

Use of Pipettes

Pipettes are used to accurately deliver specific volumes of fluids. They are made of glass or plastic and are graduated in milliliter units. The most commonly used pipettes are calibrated into 1.0-, 5.0-, or 10.0-ml units. Each pipette is marked to indicate the total volume and the smallest measurable unit. With a 1.0-ml pipette, the total volume and the smallest volume deliverable is indicated by the "1 in 1/100" designation. This indicates that the maximum deliverable volume is 1.0 ml and the smallest unit deliverable is 0.01 ml. Note that graduated pipettes can be used to deliver various volumes, not just the total volume of the pipette. Analyze the calibration for a 5.0-ml pipette. Take time to become familiar with the graduations on the pipettes. Two types of pipettes are commonly encountered in a microbiology laboratory. The first type is called a serological pipette and must be emptied completely (draining it and then "blowing out" the last drop) to deliver the volume specified. The second type (e.g. a Mohr style pipette) is not calibrated to the tip and delivers the volume specified by the graduations. Stopping the fluid beyond the last line of a Mohr pipette results in an unknown volume being dispensed. Mistakes in pipetting commonly occur with this second type of pipette when fluids are allowed to run out of the pipette beyond the calibration point. Be careful to note the type of pipette being used before beginning work.

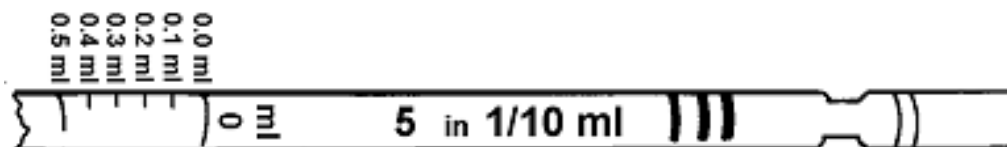
Aseptic technique must be employed when using pipettes in the microbiology laboratory. Pre-sterilized pipettes, either glass or plastic, may be available. They are packaged either individually or in bulk packages. Reusable glass pipettes must be placed with the delivery end at the bottom in metal or glass canisters (or in packets) and then steam sterilized. Canisters should be dried by using the drying cycle of the sterilizer or, alternatively, by placing them in a dry-heat oven at 150 to 170°C for 1 to 2 hr.

Never pipette any material by using your mouth. Use one of the pipetting devices described here. Two of the most commonly used are (a) a cylindrical, thumb wheel device (plastic pump), and (b) a rubber bulb aspirator. When using the thumb wheel device, carefully attach the pipette to the end opposite the wheel. Be careful not to contaminate the sterilized pipette. Exercise caution in gently attaching the pipette, for excessive force may cause the pipette to break and cause injury. By turning the thumb wheel, liquid can be drawn into the pipette, and then by turning the wheel in the opposite direction, the fluid can be delivered. When using the bulb aspirator, first expel all of the air from the bulb by simultaneously squeezing the bulb and the A valve. Then attach the pipette to the end of the bulb containing the S valve. Squeeze the S valve slowly to draw fluid into the pipette. ***Be careful not to draw fluid into the bulb.*** To release the fluid, squeeze the E valve. Let go of the E valve to stop fluid flow when the desired volume has been delivered. **It is a good idea to check your pipetting device on an extra pipette with water prior to using it on your actual sample to make sure there are no leaks that could lead to inaccurate volumes or accidental contamination.**

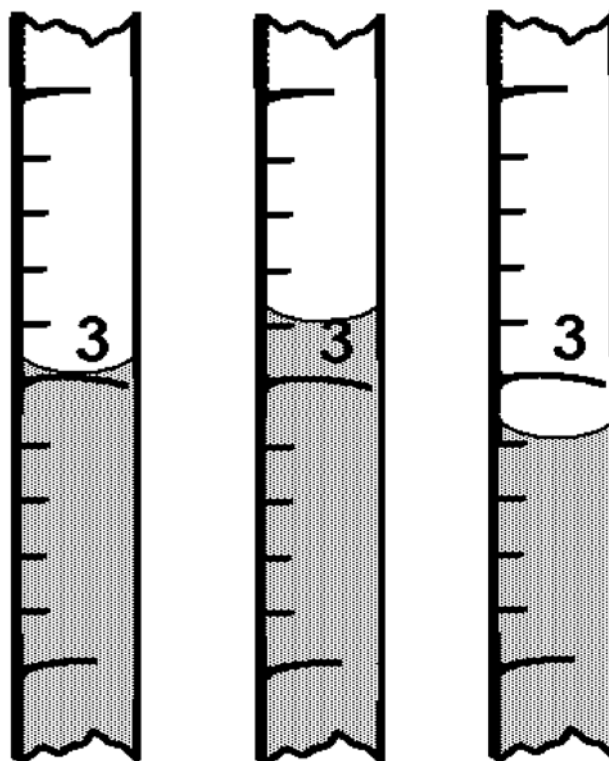
Extreme care must be exercised when using sterile pipettes. Remember all environmental laboratory surfaces, air, and skin harbor microorganisms which could be sources of contamination. Keep pipettes in canisters or packets and remove one at a time as needed. With one hand, remove a pipette from the canister, being careful not to touch the pipette tip. Using your other hand, pick up the tube or flask to be sampled and remove the stopper with the little finger of the hand holding the pipette. Flame the lip of the tube or flask, fill the pipette, re flame the tube or flask, and replace the stopper. Repeat the process to deliver the sample to a new tube or flask. Be sure to keep the pipette in an upright position, for if inverted some of the fluid may run into the pipetting device. Used pipettes must be placed in containers filled with disinfectant. If pipetting a bacterial culture be careful not to allow any to drop from the pipette before disposing of it in the disinfectant container. If

disposable pipettes are used, they should be discarded into the appropriate biohazard containers. Never place a used pipette on the lab bench or on your lab book or on any other surface. This pipetting technique involves some manual dexterity, and it is recommended that the technique be practiced using water and non-sterile pipettes in order to become more comfortable with the procedure.

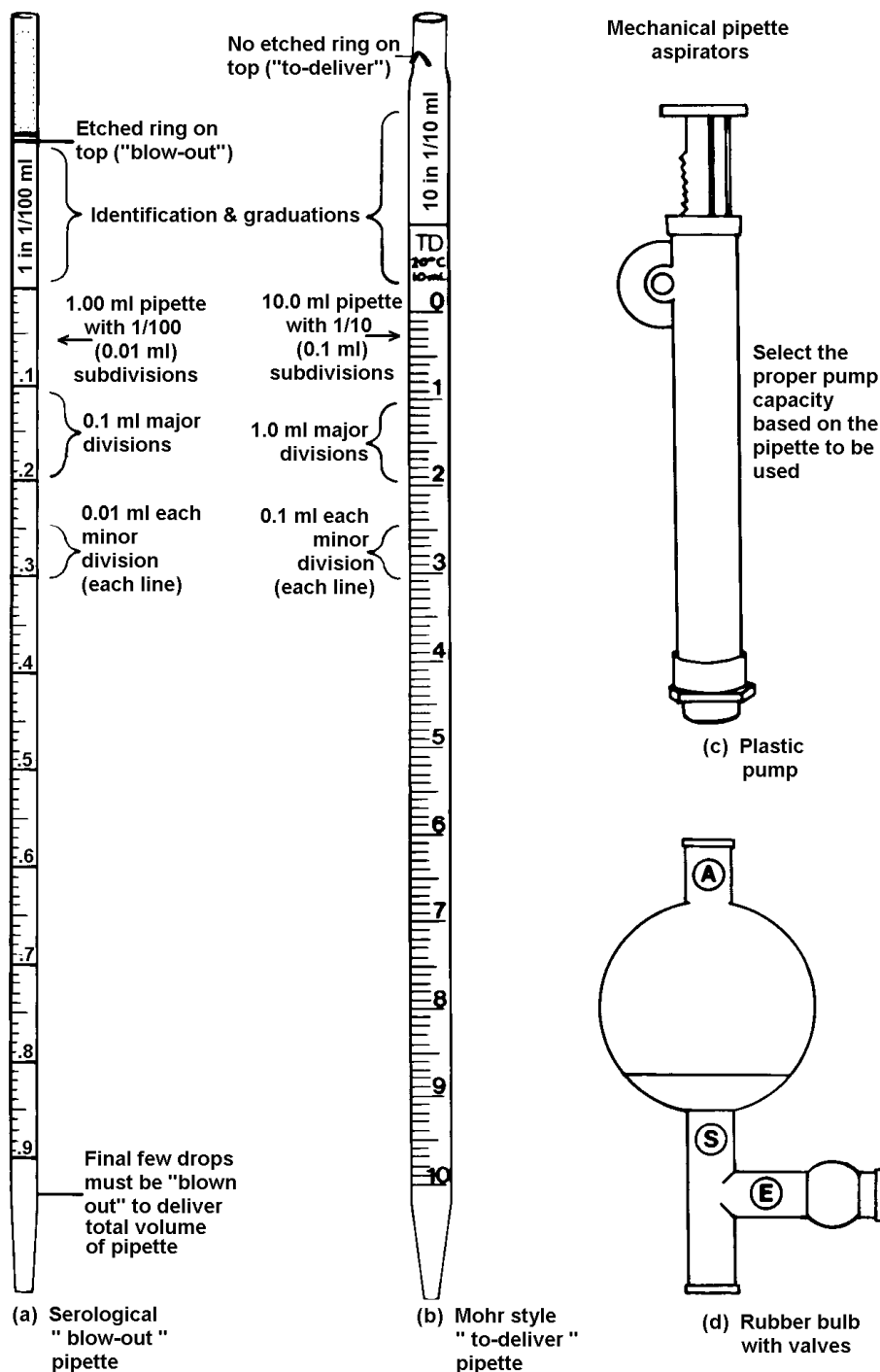
Good judgment is called for in determining the size of pipette to use in a given situation. To accurately pipette volumes between 0.1 and 1.0 ml, use a 1.0-ml pipette. For volumes larger than 1.0 ml, use a 5.0- or 10.0-ml pipette. When volumes less than 0.1 ml need to be delivered, a micropipette should be used. Micropipettes are available in various ranges. They are expensive, carefully calibrated instruments and extreme care should be exercised when using them. The use of micropipettes will be discussed later in the course.



Pipette Calibration. The numbers (5 in 1/10 ml) indicate the pipettes *total volume* and its *smallest calibrated increments*. This is a 5.0 ml pipette divided into 0.1 ml increments.



Read the base of the meniscus. When reading volumes, use the base of the meniscus. The volume in the left pipette is read as exactly 3.0 ml because the meniscus is resting on the 3.0 ml line. The middle pipette is read as 2.9 ml and the right pipette is read as 3.1 ml. (0 ml is always at the pipette's top). Although the difference in volume may seem negligible, it may be enough to introduce substantial error into your work, especially when performing multiple pipetting operations.



Pipetting

- a) A "blow-out" (serological) pipette.
- b) A "to-deliver" Mohr-style pipette.
- c) A plastic pump. The pump is attached to the pipette and the wheel turned to move fluid either up or down the pipette.
- d) A pipette bulb. The bulb is attached to the pipette below S. While pressing A valve on both sides, squeeze the rubber bulb and it will collapse. To draw fluid into the pipette, slowly press the S valve on both sides; to release fluid, press the E valve on both sides. To stop releasing fluid, let go of the E valve.