

Table 20.2. TOLERANCES FOR VOLUMETRIC CLASS A AND CLASS B PIPETTES COMPARED WITH GENERAL PURPOSE SEROLOGICAL PIPETTES

Capacity (mL)	Volumetric Class A Tolerance	Volumetric Class B Tolerance	Glass Serological Tolerance
0.1	—	—	0.005
0.2	—	—	0.008
0.5	0.006	0.012	0.01
1.0	0.006	0.012	0.02
2.0	0.006	0.012	0.02
3.0	0.01	0.02	—
4.0	0.01	0.02	—
5.0	0.01	0.02	0.04
6.0	0.01	0.03	—
7.0	0.01	0.03	—
8.0	0.02	0.04	—
9.0	0.02	0.04	—
10.0	0.02	0.04	0.06
15.0	0.03	0.06	—
20.0	0.03	0.06	—
25.0	0.03	0.06	0.10
50.0	0.05	0.10	—
100.0	0.08	0.16	—

Tolerances are expressed as \pm mL.

Information from ASTM Standard E 969-02 "Standard Specification for Volumetric (Transfer) Pipets" and ASTM Standard E 1044-96 (reapproved 1990) "Standard Specification for Glass Serological Pipets (General Purpose and Kahn)."

IV. MICROPIPETTING DEVICES

A. Positive Displacement and Air Displacement Micropipettors

Glass and plastic pipettes are typically used in biology laboratories to measure volumes as small as about 1 mL. **Micropipettors** are devices commonly used to measure smaller volumes, in the 1 to 1000 μ L range. These volume-delivering devices go by many names in addition to micropipettor, such as **microliter pipette**, **piston or plunger operated pipette**, and, simply, **pipettor**.

There are two distinct designs of micropipettor: **positive displacement** and **air displacement**, see Figure 20.11. **Positive displacement micropipettors** include syringes and similar devices where the sample comes in contact with the plunger and the walls of the pipetting instrument. Positive displacement devices are recommended for viscous and volatile samples. Syringes are commonly used to inject small volume samples into instruments for chromatographic analysis.

Air displacement micropipettors are designed so that there is an air cushion between the pipette and the sample such that the sample only comes in contact with a disposable tip and does not touch the micropipettor itself. Disposable tips reduce the chance that material from one sample will contaminate another or that the operator will be exposed to a hazardous material. Air displacement micropipettors accurately measure the volume of aqueous samples and are among the most common instruments currently used in biotechnology laboratories.

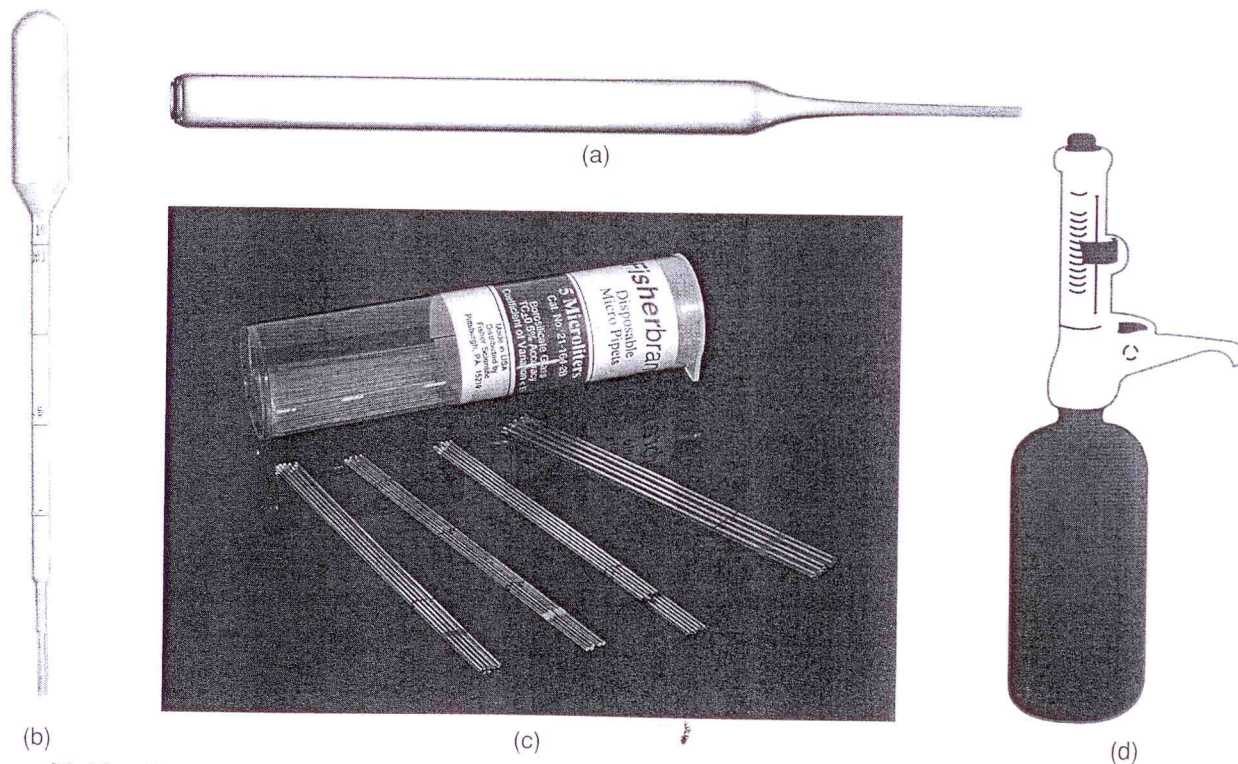


Figure 20.10. Examples of Various Types of Pipette and Liquid Dispensing Devices. a. A Pasteur pipette. b. Disposable plastic pipette to measure microliter volumes with an accuracy of about $\pm 10\%$. c. Glass capillary pipettes for measuring microliter volumes. d. A manual dispenser for a reagent bottle. (a–c courtesy of Fisher Scientific, Pittsburgh, PA.)

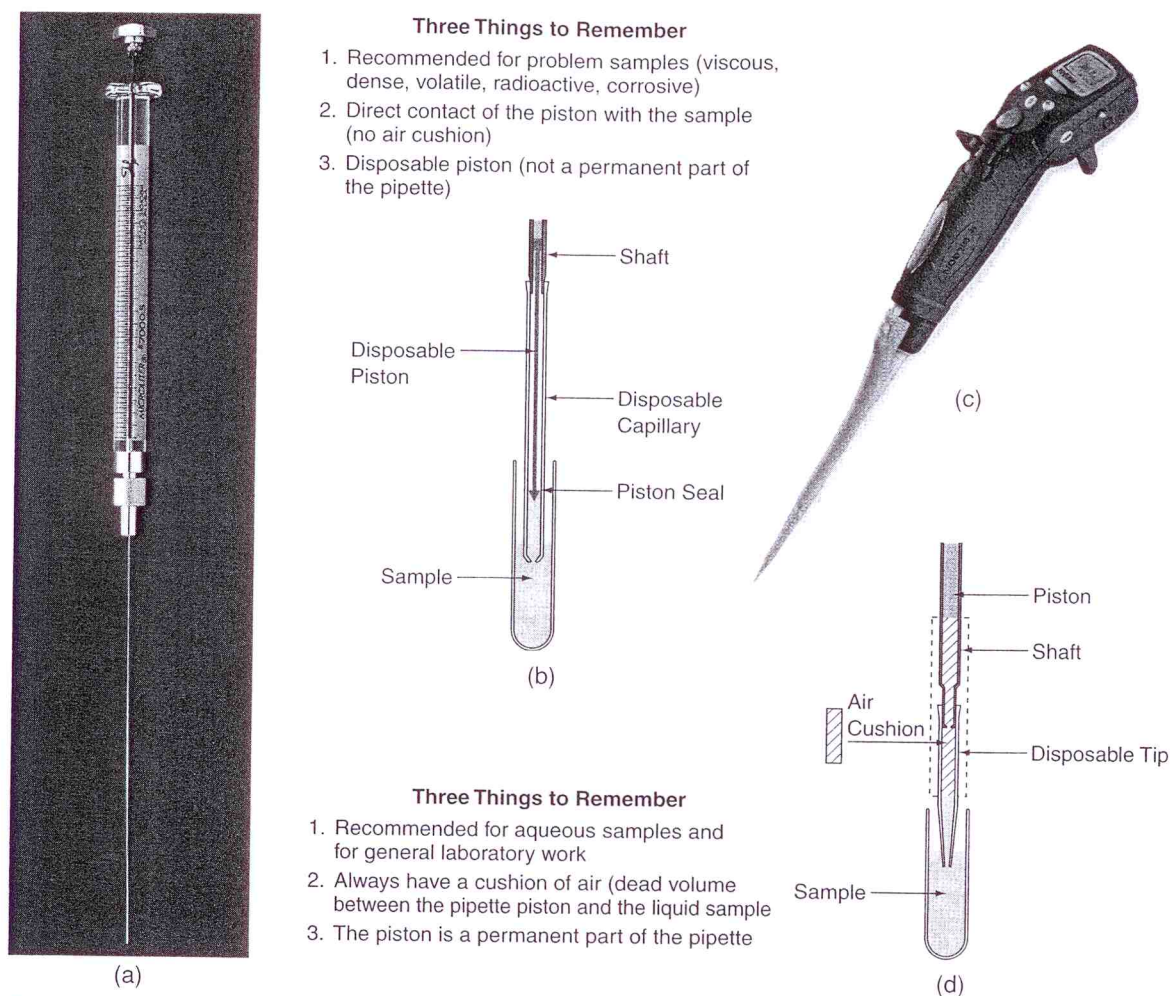


Figure 20.11. Positive Displacement versus Air Displacement Micropipettors.

a. Syringe used to inject small volume samples into chromatography instrument; positive displacement. (Courtesy of Fisher Scientific, Pittsburgh, PA) **b.** Diagram of a positive displacement device. **c.** Air displacement micropipettor. **d.** Diagram of an air displacement device. (Illustrations b, c, and d are provided courtesy of Gilson, Inc.)

Air displacement micropipettors come in an assortment of styles, see Figure 20.12 on p. 356. Some deliver only one volume, such as 100 μL ; others, called **digital microliter pipettors**, can be adjusted to deliver different volumes over a range, such as 10–100 μL . Micropipettors can be manually or electronically driven, and some are microprocessor controlled. Repetitive pipetting can lead to injury, so, in laboratories where repetitious pipetting is performed, it is worthwhile to investigate micropipettors designed to reduce operator fatigue.

B. Obtaining Accurate Measurements from Air Displacement Manual Micropipettors

i. PROCEDURE FOR OPERATION

It is important to operate micropipettors properly or they will not deliver the correct volumes. Manual micropipettors have **plungers** by which the operator controls the uptake and expulsion of liquids. As the operator depresses the plunger, different “stop” levels can be felt. Although the detailed operating directions vary depending on the type and brand of micropipet-

tor, the general operation of most manual micropipettors is similar and is summarized in Figure 20.13 and in Box 20.3, both on p. 357.

ii. FACTORS THAT AFFECT THE ACCURACY OF MANUAL MICROPIPETTORS

A variety of factors affect the accuracy of volume measurements by micropipettors:

1. **The operator’s technique is the most important factor affecting the performance of a manual micropipetting device.** If the operator does not operate the micropipettor consistently, smoothly, and correctly, it will not accurately deliver the specified volume. (See Box 20.3 on p. 357.)
2. **The physical and chemical properties of the liquids being measured will affect the volumes delivered.** (See Table 20.3 on p. 359.)
3. **Measurements are affected by the environment in which they are made.** (See Table 20.3 on p. 359.)
4. **The condition of a micropipettor will affect its performance.** Basic micropipettor maintenance will be discussed shortly.



(a)



(b)



(c)

Figure 20.12. Various Types of Air Displacement Micropipettors and Tips.

a. A multichannel micropipettor used to simultaneously deliver the same volume to 12 wells of a 96-well plate. **b.** An electronic, motorized, microprocessor-controlled micropipettor. This device reduces operator fatigue and allows the user to program the device, for example, to repeatedly dispense particular volumes. The pipette's internal software also allows the user to set a maintenance interval with an alarm that sounds when it is time for service. A computer can be used with this micropipettor to store and organize maintenance reports and data, thus assisting in adhering to GLP requirements. **c.** Disposable tips are stored in racks inside autoclavable plastic boxes; racked tips do not need to be touched with one's fingers to attach them to a micropipettor. (Photos provided courtesy of Gilson, Inc.)

iii. TIPS

Air displacement micropipettes require a disposable tip; the choice of tip can have a major impact on pipetting results. Some items to consider when choosing tips:

- There are different sizes of tips to match different models of micropipettor; be sure to choose the right one. Some manufacturers color code their tips and micropipettors to avoid confusion.
- Micropipettor manufacturers sell tips to match their devices. It is possible to purchase less-expensive, generic tips in bulk, but these may not seal properly on every model of micropipettor, may leak, and may not dispense liquids as accurately as tips recommended by the manufacturer.
- Special wide-bore tips are useful to reduce shearing when pipetting DNA and other large molecules and intact cells. These tips may, however, be susceptible to error due to changes in barometric pressure. Smaller bore tips may be better for reaching inside small tubes or vials.
- Tips can be purchased loose in bags, or mounted in racks that sit inside plastic boxes, see Figure 20.12c. Racked tips can be mounted on the end of a micropipettor without touching them, which is desirable to avoid contamination.
- It is common practice to autoclave boxes of tips to sterilize them. When removing an autoclaved tip from its box, quickly remove the tip and shut the box to reduce the exposure of the remaining tips to the environment.
- Longer tips are available with small flat ends that are especially designed for loading samples into electrophoresis gels.

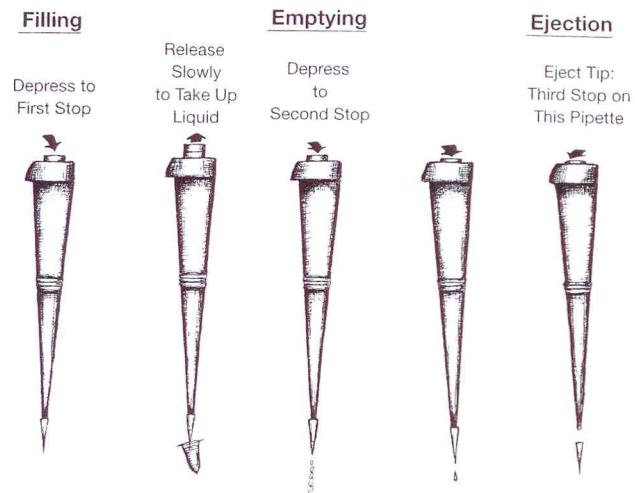


Figure 20.13. Using a Manual Micropipettor. Note that not all micropipettors have a third stop to eject the tip. (Sketches by Sandra Bayna, a biotechnology student.)

Box 20.3. PROCEDURE TO OPERATE A MANUAL MICROPIPETTOR (IN THE USUAL, FORWARD MODE)

1. Set the micropipettor (if it is adjustable to different volumes) to the desired volume.
2. Attach a disposable tip to the micropipettor shaft. Press firmly to ensure an airtight seal.
3. **Optional: Prewet the tip by aspirating and dispensing the solution to be measured into a waste container or back into the original solution.**

Note: Prewetting leaves a thin film of the liquid to be measured on the inside of the tip. Some operators prewet the tip, others do not. There is evidence that better accuracy is obtained with a prewetted tip. Since prewetting the tip will affect its performance, it is important that operators are consistent in whether they prewet the tip or not.

4. **Hold the micropipettor vertically and, while observing the tip and the sample, depress the plunger to the first stop, and place the tip in the liquid.**

Note: For the utmost accuracy, it is recommended that the tip be immersed into the sample a specified distance. This distance may be specified by the manufacturer. Otherwise, general guidelines are:

for volumes of 1–100 μL , immerse the tip 2–3 mm

for volumes of 101–1000 μL , immerse the tip 2–4 mm

for volumes of 1001–10,000 μL , immerse the tip 3–6 mm

5. **Allow the plunger to slowly return to its undepressed position as the sample is drawn into the tip.**

Wait 1 second before removing the tip from the liquid.

Note 1: *Never allow the plunger to snap up!* (If the plunger “snaps,” fluid can be aspirated into the interior of the micropipettor. Also, the volume measured will be incorrect.)

Note 2: If any liquid remains on the outside of the tip remove it carefully with a lint-free tissue, taking care not to wick liquid from the tip orifice.

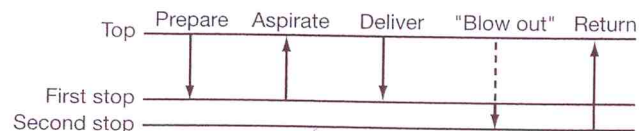
Note 3: Pull the micropipettor straight out of the container after aspirating sample. Do not allow the tip to touch the side of the container.

6. **Place the tip so that it touches the side of the container into which the sample will be expelled; depress the plunger to the second stop.** Watch as the sample is expelled to the container. Wait about 2 seconds and be certain all the liquid is expelled. Remove the tip from the vessel carefully, *with the plunger still fully depressed*.

Note: It is also correct to dispense the sample directly into a solution already in the tube and to rinse the tip with the solution. Discard the tip after such use.

7. **Eject the tip using the third stop, tip ejector button, or other mechanism.**

The procedure outlined in this box is the most common method of pipetting, called *forward mode* pipetting. Forward mode pipetting is recommended for aqueous samples. There is also *reverse mode* pipetting, which is less commonly used but is recommended for volatile and viscous samples. Reverse mode pipetting is described in Table 20.3. The following diagram shows the steps in forward mode pipetting.



(Figure modified from one in ASTM E 1154-89 (reapproved 1993) “Standard Specification for Piston or Plunger Operated Volumetric Apparatus.”)

