GUIDE TO PIPETTING

Third Edition

GILSON®

HOW THE PIPETTE STORY BEGAN...

Over a century ago, Louis Pasteur invented the glass Pasteur pipette to reduce contamination when transferring samples. The Pasteur pipette is still in use today.

The next notable advancement in pipetting occurred in the late 1950s with the introduction of a handheld, piston-operated pipette as a safe alternative to potentially dangerous mouth pipetting. The first handheld pipettes were fixed volume pipettes, meaning that they had a pre-established volume setting. After fixed volume pipettes, variable volume pipettes were introduced, which provided a more flexible stepper volume setting.

In 1972, Dr. Warren Gilson invented the first adjustable volume pipette. As an original error-preventing feature, the selected volume was now clearly displayed on the Gilson pipette (direct digital readout). Today, Gilson precision pipettes are still the world standard for accuracy, precision, and reliability.

Today, Gilson continues its creative efforts and offers innovative, robust, and reliable pipettes to help scientists in their daily work.

The pipetting system is our core expertise, and we truly enjoy sharing this knowledge and experience with you, and other pipette users, to help achieve your goals.

Filled with tips and information from our pipetting experts, this guide covers all aspects of pipetting, from selection to proper maintenance. Examples given are based on our own pipette range, but the techniques described are equally applicable to other brands of pipettes and tips.

reddot design award
winner 2015

Gilson is one of the winners of the Red Dot Award: Product Design for MICROMAN® E.

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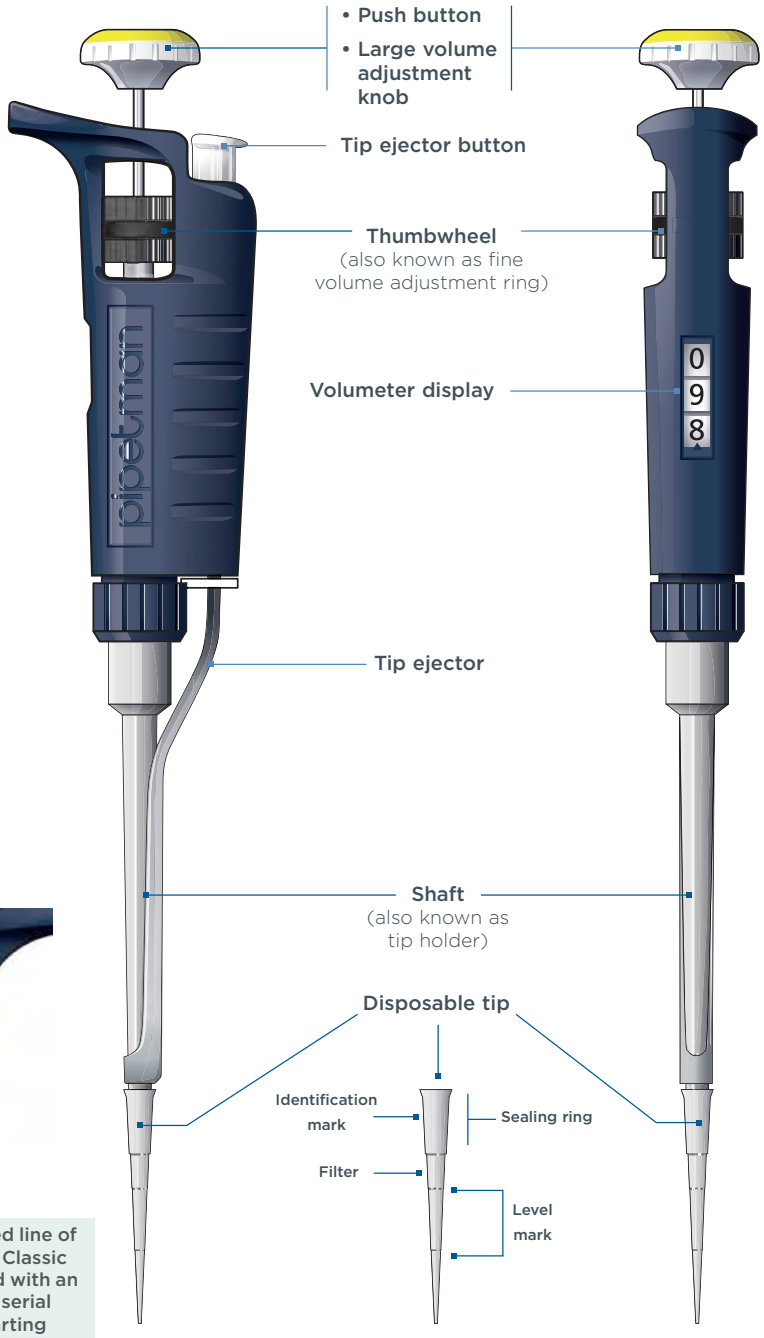


Serial number

NOTE

The updated line of PIPETMAN Classic is identified with an underlined serial number starting from **QG**.

Figure 1
PIPETMAN® diagram*

*Pipettes with different designs are available. For more information, visit **www.gilson.com**.

Positive-Displacement Pipette

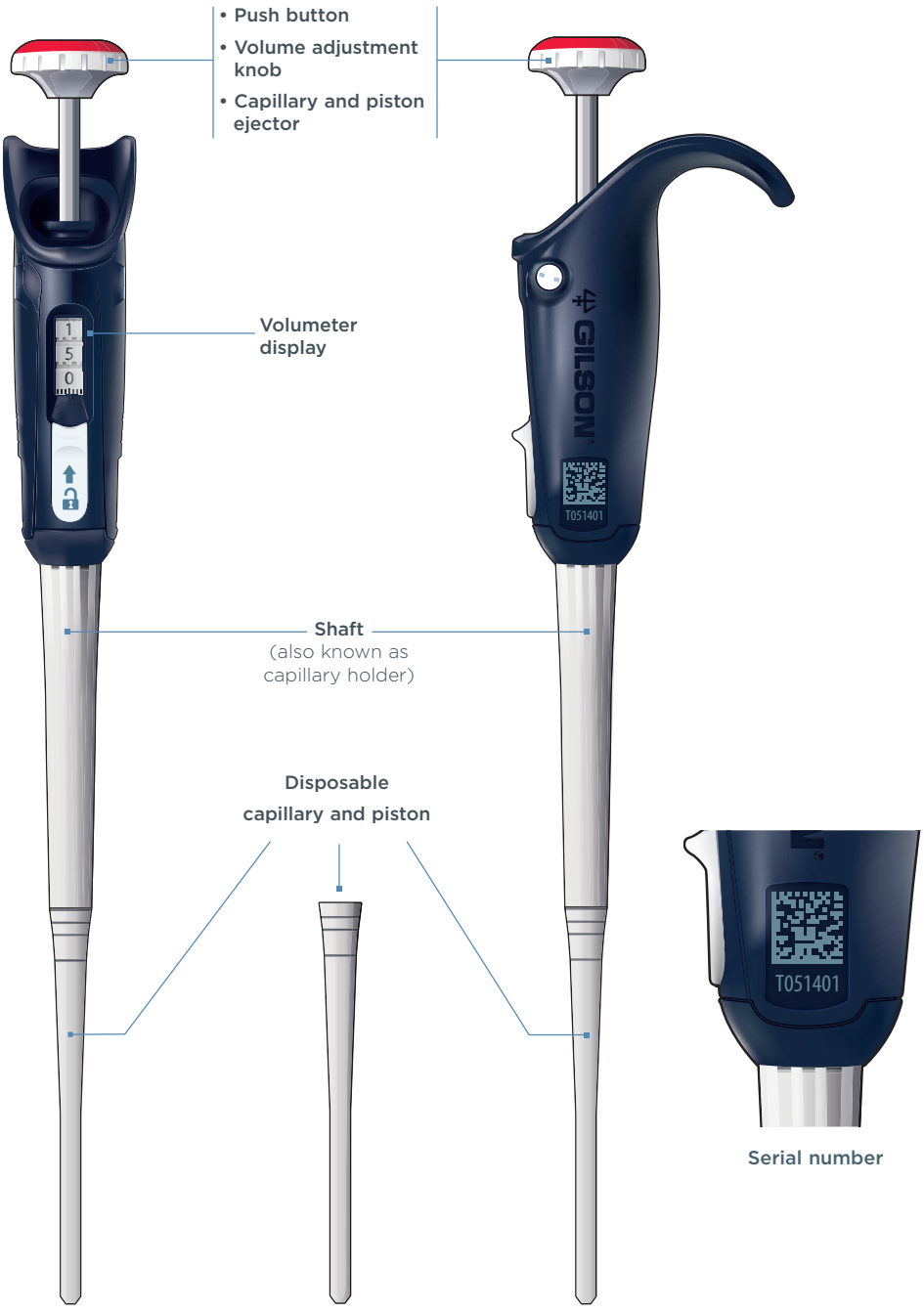


Figure 2
MICROMAN® E diagram*

SELECTING THE RIGHT PIPETTE

SELECTING THE RIGHT PIPETTE

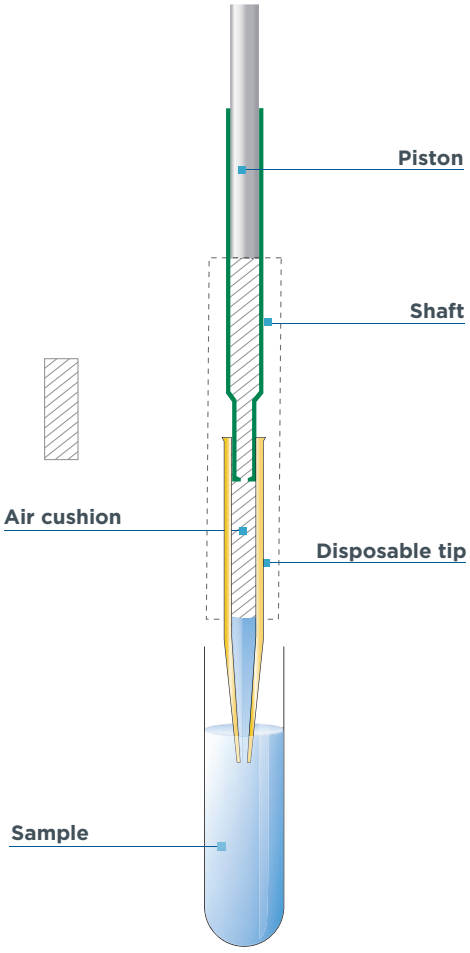
Working Principle of Pipettes

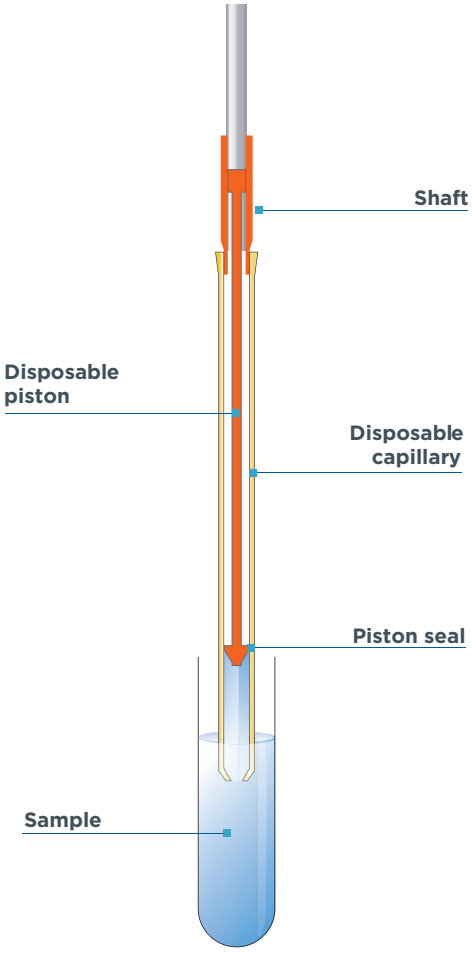
Air-Displacement Pipettes

- Recommended for aqueous samples and for general laboratory work.
- Always have a cushion of air (dead volume) between the pipette piston and the liquid sample.
- The piston is integrated into the lower part of the pipette.

Positive-Displacement Pipettes

- Recommended for non-aqueous samples (viscous, dense, volatile, radioactive, corrosive, contaminating, hot, and cold).
- Direct contact of the piston with the sample (no air cushion).
- The disposable piston is part of the tip (not integrated into the pipette).

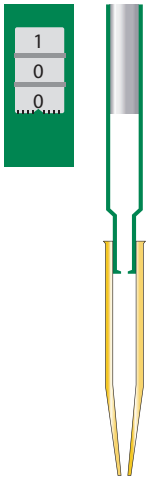




Working Principle of Air-Displacement Pipettes

When the push button is pressed on an air-displacement pipette, the piston inside the instrument moves down to let air out. This means the **air is displaced by the piston.** The volume of air displaced is equivalent to the volume of liquid aspirated.

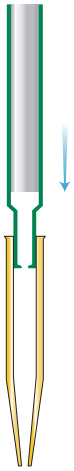
The following infographic shows how the piston determines the volume of air displaced and subsequently the volume of sample aspirated.



1

Set Volume

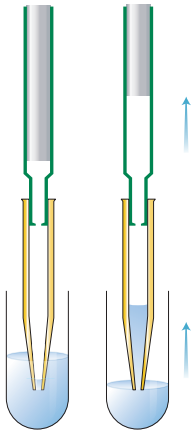
The required volume is set. The piston moves to the appropriate position.



2

Prepare for Aspiration

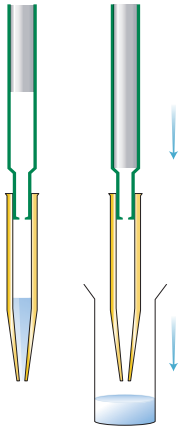
The push button is pressed prior to sample aspiration. The piston descends and expels a volume of air equal to the selected volume of liquid.



3

Aspirate Sample

As the push button is released, a partial vacuum is created inside the tip. The ambient atmospheric pressure forces the desired volume of liquid through the orifice into the tip.



4

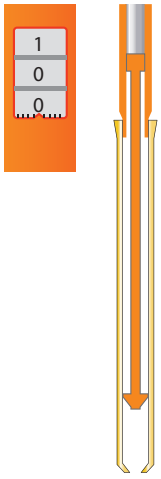
Dispense Sample

The push button is pressed again. Air pressure increases inside the shaft and the tip. The compressed air pushes the liquid out of the tip.

Working Principle of Positive-Displacement Pipettes

Positive displacement pipettes, such as MICROMAN, operate like a syringe. **There is no air cushion between the disposable piston and the sample.** With no elastic air cushion to expand or contract, the aspiration force remains constant, unaffected by the physical properties of the sample.

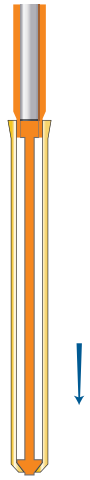
This allows the positive-displacement operator to pipette very viscous or high-density samples, such as glycerol and blood.



1

Set Volume

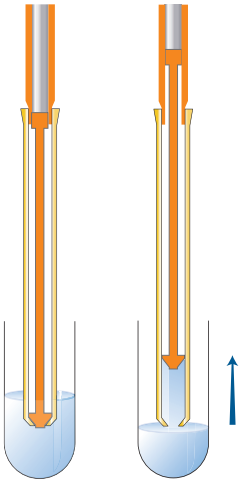
The required volume is set. The piston moves down to the appropriate start position.



2

Prepare for Aspiration

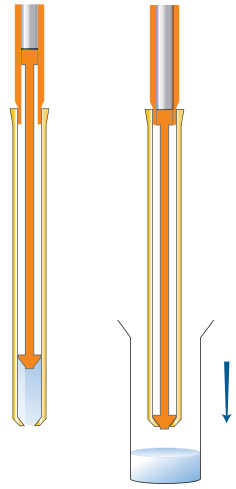
The push button is pressed prior to sample aspiration. The piston descends down to the end of the capillary.



3

Aspirate Sample

The orifice is immersed below the liquid surface. As the push button is released, the piston moves up and the ambient pressure forces the desired volume of liquid through the orifice into the capillary.



4

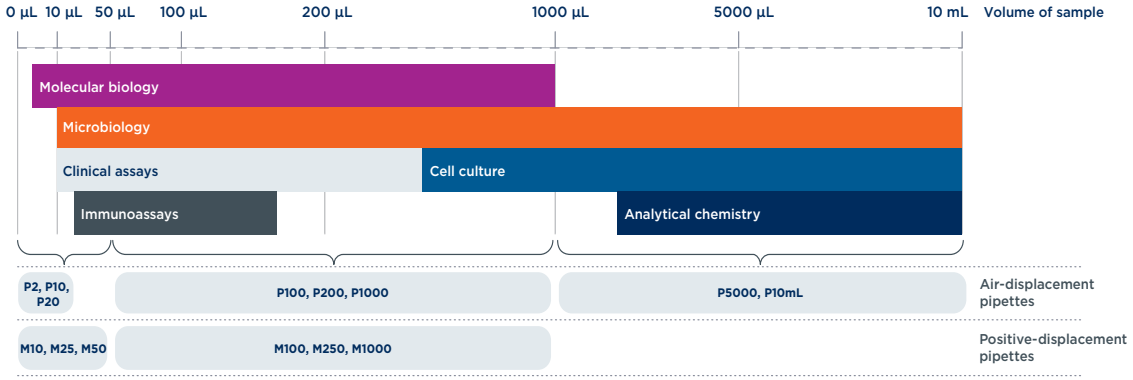
Dispense Sample

The push button is pressed again.The piston moves down and expels the liquid out of the capillary.

The Right Choice for Your Application

The type of analysis to perform, the physical properties of the liquid, and the volume to be handled will determine which pipette to use. It is recommended to select a pipette with a nominal (maximum) volume as close as possible to the desired volume to transfer.

Table 1
Recommendations for pipetting different volumes



Consider the Physical Properties of Your Sample

For volumes greater than 10 mL, it is suggested to work with a pipette filler like the MACROMAN with plastic or serological pipettes.
Regardless of the volume you require, the nature of the sample directly impacts precision and accuracy. Air-displacement pipettes are better for aqueous liquids whereas positive-displacement pipettes are used for non-aqueous samples such as viscous or volatile liquids.

SAMPLE TYPES	EXAMPLES	RECOMMENDED PIPETTES
Aqueous	Water, sucrose, Tris, buffers with a pH of 7	Air-displacement
Biological	DNA, RNA, proteins	Air-displacement with filter tips
Viscous	Glycerol, surfactants, oil	Positive-displacement
Volatile	Ethanol, hexane, formaldehyde	
Hazardous	Radioactive isotopes, blood, infectious bacteria or viruses	
Corrosive	Acids such as hydrochloric acid or sulfuric acid, bases such as ammonium hydroxide, salts such as sodium chloride	

Accuracy and Precision While Pipetting Non-Aqueous Liquids

Positive-displacement pipettes like MICROMAN are the right solution for complete and rapid pipetting of viscous and dense liquids such as oil, syrup, cosmetic cream, liquefied food, paint, glycerol, or buffers.

Positive-displacement pipettes are the unique solution to avoid leakage when pipetting high vapor pressure liquids such as acetone, chloroform, alcohol, or other solvents.

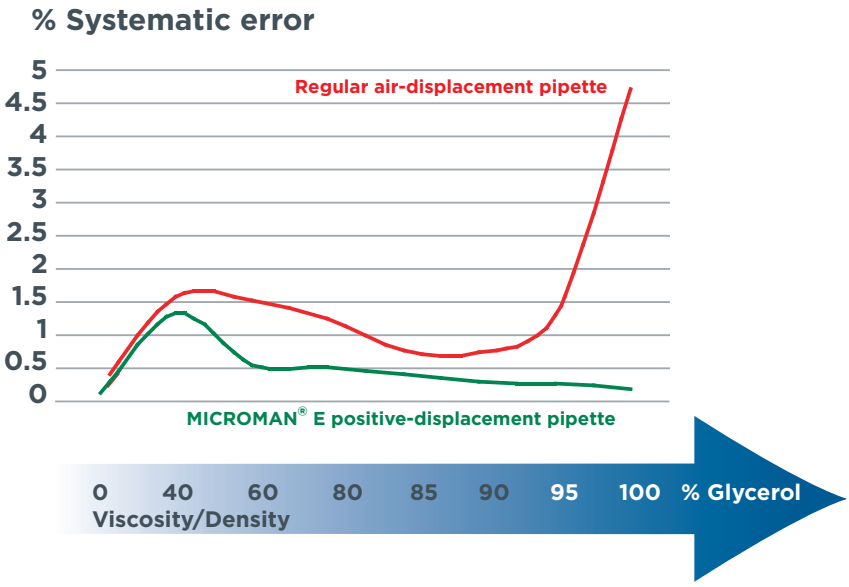


Figure 3
Systematic Error (%) when using a MICROMAN® E positive displacement pipette vs. a regular air displacement pipette

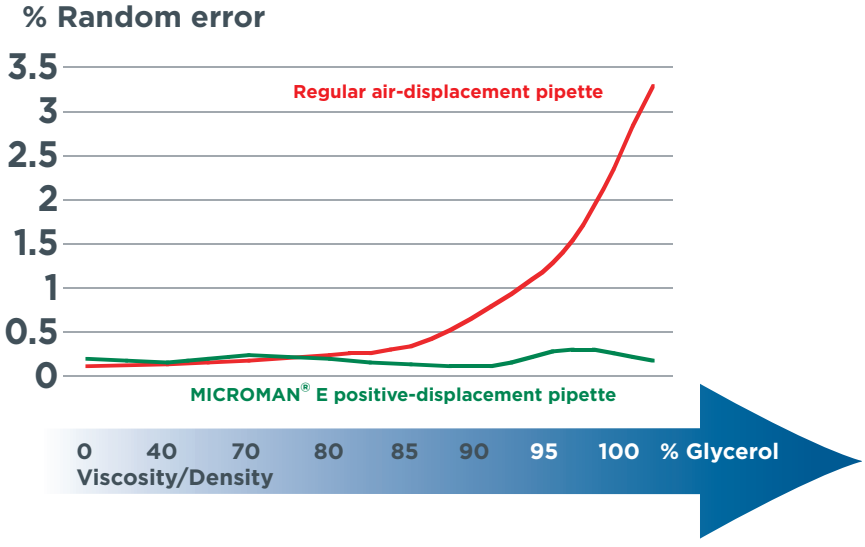
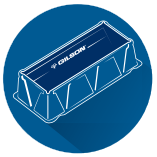


Figure 4
Random Error (%) when using a MICROMAN® E positive-displacement pipette vs. a regular air displacement-pipette

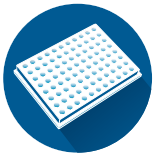
Specific Pipettes for Specific Vessels

Test tubes and centrifuge tubes are used with all single channel pipettes for sample preparations, such as qPCR templates.

Long test tubes, are used with **positive-displacement pipettes and pipette fillers** with plastic or glass pipettes. These devices are specially designed to reach the bottom of the tubes.



Reagent reservoirs are ideal for dispensing reagents, especially with multichannel pipettes.



96-well and 384-well microplates, as well as 8-well strips, are commonly used with **air-displacement, multichannel pipettes** for applications like ELISA. They are also used with single channel pipettes.

Multichannel pipettes allow transferring 8 to 12 different samples in one shot and filling a microplate 8 to 12 times faster than with single channel pipettes.

High Throughput and Repetitive Pipetting

When pipetting in a high throughput setting, it is important to have reliable results and be as efficient as possible. Reliable results means not only having reproducible results with one technician's samples, but also among all technicians in the lab. There are a variety of ways to improve reliability and efficiency, some of which include using motorized pipettes and/or repetitive pipettes.

User-to-User Variability

Motorized pipettes can help reduce variability between users. There are many factors that can affect your pipetting results, including volume setting, pipetting technique, and the rate of aspirating and dispensing. With a motorized pipette you can set the exact volume on the digital display — the motor uses the same pipetting force every time and maintains the same rate of speed when aspirating and dispensing a sample.

Aliquoting

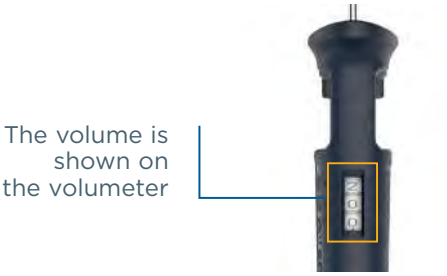
To deliver several aliquots without refilling, you may either choose the repetitive mode of **a motorized air-displacement pipette,** or **use a positive-displacement repeater.**

Repeaters enable up to 125 aliquots, whereas the number of aliquots with air-displacement motorized pipettes will depend on the pipette volume.

For operations fewer than ten aliquots, using a motorized air-displacement pipette is likely the better option.

PIPETTING TECHNIQUES

Adjust the Volume Display



Reading and Adjusting the Volume

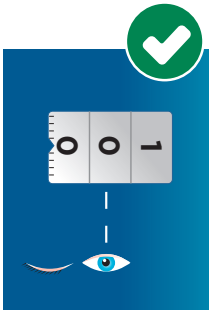
Hold the body of the micropipette in one hand and use the other hand to rotate the thumbwheel or the push button. With the push button, the volume can be easily adjusted with one hand. Push button volume adjustment is available on all MICROMAN pipettes and on PIPETMAN pipettes (except PIPETMAN L) manufactured after April 1995.

A Helpful Hint for Improving Reproducibility and Accuracy

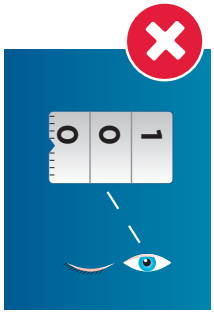
Always finish setting clockwise for best reproducibility. This is how to obtain a clockwise volume setting:

When decreasing the volume setting, slowly reach the required setting, making sure not to pass the setting.

When increasing the volume setting, pass the required value by 1/3 of a turn and then slowly decrease to reach the volume, making sure not to pass the setting.



Correct alignment: accurate reading



Incorrect alignment: error

To avoid parallax, hold the pipette in a horizontal position. Adjust the volume until the indicator is lined up with the desired volume.

NOTICE

To avoid internal damage to your pipette, never attempt to force the volume setting beyond the limits.

Air-Displacement / Forward Mode Pipetting

The forward mode is the standard way of pipetting with an air-displacement pipette like PIPETMAN.

1

Prepare

Hold the pipette in a nearly vertical position. Depress the plunger smoothly to the first stop position.

2

Aspirate

Immerse the pipette tip in the liquid.* Allow the plunger to move up smoothly to the rest position. Wait one second so that all the liquid has time to move up into the tip.

3

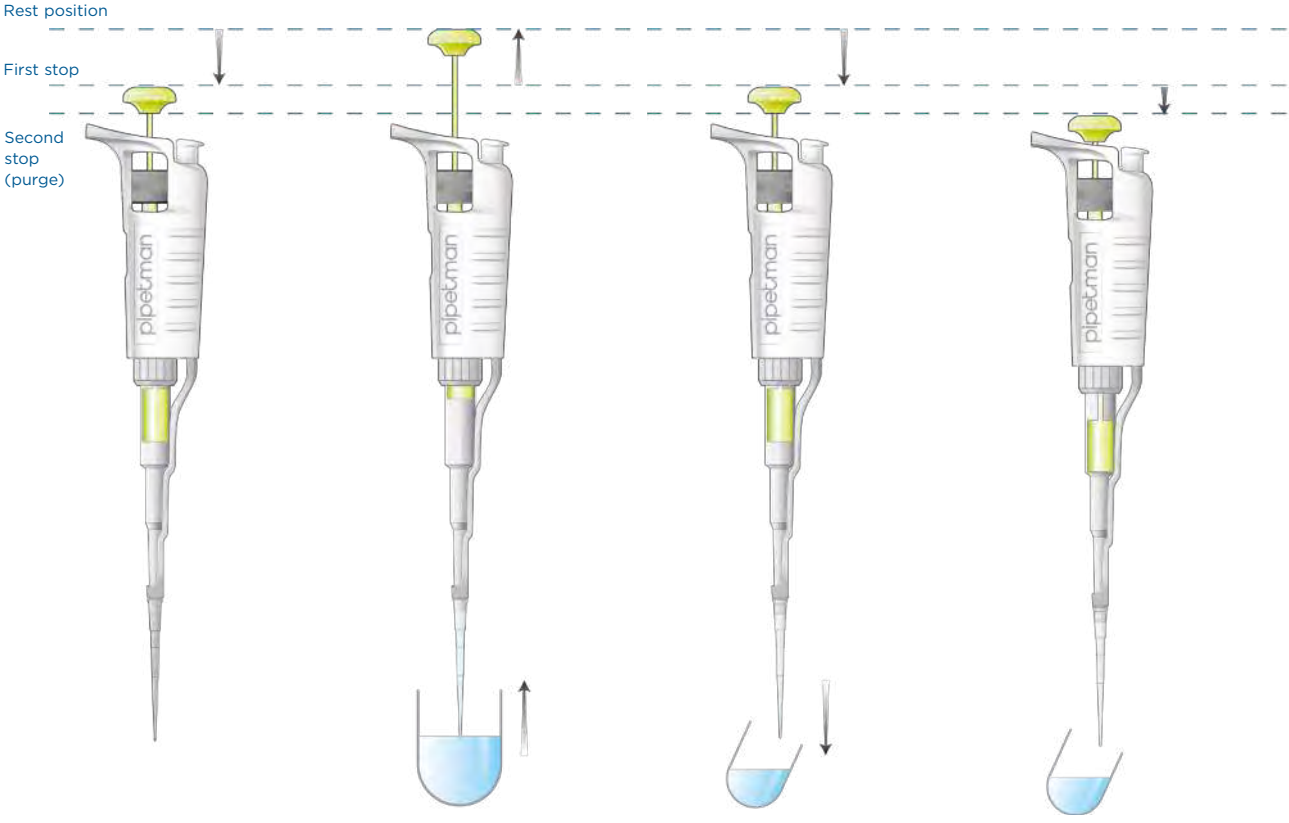
Dispense

Place the pipette tip at an angle (10° to 45°) against the inside wall of the receiving vessel. Depress the plunger smoothly to the first stop position.

4

Purge

Wait one second, then depress the plunger to the second stop position. This purge stroke removes any remaining sample from the tip. Remove pipette tip end from sidewall by sliding it up the wall.



* The immersion depth of your tip can have a significant effect on your results (for depth per model, see table above). If the tip is immersed too deeply, droplets will form on the outside of the tip and they will be deposited along with your sample. If the tip is not immersed deeply enough, vortexing will occur and your pipette will not aspirate the selected volume.

In general, precision in forward mode depends on precise draining by air pressure (air-displacement pipettes) or internal wiping of the pipette barrel (positive-displacement pipettes).

5

Home

Allow the plunger to move up to the rest position.

VOLUME	IMMERSION DEPTH
0.1 —1 µL	1 mm
1—100 µL	2–3 mm
101—1000 µL	2–4 mm
1001 µL— 10 mL	3–6 mm

Pre-Wet

To obtain greater uniformity and precision of dispensing, it is better to provide identical contact surfaces for all aliquots. This is done by pre-rinsing with the same liquid as the one dispensed.

For pre-wetting, aspirate with the tip, and then dispense back into the original reservoir or to waste.

Pre-wet again when adjusting the volume.

Air-Displacement / Reverse Mode Pipetting

The reverse mode is only possible with air-displacement pipettes. It is sometimes used to pipette slightly viscous liquids.

1

Prepare

Hold the pipette in a nearly vertical position. Depress the plunger smoothly to the second stop position.

2

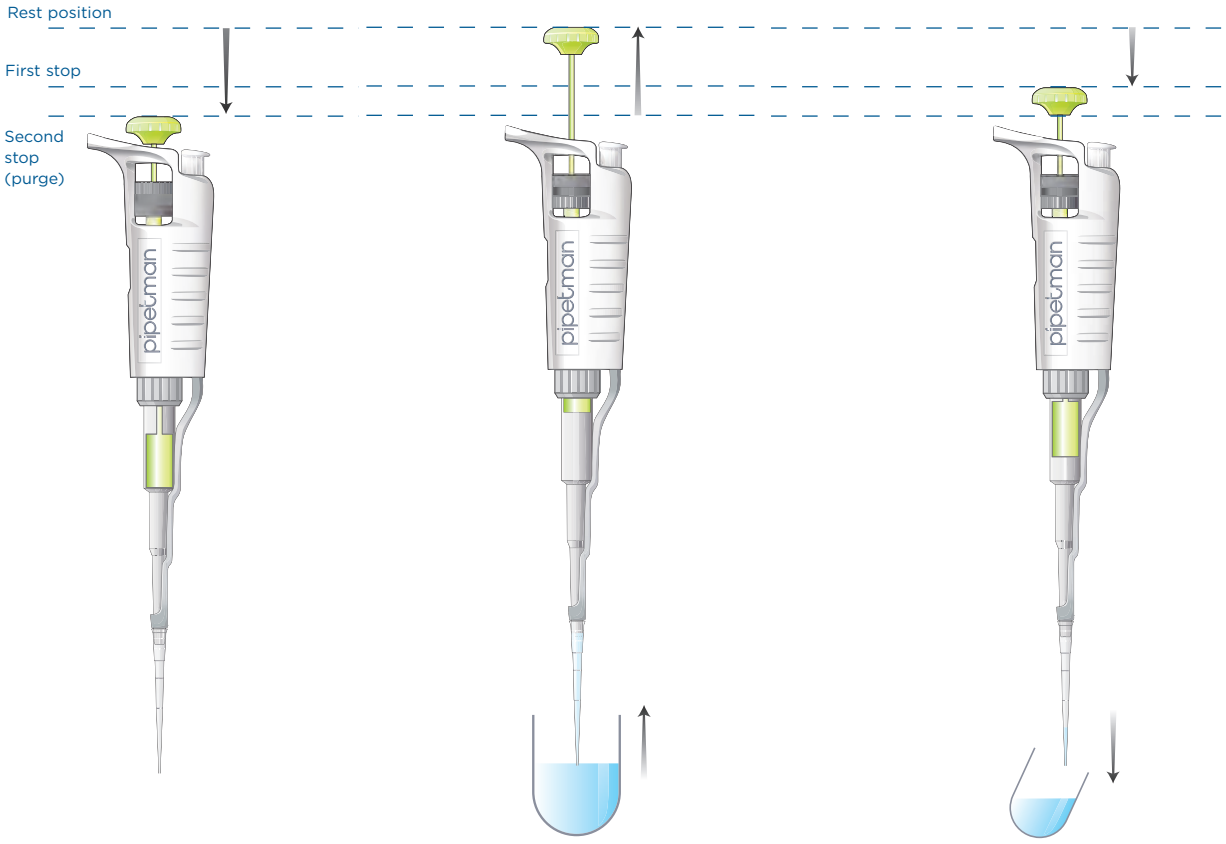
Aspirate

Immerse the pipette tip in the liquid. Allow the plunger to move up smoothly to the rest position. Wait one second so that all the liquid has time to move up into the tip.

3

Dispense

Place the pipette tip at an angle (10° to 45°) against the inside wall of the receiving vessel. Depress the plunger smoothly to the first stop position. Wait one second.



In reverse mode pipetting, the purge stroke is used during preparation. During aspiration, an amount of liquid equal to the amount of purged air is added. This amount compensates for the liquid that remains inside the tip while dispensing.

4

Re-Aspirate

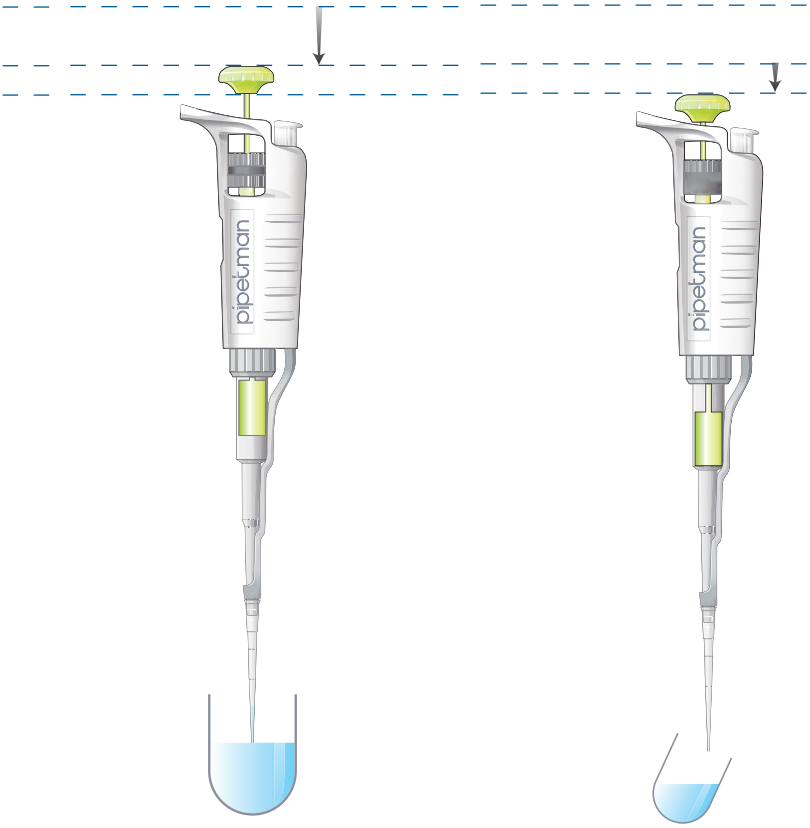
If the pipette tip is to be reused for the same sample, maintain the plunger in the intermediate position for subsequent immersion for the next pipetting cycle and restart step 2 (Aspirate).

or

5

Purge

Wait one second and then 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 / Forward Mode Pipetting

1

Prepare

Press the plunger button to the first stop. The piston moves to the appropriate position.

2

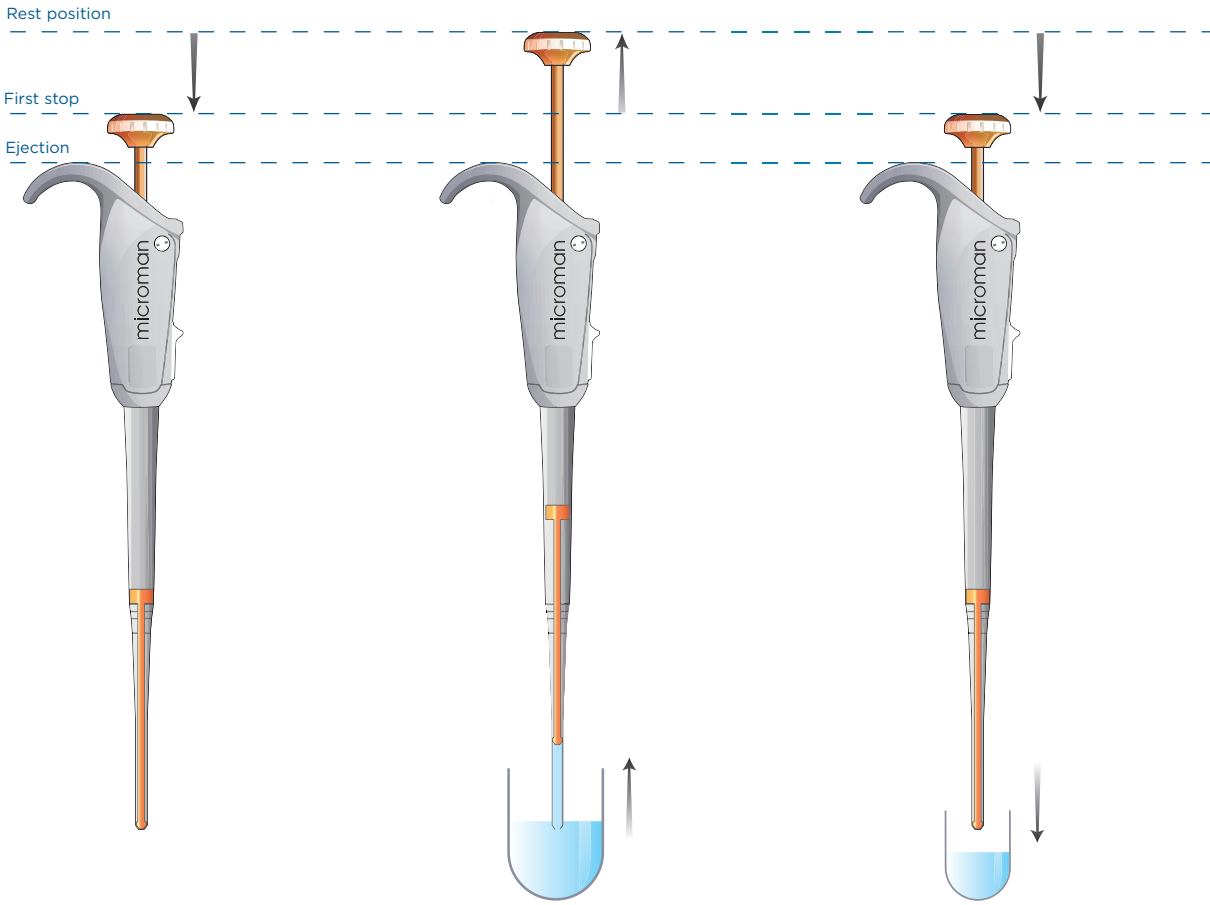
Aspirate

Immerse the capillary/piston in the liquid.* Release the plunger, letting it move up to the home position. The piston moves up and the ambient pressure forces the desired volume of liquid through the orifice into the capillary.

3

Dispense

Press the plunger button to the first stop. The piston moves down and expels the liquid out of the capillary.



* The immersion depth of your tip can have a significant effect on your results (for depth per model, see table page 20). If the tip is immersed too deeply, droplets will form on the outside of the tip and they will be deposited along with your sample. If the tip is not immersed deeply enough, vortexing will occur and your pipette will not aspirate the selected volume.

4

Eject

Press the plunger all the way down to the second and last stop. The capillary and piston are ejected without hand contact.

Wiping

To avoid distorted results linked to the volume pipetted, ensure that no liquid is on the outside part of the tip. When pipetting viscous liquids, such as cream, it may be necessary to wipe the outside of the tip or the capillary with a clean medical wipe. Do not touch the orifice. Choose a tissue that is resistant, lint-free, and inert to acids and solvents. Dispose of the tissue in a safe, hygienic manner.

NOTICE

When working with high risk specimens, do not wipe the disposable part. Make sure fluid depth penetration does not exceed the recommended immersion depth.*

Pre-Wet

To pre-wet, aspirate with the tip and then dispense back into the original reservoir or to waste.

NOTICE

When working with high risk specimens, do not wipe the disposable part. Make sure fluid depth penetration does not exceed the recommended immersion depth.

Tips for Mistake-Free Pipetting

How to Avoid Typical Pipetting Mistakes

MANY FACTORS MAY IMPACT PIPETTING ACCURACY

INFLUENCING PARAMETERS AND EFFECTS	CORRECTIVE MEASURES
Leaky/poorly seated pipette tips may affect accuracy by 0.5% to 50%	Use PIPETMAN® DIAMOND Tips or recommended pipette tips
Reuse of pipette tips may affect accuracy by up to 4%	Use pipette tips only once
The straightness of pipette tips may affect accuracy by up to 10%	Use appropriate tips only
The difference in vapor pressure of the liquid to be pipetted versus that of the water used for adjustment may affect accuracy by up to 2%	Pre-wet pipette tips
Failure to wipe pipette tip on the vessel wall can affect accuracy by up to 3%	Wipe of the pipette tip on the vessel wall (wiping distance 8 to 10 mm)*
Pipette tip immersion depth and handling angle during pipetting may affect accuracy by up to 1%	Hold pipette in a vertical position while pipetting
Irregular rhythm and timing during pipetting can affect accuracy by up to 1.5%	Apply a consistent pipetting technique
A leaky piston system can affect accuracy by 1% to 50%	Check the pipette and the volume aspirated regularly
Uneven piston movement can affect accuracy by up to 0.5%	Smooth pipetting of piston

Information extracted from ISO 8655-2 - Appendix B

* Gilson recommends touching the tip to the vessel wall at an angle of 10° to 45°.

Pipetting Ergonomics

Take a Few Minutes to Get Organized and Ensure You Have:

1. **An appropriate posture**
2. **The right equipment**
   Gilson offers various pipettes with forces adapted to user preferences. The forces of PIPETMAN L are some of the lowest.
3. **The appropriate technique**
4. **A good work organization and environment**

A good test for proper ergonomics is to see if you can rest your elbow comfortably on the work surface. If not, your receptacle may be too low or too high—find the right height.

Download the Gilson Ergonomic Poster on **www.gilson.com**

Take Time to Relax

1. If possible, try to switch periodically between different types of work.
2. Keep an appropriate, unrushed working speed. Let go of the pipette from time to time and give your fingers/hand a (micro) break.
3. Take frequent short breaks. Change your sitting position. Lean back and relax your shoulders and arms.

Ensure Smooth Pipetting

1. To facilitate uniform timing and motion, keep all necessary items within arm's reach.
2. Place **the most frequently used objects** in front of you. The more rarely used items can be placed a little further away from you.
3. The opening of the receptacle for used tips should be at the same height as the end of your pipette.

Use a Pipette Holder

Protect your pipette and always store it vertically on a pipette holder. Pipettes left on a workbench or stored in a drawer can easily come into contact with samples and become contaminated.

Figure 5
SINGLE® pipette holder

Figure 6
POWER CAROUSSEL® stand