inside wall of receiving vessel. Do not allow the pipette tip to touch any fluid in the receiving vessel.
12. Deliver the sample to the receiving vessel: depress the plunger knob to the first/upper stop, pause slightly, and then depress the plunger knob to the second/lowest position (this secondary movement is used to **BLOW OUT** the contents of the pipette tip and is similar to the blow out step used for serologic pipettes)
13. With the plunger knob still held in its lowest position, withdraw the pipette from vessel (this is done to eliminate aspirating some of the sample from the receiving vessel back into the plastic tip resulting in delivering a falsely low volume)
14. Return plunger knob to the release position; remove and properly discard plastic tip



'MLA' pipette:

1. This is a **TD** (to deliver) pipette, which has only 1 plunger stop position. The same plunger stroke is used for aspiration and delivery of sample
2. This type of pipette is most often used in clinical laboratories due to its ease of use

TO OPERATE the MLA pipette:

1. Apply a clean plastic tip firmly to the pipette
2. Depress fully the plunger knob
3. Immerse the tip approximately 1/8" into the solution
4. Return the plunger knob to the release position, aspirating the sample into the pipette tip
5. Slightly remove the plastic tip from solution and return the sample to the original container: fully depress the plunger knob
6. Return plunger knob to its release position
7. Depress fully the plunger knob
8. Immerse the tip approximately 1/8" into the solution
9. Return the plunger knob to the release position, aspirating the sample into the pipette tip
10. Remove the plastic tip from solution: and while removing the plastic tip from the solution, gently 'swipe' the tip against the side of the tube to avoid delivering any fluid adhering to the outside of the plastic tip into the receiving vessel
11. Place plastic tip inside the receiving vessel without touching the inside wall of receiving vessel. Position sample tip so that delivery of sample does not result in the sample dribbling down the entire length of the inside wall of receiving vessel; try to deliver the sample near the bottom of the vessel while not touching the fluid that is already in the receiving vessel) Do not allow the pipette tip to touch any fluid in the receiving vessel.
12. Deliver the sample to the receiving vessel: fully depress the plunger knob
13. With the plunger knob fully depressed, withdraw the pipette from vessel (this is done to eliminate aspirating some of the sample from the receiving vessel back into the plastic tip resulting in delivering a falsely low volume)
14. Return plunger knob to the release position; remove and properly discard plastic tip

This is the pre-wetting step that is required when using plastic pipette tips