



INTRODUCTION TO PIPETTES



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Revised by:

**Angela Nolte, Dawn Wisniewski,
Saadia Yunus and Ruth Dusenbery**

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.

USE OF MICROPIPETTES



Automatic micropipettes are usually used for volumes between 1 microliter and 1 milliliter.

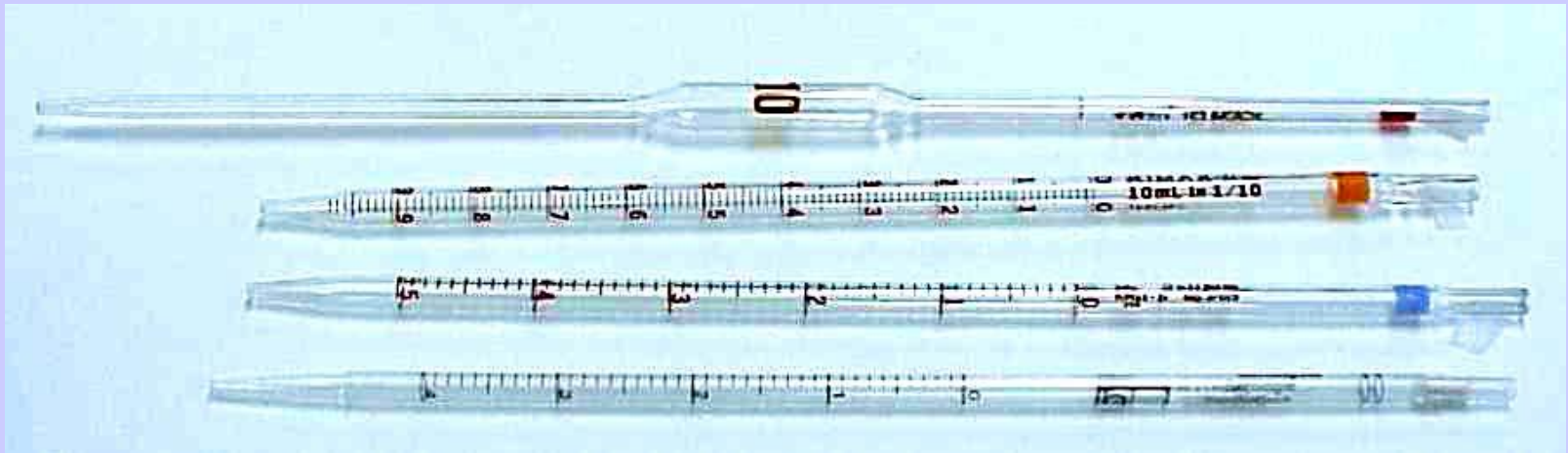
Refer to the SLC Module D11, Automatic Micropipette, for instructions on how to use these instruments.

PURPOSE



- The purpose of this module is to demonstrate how to properly use and handle volumetric and measuring pipettes.
- By the time you finish this module you should know:
 - how to choose the proper type of pipette
 - what the specifications on a pipette mean
 - how to properly handle and dispose of pipettes
 - how to use pipette fillers

TYPES OF PIPETTES




- Volumetric
- Measuring
 - Mohr
 - Serological

VOLUMETRIC PIPETTES

- **Used to deliver a single specific volume of liquid, usually between 1 and 100 ml.**
- **Shaped like rolling pins with a large belly, one blunt end, the neck, and one tapering end, the tip.**



VOLUMETRIC PIPETTES

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- Used for accurate measurements, since it is designed to deliver only one volume and is calibrated at that volume.
 - Should be used when accuracy and reproducibility are crucial, because these can achieve accuracy to four significant figures.

SPECIFICATIONS ON A VOLUMETRIC PIPETTE

On a volumetric pipette, the specifications indicate

- how much liquid will be transferred if the liquid is drawn up to the calibration line on the neck
- the temperature at which the calibration was made
- whether it is a TD or TC pipette




SPECIFICATIONS ON A VOLUMETRIC PIPETTE



- When emptying a volumetric pipette, the liquid is allowed to drain out. It is **NOT** forced out.
- After it is emptied, the small amount of liquid which remains in the tip should not be blown out.
- Volumetric pipettes are **NOT** blow-out pipettes

MEASURING PIPETTES

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- **They are straight glass or plastic tubes with one tapering end.**
 - **Calibrated into small divisions so that various amounts of liquid can be measured with the same pipette.**
 - **Usually used to measure any amount between 0.1ml and 25.0ml.**
 - **They are not as accurate due to the fact that any imperfection in their internal diameter will have a greater effect on the volume delivered.**

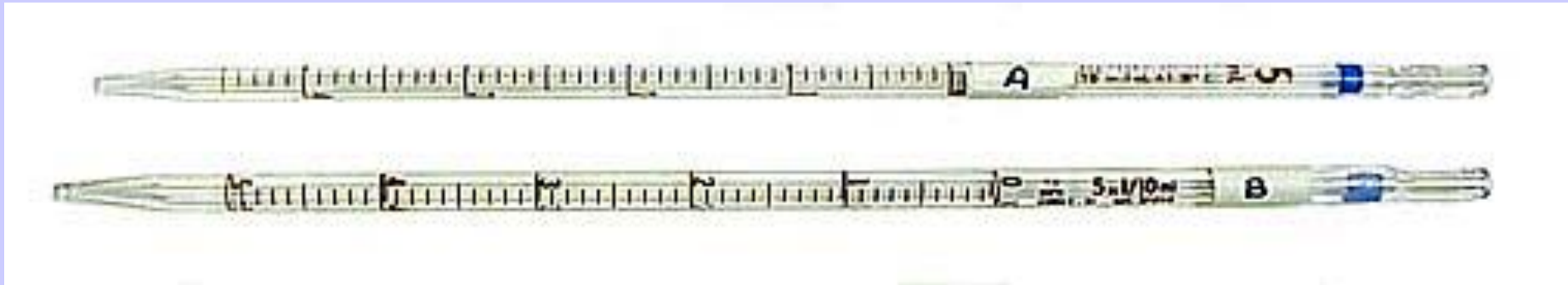
MOHR AND SEROLOGICAL PIPETTES

Measuring pipettes are divided into:

- **MOHR PIPETTES**
 - **the graduations on these always end before the tip**
- **SEROLOGICAL PIPETTES**
 - **the graduation marks continue to the tip**



Examine pipettes A and B.
Which is the serological and which is the
Mohr?



SPECIFICATIONS ON A MEASURING PIPETTE



Printed on the neck of the pipette are the specifications that indicate:

- the maximum volume of liquid that can be transferred
- the size of the divisions on the pipette
- the temperature at which calibrations were made
- if the pipette is a “**to deliver**”(TD) or “**to contain**”(TC) pipette.

5 in 1/10 ml
TD 20°C

Specifications on a pipette as shown above indicate that the pipette is calibrated in 1/10ml divisions and will deliver up to 5.0 ml within published tolerance levels at 20°C.



1 ml in 1/100


TD 20°C

These specifications indicate that the pipette is calibrated in 1/100 ml divisions and it will deliver up to 1.00 ml within published tolerance levels at 20°C.



BELLCO T.D. 1x.01ml
V. I. P. 20°C

BLOW OUT PIPETTES



If a serological pipette has a frosted band or two thin rings around the neck, then it is a blow-out pipette.

This means that after all the liquid has been allowed to drain out by gravity, the last drop remaining in the tip is also forced out.

BLOW OUT PIPETTES

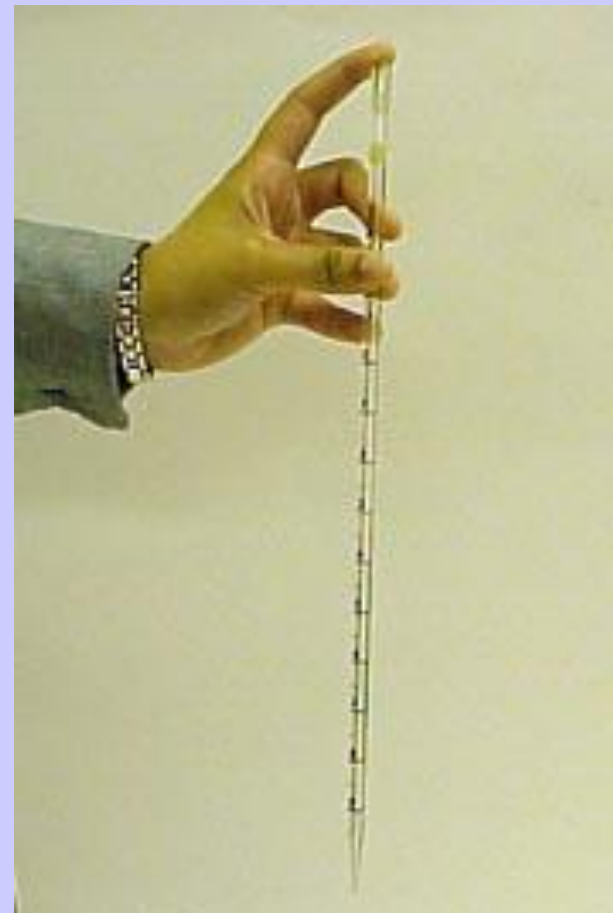
The frosted band should not be confused with thicker colored rings or colored dots, which are a manufacturer's code for the maximum volume of the pipette.

Remember, only blow-out a serological pipette if it has a frosted band or two thin rings.



HANDLING AND DISPOSING OF PIPETTES

- Chipped and cracked pipettes should be replaced as they are unsafe and may affect the accuracy of measurements.
- **NEVER** mouth pipette.
- Hold the pipette by the upper third of the tube and keep the tip from touching anything.

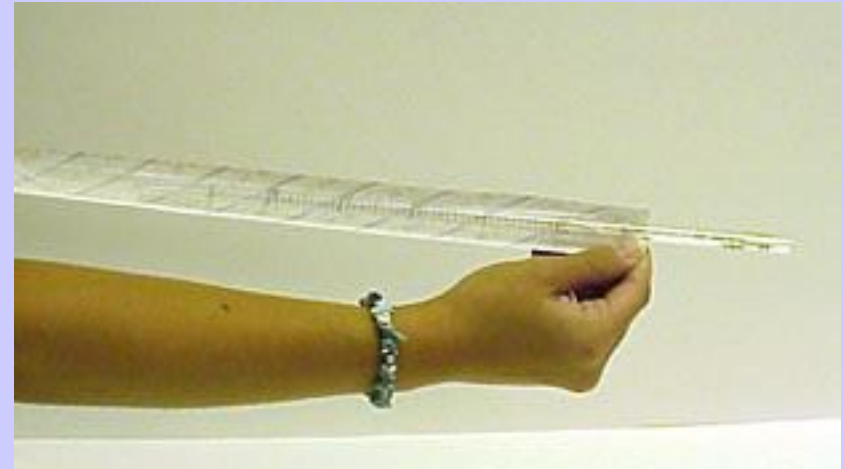


Dispose dirty pipettes by placing in soapy water solution in a tray. Place disposable pipettes in a cardboard holder. Do not leave pipettes on counters or sinks. If you are working with radioactive materials be sure to dispose pipettes in a properly marked container.



HANDLING STERILE PIPETTES

When using sterile pipettes, be sure to use proper sanitary techniques. If you have a sterile package of disposable pipettes, tear only a small corner of the package open and push one pipette out of this opening, then immediately close the package to prevent contamination.



If you are using sterile pipettes in a pipette canister, place the canister on its side, slide off the cover, pull out one pipette and replace the cover immediately.



TRANSFERRING A PRECISE VOLUME OF LIQUID

A pipette bulb is used to draw liquid up into the pipette. There are many types of pipette bulbs.

The use of 3 types will be demonstrated in this module. The first to be demonstrated will be the common rubber bulb.

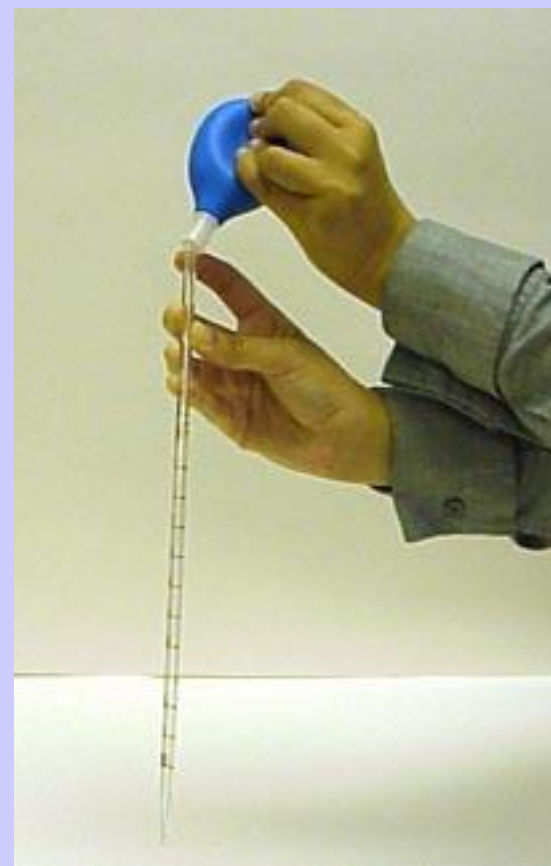
When in the SLC, perform the steps as they are described, using the serological pipette labeled C.



USING THE COMMON RUBBER BULB

Hold the pipette about 8 cm below the mouthpiece with one hand. Then with your other hand squeeze the bulb and touch the opening to the mouth of the pipette.

Insert no more than one-half cm of the pipette into the bulb.



Place the tip into the colored liquid and slowly release the pressure on the bulb.

The liquid will be drawn up into the pipette and will form a curved surface against the glass.

This surface is called the meniscus. Pull the bottom of the meniscus up about 1 cm past the desired level.



Then quickly, but carefully, remove the bulb as you slip your free index finger over the tip of the mouthpiece hole.

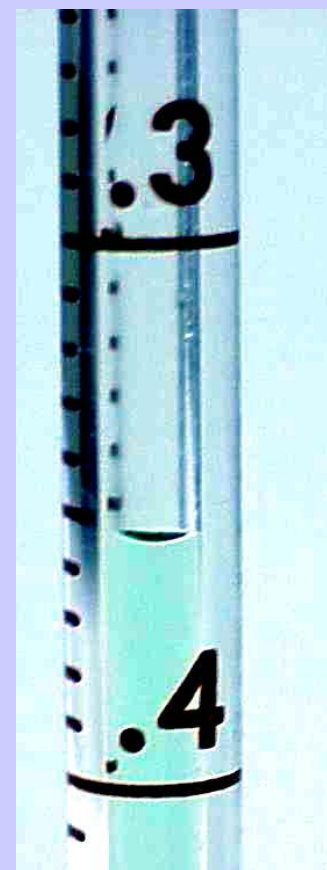
Never use your thumb--your index finger will allow better control and will also enable you to hold other items with your free fingers when necessary.

Then with your finger still on the end of the pipette, gently lift the pipette out of the solution.



Then raise your finger just enough to allow the bottom of the meniscus to line up with the desired graduation mark. You should observe the meniscus at eye-level while doing this.

When the meniscus is at the desired level, touch the tip of the pipette to the inside of the container holding the colored water, to remove any drops of liquid on the end of the pipette. Now, there is precisely (0.645 ± 0.001) ml of colored water in your pipette.



Keeping your finger on the end of the pipette, gently move it to the waste container.

Touch the tip to the inside of the container, lift your finger off the end and allow the liquid to drain out of the pipette.

Hold the pipette in this position for a few seconds after it stops draining.






When you are at the Pipette station in the SLC:

use pipette F, and draw precisely (2.5 ± 0.1) ml of colored water into the pipette following the procedures described above, except that you must blow out this pipette after the liquid drains out, since you are using a serological pipette.

SUMMARY

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- Squeeze bulb and touch it to the mouth of the pipette.
 - Place other end of the pipette in liquid to be transferred and slowly release pressure on bulb.
 - Draw liquid up past desired level, quickly replacing bulb with index finger.
 - Let liquid drain until bottom of meniscus lines up with desired level on pipette.
 - Touch tip of pipette to inside of beaker to remove any adhering drops.
 - Transfer liquid to second beaker and touch tip to inside of beaker and let liquid drain out of pipette.

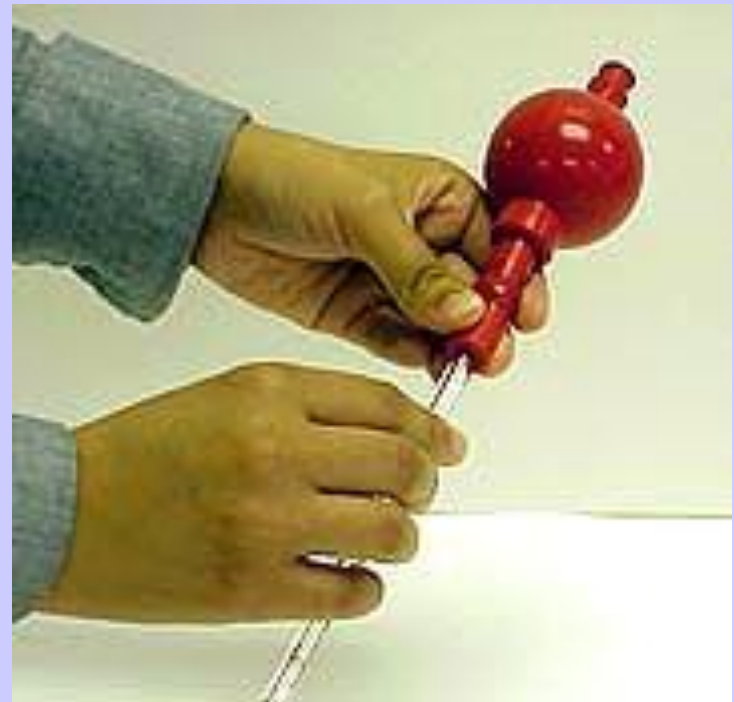
USE OF SAFETY PIPETTE FILLER

In this part of the module, the use of safety pipette filler (Propipette) will be demonstrated.

When at the Pipette station in the SLC, perform the steps as they are described using any of the pipettes A - D to transfer a specified amount of colored water into the waste container. If you use a measuring pipette, you are free to choose the amount of liquid to be transferred.



With a gentle twisting motion, insert the pipette into the Safety Pipette Filler about 1/2 cm.



To produce a vacuum for aspiration, squeeze valve “**A**” with your thumb and index finger of one hand while using your other hand to squeeze the bulb. “A” stands for “**air**” or “**aspirate.**”



(Click on the picture to see a movie on the SLC computer)

To “pull” the liquid up into the pipette place the pipette into the liquid and squeeze the “**S**” or “**suction**” valve until the liquid reaches the desired level.

Touch the tip of the pipette to the side of the vessel containing the liquid to remove any adhering drops.



To “**expel**” the liquid,
squeeze the “**E**” valve.
This will allow the liquid
to flow out of the pipette
except for the last drop.



If the last drop of liquid that remains in the tip must be expelled, squeeze valve “E” with your thumb and forefinger, cover the opening in the small bulb with your middle finger and squeeze the small bulb.




PIPETTING AIDS

The pipetting aid has a plunger at one end and a rubber ring at the other end into which the pipette is inserted. The plungers are color coded depending on the volume of liquid that they can pull up.



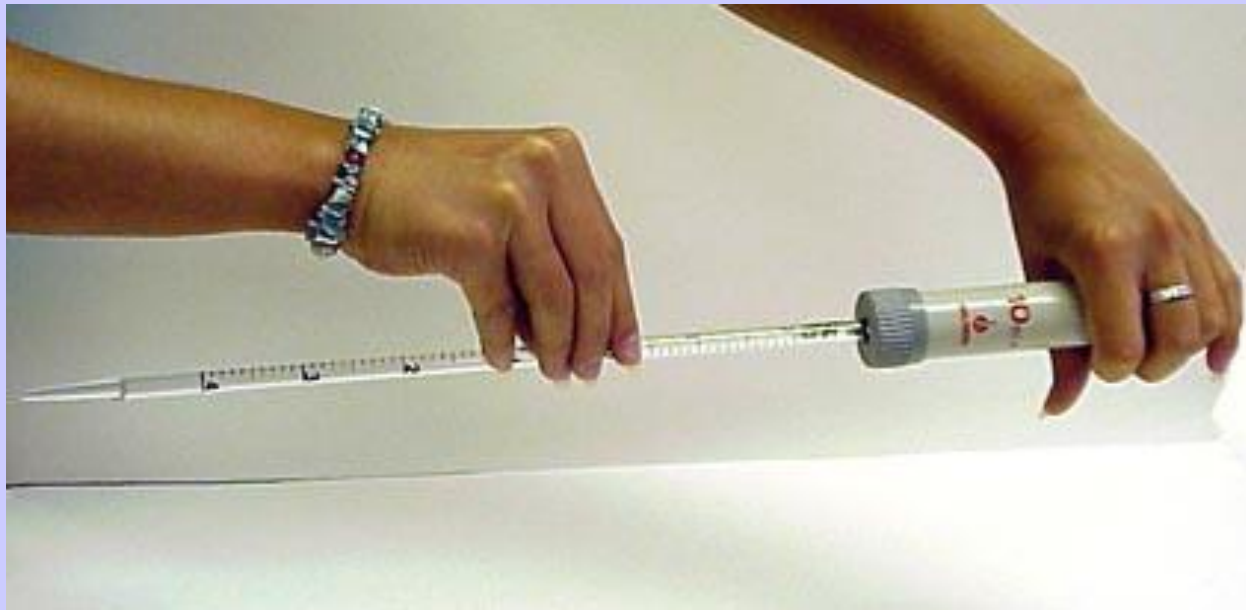
PIPETTING AIDS



When at the Pipettes station in the SLC, perform the steps as they are described using any of the pipettes A - D to transfer a specified amount of colored water into the waste container.

If you use a measuring pipette, you are free to choose the amount of liquid to be transferred.

Insert the pipette into pipetting aid. Place your index finger over the hole in the plunger and push it down completely and hold it there.



Place the tip of the pipette into a beaker containing colored water and allow the plunger to rise all the way up, keeping your finger firmly on the hole in the plunger.

Adjust the level of the meniscus by lifting your finger. If the meniscus level isn't high enough, depress the plunger completely and allow it to rise again.



When the pipette is filled to the desired level, touch the tip against the side of the beaker and move the pipette over the waste beaker.

Lift your finger off of the hole in the plunger, and allow the pipette to drain.

If you need to blow-out the last drop of liquid in the tip, place your finger over the hole in the plunger and depress it again.



OTHER PIPETTE BULBS

Other pipette bulbs that are often used include the Vadosa pipette filler, seen on the left, and the Pipette Pumper, on the right. Your lab instructor will demonstrate their use if necessary.




OTHER PIPETTE TYPES

Transfer of uncalibrated volumes up to 2.5 ml can be accomplished using glass “transfer” or “Pasteur” pipettes shown below. These may be sterilized before use.

Roughly calibrated volumes of 1 and 2 ml can be transferred with the one piece plastic transfer pipettes which may be purchased as sterile or non-sterile units.





If you feel you have mastered the objectives of this module, please obtain a posttest from the Science Learning Center personnel. If you don't feel confident with the use of pipettes, repeat the module. Next, obtain a Mastery Exercise. If you do not pass the Mastery Exercise, you may return as many times as you need to and review the unit and try the exercise again. When you have successfully completed it, make sure your name is recorded.