Gilson guide to pipetting

PRECISION

microliter plates

VISCOSITY

AQUEOUS SAMPLES

CONTAMINATION

RNA

DNA

POSITIVE-DISPLACEMENT

Forward Mode

PERFORMANCE

specifications

ACCURACY

Second Edition

Louis Pasteur
the father of modern biology
1822-1895

Over a century ago, Louis Pasteur gave glass pipettes a new form to prevent liquid reflux. The Pasteur pipette is still in use today.

The next significant improvement on pipettes came in the late 50's with the introduction of a hand-held, piston-operated pipette as a safe alternative to potentially dangerous mouth pipetting. The first hand-held pipettes had a pre-established volume setting (fixed volume pipettes). Further improvement came with the introduction of a more flexible, stepper volume setting (variable volume pipettes).

Pipetting since Louis Pasteur

In 1972, Dr. Warren Gilson introduced a high precision pipette which could be adjusted for any volume within its range… even fractional (continuously adjustable). Its originality… to avoid error, the selected volume was clearly displayed on the Gilson pipette (direct digital readout).

Twenty-five years later, Gilson precision pipettes are still the world standard for accuracy, precision and reliability.

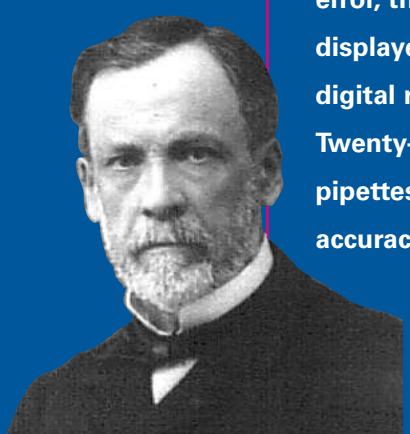


Photo courtesy of the Institut Pasteur.

Chapter 1
Selecting the best pipette for your application

Chapter 2
Selecting the right tip for your pipette

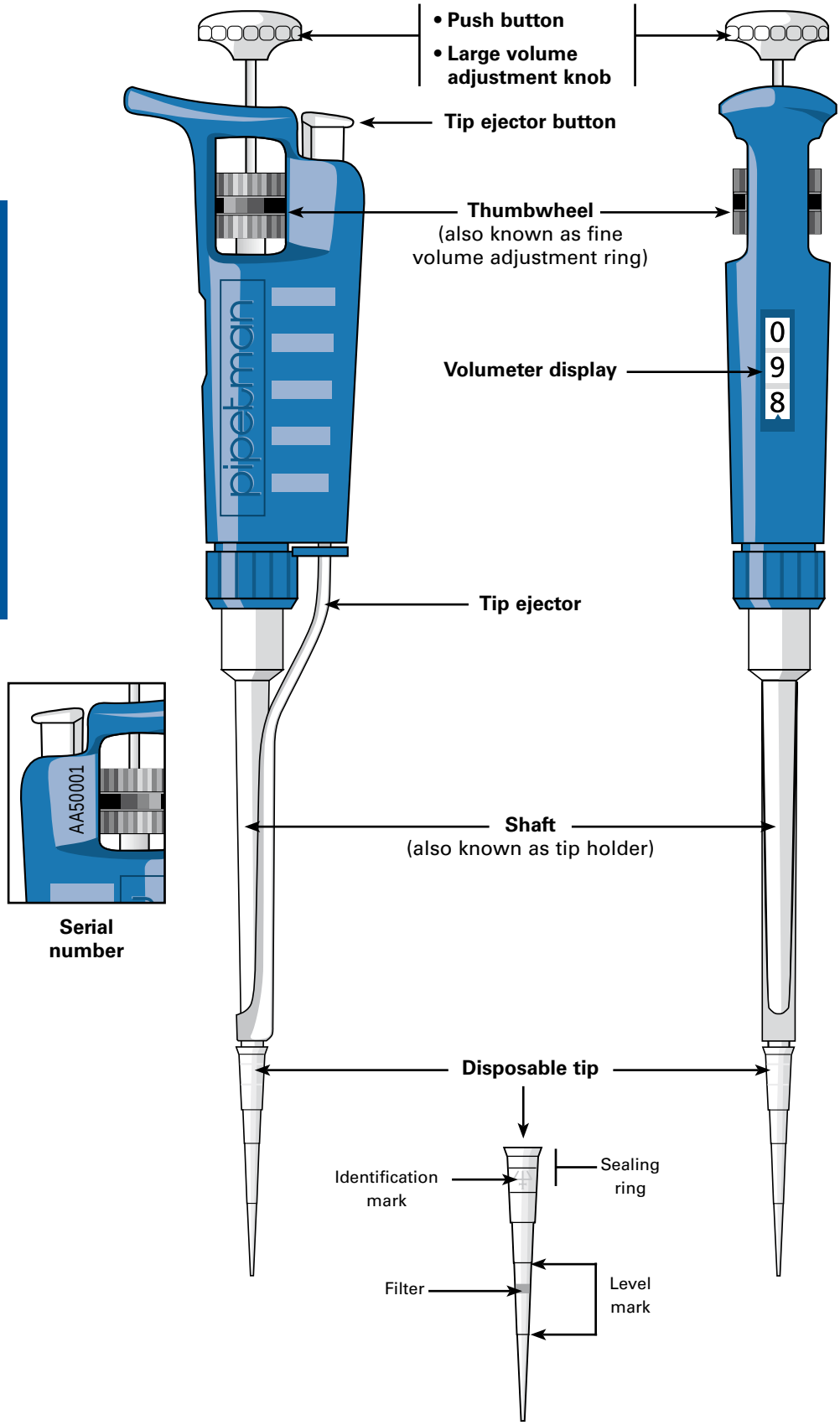
Chapter 3
Correct operation

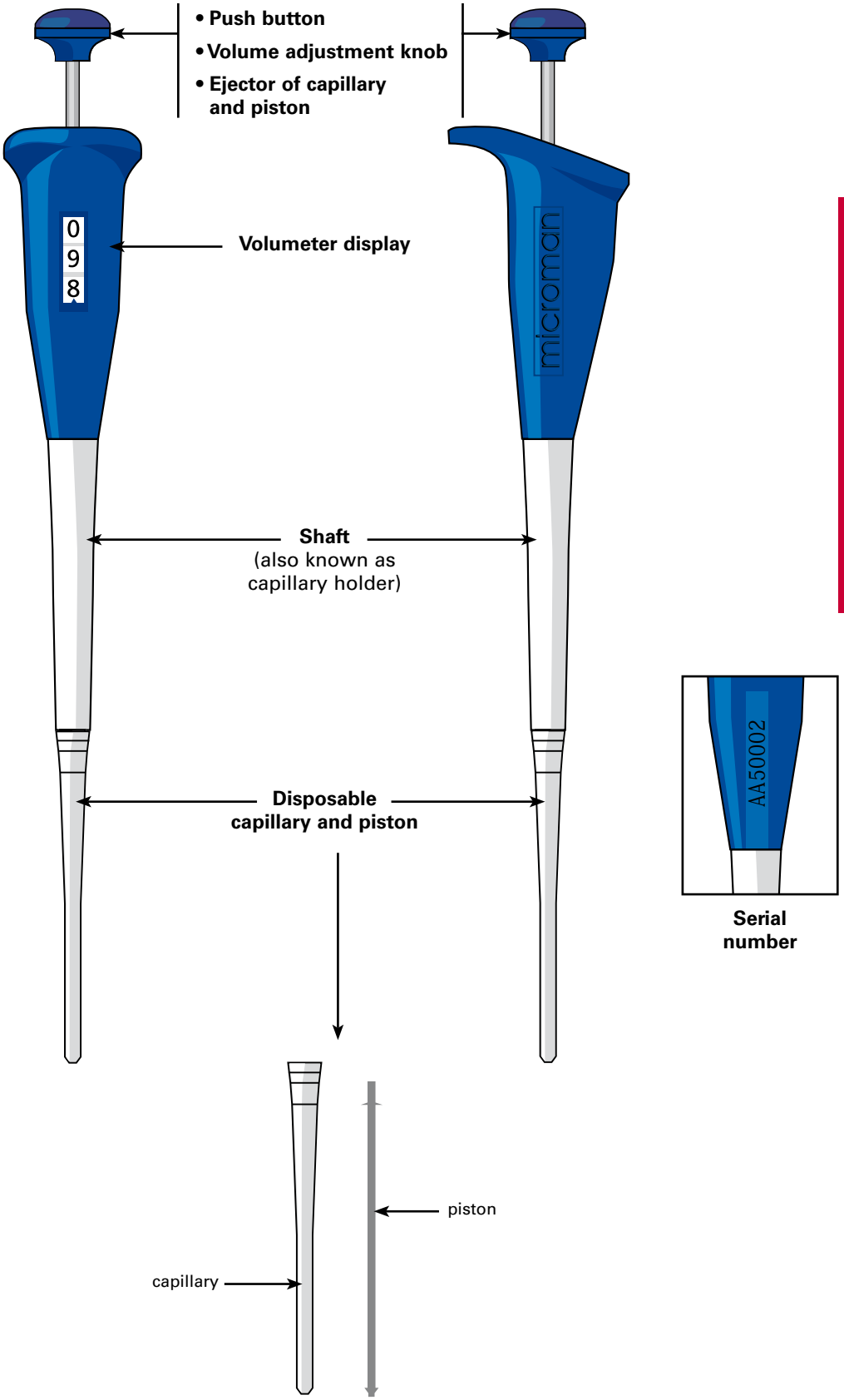
Chapter 4
Preventing contamination

Chapter 5
Caring for your pipette

Chapter 6
Control and calibration

Air-displacement pipette
PIPETMAN





Serial number

MICROMAN
positive-displacement pipette

Selecting the best pipette for your application

Chapter 1

Today, there is a pipette for virtually every requirement... aqueous solutions, dense, viscous, or radioactive compounds, DNA sequencing, distributing aliquots, filling microtiter plates, etc.

The type of analysis you are performing, the physical properties of the liquid and the volume range of the pipette will determine which pipette and tip you should use.

Aqueous samples

Viscous samples

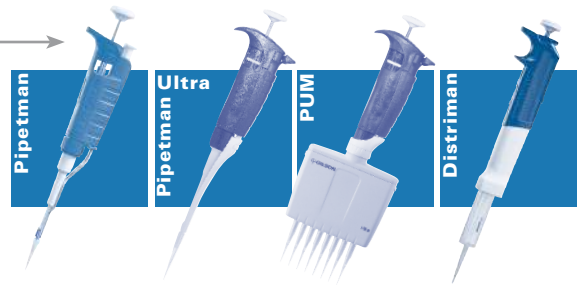
Radioactive corrosive... samples

DNA - RNA biological samples

1.1 Consider the physical properties of your sample

Aqueous samples

Correctly operated and maintained, **Pipetman®** pipettes will give precise results with most liquids commonly found in the laboratory. If you have a large number of fractions to dispense, use a **Distriman™** repetitive pipette.



Viscous samples

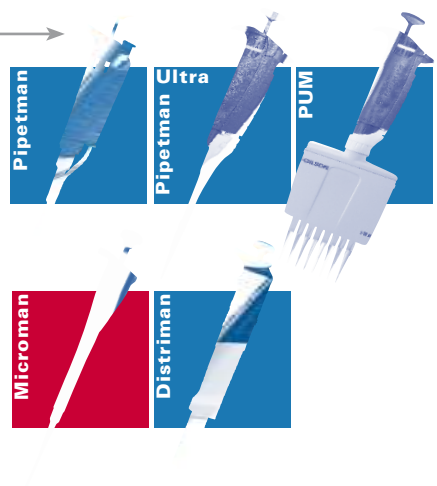
You may find it easier to use a Gilson Microman® pipette. Microman gives reproducible results with highly viscous samples like glycerol, detergent and honey. Microman is also recommended for highly volatile (chloroform) or dense liquids (mercury) which are often difficult to aspirate.

Radioactive and corrosive samples

Microman pipettes are specially designed to handle aggressive liquids without danger for the user or damage to the instrument.

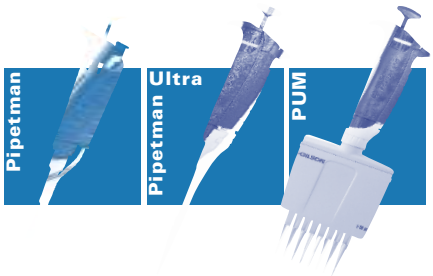
DNA, RNA, biological samples

The choice is yours. You can use either a **Pipetman** with **Diamond®** autoclavable filter tips (0.1 µl - 1 ml) or an autoclavable **Microman** (Model M10 and M100) with sterilized capillaries and pistons. Properly used, these instruments will assure precise, contamination-free results. For multiple dispensing, opt for a **Distriman** repetitive pipette with a **DistriTip™** gamma-sterilized micro syringe.



1.2 The working principle of air-displacement and of positive-displacement pipettes

The most important difference between Pipetman, Microman and Distriman is their working principle.



Pipetman (single and multi-channel) are **air-displacement** pipettes.

Microman and Distriman are **positive-displacement** pipettes.

Important

Piston perfect

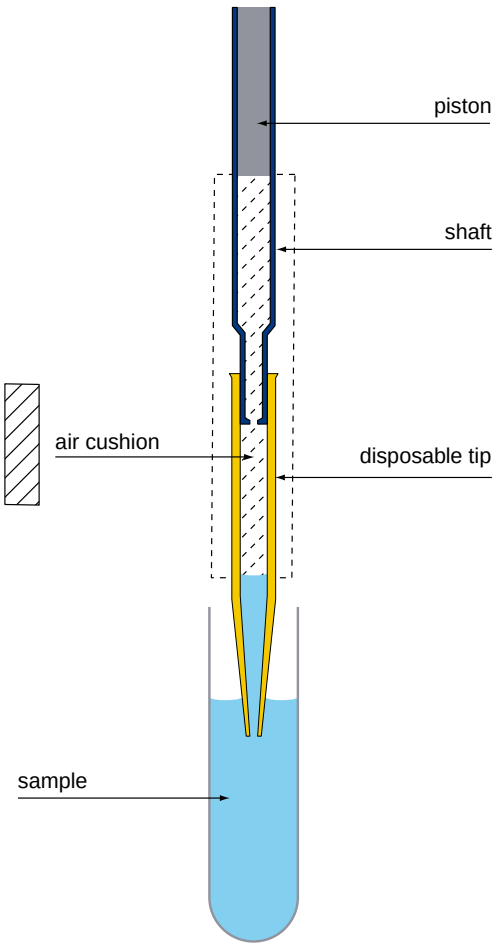
When you set the volume on an air-displacement pipette, the piston regulates the volume of the air cushion, which, in turn, determines the volume of liquid which is to be aspirated. The piston has to be perfect if the sample volume is to correspond exactly to the selected volume. That's why Pipetman pistons are examined individually to make sure there are no flaws. They are even cleaned individually to make sure there are no dust particles.

Since the piston is a permanent part of Pipetman pipettes, a perfect piston means perfect measurement every time.

What are air-displacement pipettes?

Three things to remember

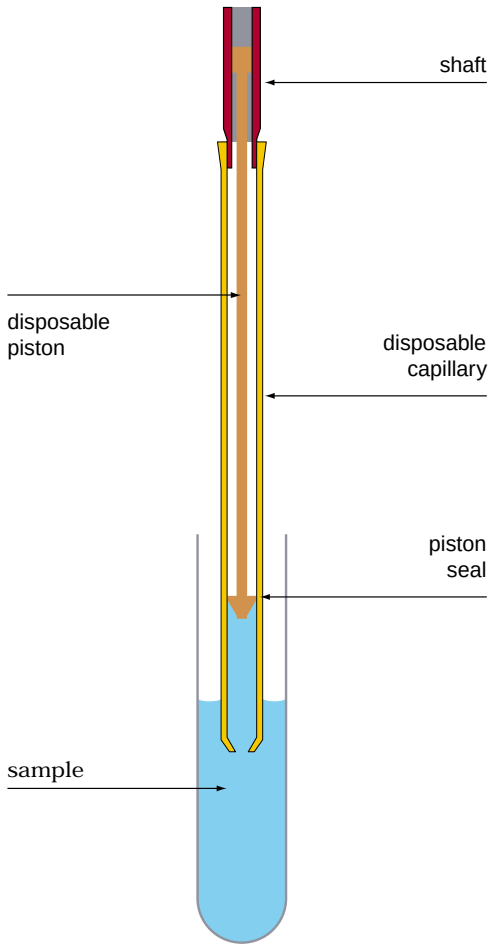
1. Recommended for aqueous samples and for general laboratory work
2. Always have a cushion of air (dead volume) between the pipette piston and the liquid sample
3. The piston is a permanent part of the pipette



What are positive-displacement pipettes?

Three things to remember

1. Recommended for problem samples (viscous, dense, volatile, radioactive, corrosive)
2. Direct contact of the piston with the sample (no air cushion)
3. Disposable piston (not a permanent part of the pipette)



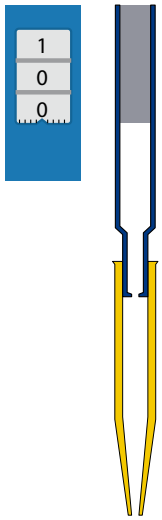
How do air-displacement pipettes work?

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

The schematic drawings (on the right) show how the piston determines the volume of air displaced and subsequently the volume of sample aspirated.

1 Set the volume

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



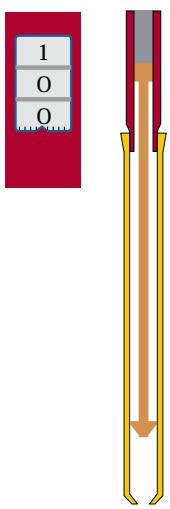
How do positive-displacement pipettes work?

Positive-displacement pipettes work 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 Microman operator to pipette very viscous or high density samples, such as mercury or toothpaste.

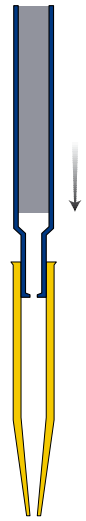
1 Set the volume

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



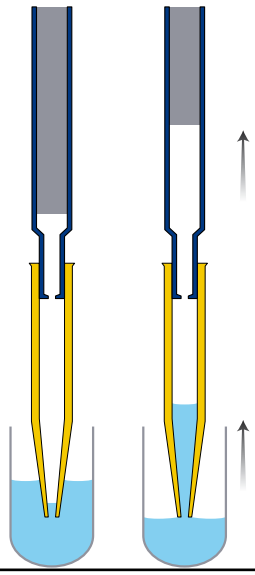
2 Prepare for aspiration

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



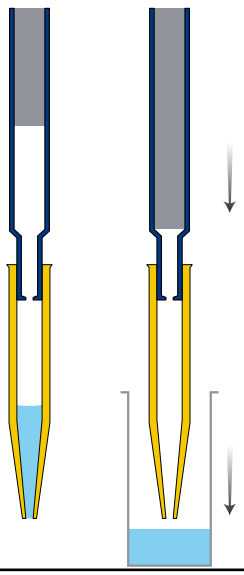
3 Aspirate the sample

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.



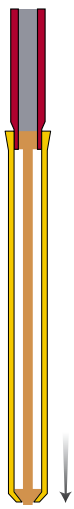
4 Dispense the sample

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.



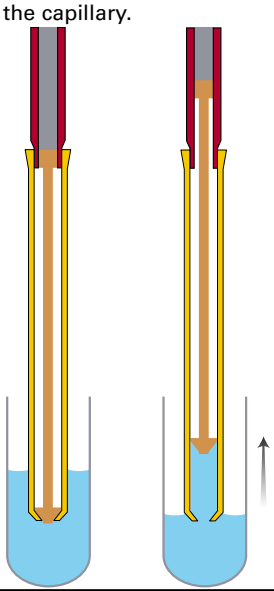
2 Prepare for aspiration

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



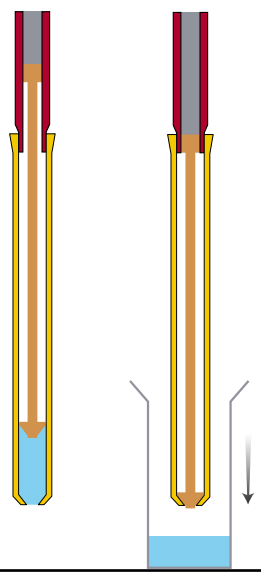
3 Aspirate the sample

The orifice is then 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 the sample

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

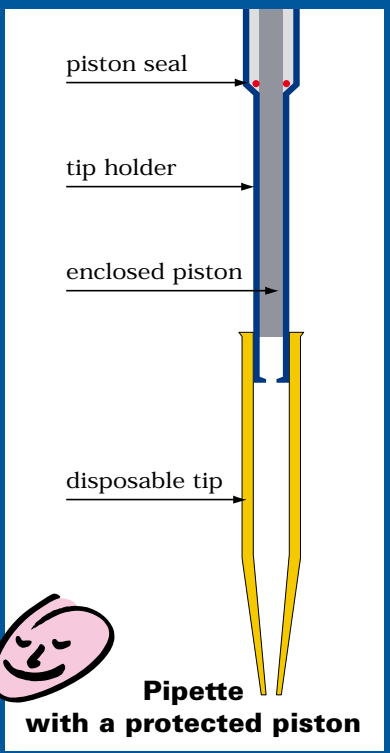


All air-displacement pipettes are not the same

For applications such as DNA sequencing and biochemical procedures that require tiny, valuable, samples, two Pipetman models provide exceptional accuracy and precision, from 0.1 to 2 µl for Model P2 and 0.5 to 10 µl for Model P10.

Minimum air space between the piston and sample makes performance less sensitive to variations in temperature and liquid properties such as vapor pressure and density.

Select a pipette with a protected piston, so there is no risk of sample contact or cross-contamination.



Pipette with a protected piston

Grease-free Pipetman requires less maintenance, reduces the risk of leaks and contamination.

Important

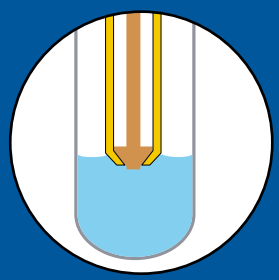
To grease or not to grease?

Unlike most pipettes with greased pistons, Pipetman uses "dry seal" technology. Greased pistons must be regreased regularly or leaks may occur. The grease may be altered by vapors from liquids pipetted... another cause of leaks. Grease can also introduce contaminating particles into the body of the pipette.

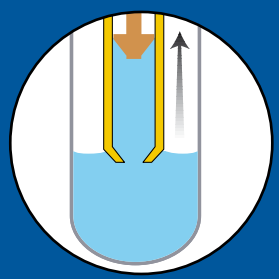
You should never grease a Gilson Pipetman.

Mistake-free pipetting

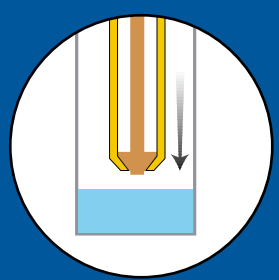
With Microman pipettes, the piston comes into direct contact (positive-displacement) with the sample.
There is no air cushion to expand or contract in response to the density or the temperature of samples.



The desired volume is aspirated completely.
The piston seal prevents biohazardous, radioactive or corrosive aerosols from entering the instrument.

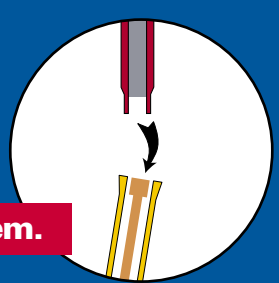


During dispensing, the piston wipes the internal capillary wall, assuring accurate dispensing of almost any viscous sample, from glycerol to glue.



To prevent contamination, the capillary and piston may be changed after each sample. They are ejected automatically so...

... you do not have to touch them.



1.3
Consider the volume of liquid you want to transfer

The eight models of the Gilson Pipetman and Pipetman Ultra are designed to transfer precise volumes from 0.1 µl to 10 ml. Pipetman is also available in fixed volumes from 2 µl to 1 ml.

model	volume range
P2 / U2	0.2 - 2 µl*
P10 / U10	1 - 10 µl*
P20 / U20	2 - 20 µl
P100 / U100	20 - 100 µl
P200 / U200	50 - 200 µl* / 20 - 200 µl
P1000 / U1000	200 - 1000 µl
P5000 / U5000	1 - 5 ml
P10ml / U10ml	1 - 10 ml

* With a precise pipetting technique P2 and U2 may be used to aspirate volumes down to 0.1 µl, P10 and U10 at 0.5 µl, and P200 at 30 µl.

The six models of the Gilson Microman speciality pipettes are designed for the precise transfer of problem liquids in volumes from 1 µl to 1000 µl.

model	volume range
M10	1 - 10 µl
M25	3 - 25 µl
M50	20 - 50 µl
M100	10 - 100 µl
M250	50 - 250 µl
M1000	100 - 1000 µl

The Distriman repetitive pipette is available with three different syringes (micro, mini and maxi), so you can distribute aliquots in any volume (even fractional)
from 1 µl to 1.25 ml.

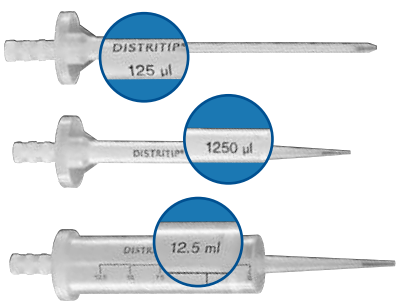
syringe (total syringe volume)	**volume range of aliquots**
Micro (125 µl)	1 to 12.5 µl
Mini (1250 µl)	10 to 125 µl
Maxi (12.5 ml)	100 µl to 1.25 ml

Important

The model and the useful volume range on **Pipetman, Pipetman Ultra** and **Microman** pipettes are shown on the top of the push-button

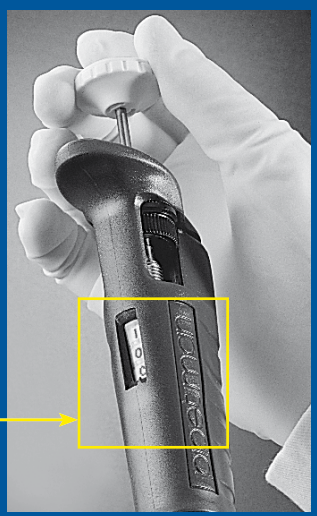
Important

The total volume that can be aspirated is shown on the side of each **Distriman** syringe

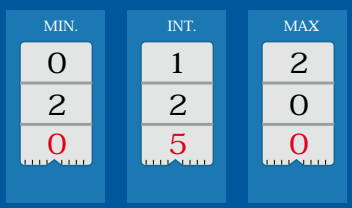


Q How do I read the volume?

A The volume is shown on the digital volumeter



Pipetman P20



Pipetman P200

Pipetman P1000

Examples of volume settings for the Pipetman

The volume is displayed as three digits which are read from top to bottom. The figure on the left shows the minimum (MIN.) and maximum (MAX.) volume settings. It also shows examples of intermediate volume settings (INT.).
The numbers in red represent tenths of microliters (µl) on the P20 and milliliters (ml) on the P1000. For the correct correspondance on the other models, consult your instruction manual.

Examples taken from the most commonly used Pipetman pipettes, models P20, P200 and P1000.

Selecting the right tip for your pipette

Chapter 2

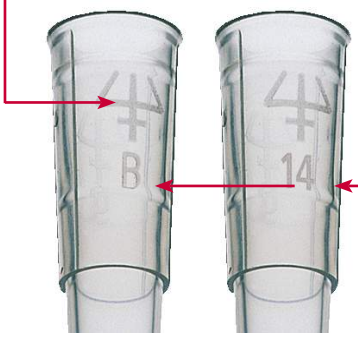
Micropipettes such as Pipetman and Microman must always be used with a suitable tip attached. Tips for Microman are also known as "capillaries". Tips for Distriman are also known as "syringes".

Used correctly, all of these pipettes provide guaranteed accuracy and precision provided that proper Gilson tips are used.

Safety first! Gilson capillaries and pistons for Microman are unbreakable. The risk of personal injury associated with glass capillaries is totally eliminated.

GUARANTEEING MANUFACTURING QUALITY

Every Diamond® precision tip is individually marked with the Gilson logo and with an identification number. With this number, it is possible to identify the mold and even to locate the exact cavity which produced the tip.



GUARANTEEING TRACEABILITY

The batch number on every box and bag makes it possible to trace the itinerary of the tips from packaging to delivery to the laboratory.

2.1 Evaluating tips

Although they may look alike, all tips are not the same. The choice of a poor quality tip will jeopardize your results. Prefer a tip recommended by the pipette manufacturer and always check the following points:

Tip Evaluation Sheet

Physical Aspects	***yes***	***no***
Clean and free of dust particles?	☐	☐
Perfectly formed collar? (for air-tight fit)	☐	☐
Smooth and regular surface? (minimal retention of liquid)	☐	☐
Fine point finish? (prevents formation of droplets)	☐	☐
Translucent (no contaminating additives or dyes)	☐	☐
Chemical resistance	☐	☐

Tip manufacturer's Guarantee	***yes***	***no***
Brand marked on the tip?	☐	☐
Identification number on the tip?	☐	☐
Batch number on the box?	☐	☐
Quality certificate?	☐	☐

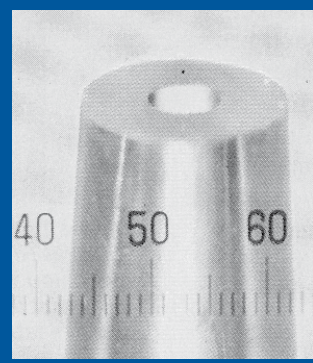
Important

Gilson Diamond tips are supplied with typical values for trace metal release. These concentrations are lowered to the analytical noise level after 5 to 10 rinsings with concentrated acid.

Learning more

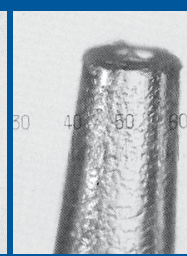
More than meets the eye

These photos taken under the microscope show some of the differences between good and poor quality tips.



perfect point

imperfect point aperture



rough surface

excess plastic

2.2
Choosing the best tip for your application

Gilson tips are available in a variety of formats:

Loose in bulk packaging

An economical solution for routine. May be hand loaded in empty tip racks for convenience or for autoclaving in the laboratory.

Racked for easy mounting with no hand contact

Hinged lids protect against dust. Convenient 96-well format for filling microtiter plates with a multichannel Pipetman. Color-coded for easy identification. Ready for autoclaving in the laboratory. Tip-racks may be reused.

Racked and pre-sterilized for working in sterile conditions

Factory irradiated and delivered in a sealed tip rack.

Racked and with autoclavable filter

Tips with a filter prevent contaminating aerosols from entering the pipette. Gilson Diamond filter tips can be successfully autoclaved in the laboratory.

Racked and pre-sterilized with filter

Factory irradiated filter tips delivered in a sealed tip rack.

Individually wrapped and pre-sterilized

Opened just before use so the benefit of sterilization is assured right up to the last minute. A good solution when you only need a few tips.

Learning more

How to prevent aerosol contamination?

It is indispensable to prevent aerosol contamination when you are using PCR and other amplification methods, or when you are pipetting DNA/RNA solutions, infectious materials, radioactive samples, etc. Gilson offers two solutions:

1 Use a Pipetman pipette with Diamond pre-sterilized filter tips when you have to face at least two of the following problems at the same time:

- working in sterile conditions
- pipetting aqueous samples
- avoiding cross-contamination.

2 Use a Microman pipette with pre-sterilized capillaries and pistons when you have to face at least two of the following problems at the same time:

- working in sterile conditions
- pipetting viscous samples
- avoiding cross-contamination.

For more information, see page 35.

Correct operation

Chapter 3

To optimize your results, the pipette is important but don't forget to use it properly.

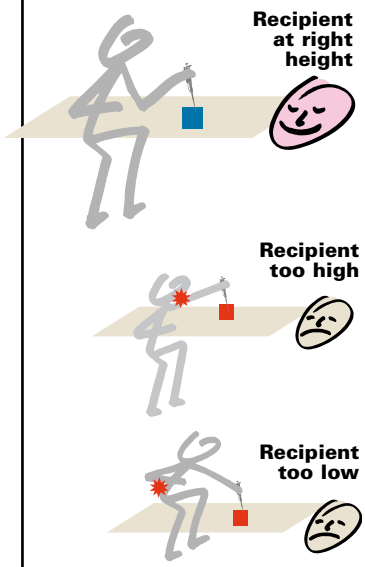
1. **First organize your work station for maximum efficiency and minimum fatigue.**
2. **Make sure the tip is properly mounted and fits well before you set the volume.**
3. **Adjust the volume.**
4. **Choose the mode of pipetting (reverse or forward mode) adapted to your sample.**
5. **Eject the used tip and store the pipette in an upright position to avoid damage and cross-contamination.**

Take a few minutes to get organized

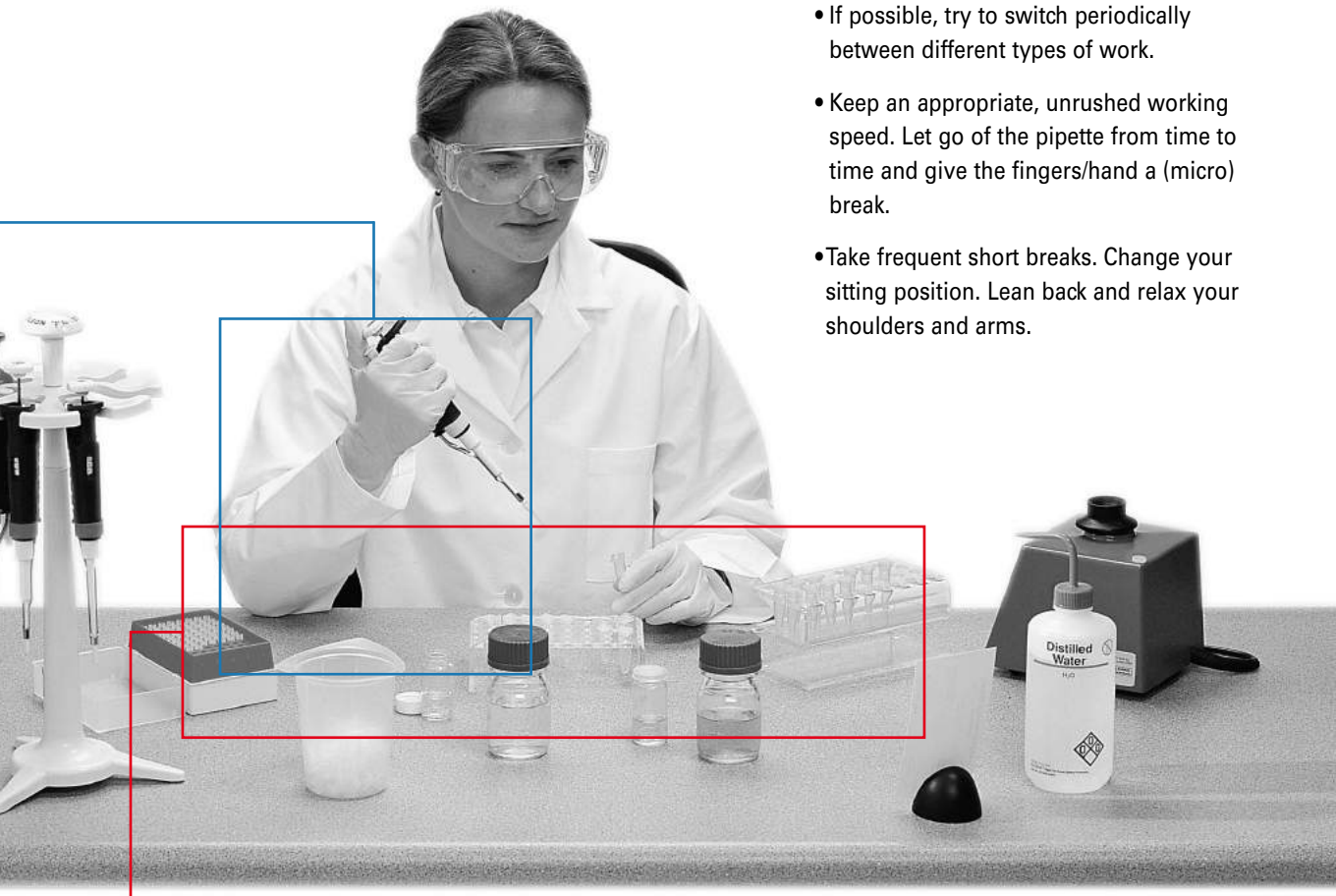
- Adjust your **chair or stool** so that the **work surface** is at the right height when you are sitting straight
- If possible, always try to work with your **hands below shoulder height**
- Try to evaluate if you can reduce the height of applications such as gel loading
- Adjustable tables/workbenches are a good solution

Important

A good test is to see if you can rest your elbow comfortably on the work surface



3.1 Organize your work station



Take time to relax

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

Special attention should be paid to smooth pipetting

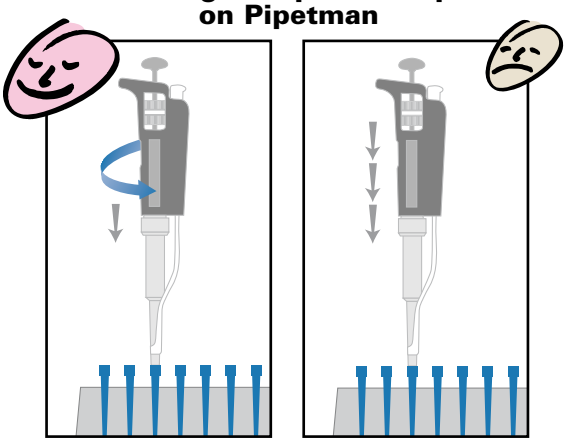
- To favor uniform timing and motion, have all necessary objects within easy arm's reach
- Place **the most frequently used objects** in front of you. The more rarely used items can be placed a little further away from you
- The opening of the recipient for used tips should be at the same height as the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

3.2
Fit a disposable pipette tip

To fit a disposable tip on a Pipetman, hold the micropipette in one hand and use a slight twisting movement to seat the tip firmly on the shaft of the micropipette and to ensure an air-tight seal.

Diamond tips for Pipetman are available in TIPACK racks for easy mounting with no hand contact.

Fitting a disposable tip on Pipetman



Press down with rotation motion

Avoid stabbing the tip as though the pipette were a knife

To mount DistriTips on a Distriman, consult the instruction manual.

To fit a capillary and piston on a Microman, press the plunger button to the second stop. The jaws on the pipette will open automatically and seize the piston, mounting the capillary and piston in the correct position.

For maximum protection against contamination, capillaries and pistons for Microman are available pre-assembled, racked and pre-sterilized.

To fit tips on a Pipetman Ultra 8X300 multichannel pipette. When you are using a multichannel pipette with a standard flat-bed tip rack, it is not always easy to mount all eight tips at the same time. You have to push down hard, or even hammer and pound. The patented G-F.I.T. *system*™ provides a firmer fit of the Gilson Diamond® Tips and most standard tips while decreasing the force to fit and eject, pick-up is fast, and tips won't fall off.

Important

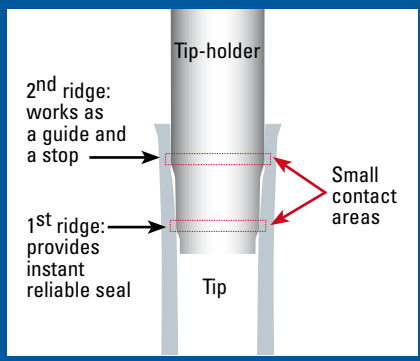
Always choose a tip recommended by the pipette manufacturer (see page16).

Important

Never attempt to use a micropipette without a suitable tip attached.

Learning more

How to mount tips on a Pipetman Ultra multichannel pipette?



Small contact areas

=

instant reliable seals

+

low ejection forces

The patented G-F.I.T. System™ provides instant reliable seals and low ejection forces for Pipetman Ultra pipetttes.

G-F.I.T. System is compatible with most standard tips.

The force required to fit and eject Diamond tips using the G-F.I.T. System can be reduced by as much as half of that of regular tip-holders. Above 40 N, the ejection force remains at a steady level around 17 N.

Exert a light vertical force followed by a slight lateral rocky movement to secure the tip fitting

3.3
Adjust the volume setting

To adjust the volume

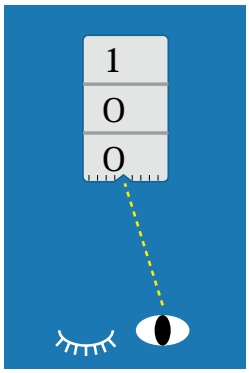
Hold the body of the micropipette in one hand and use the other hand to rotate the thumbwheel - or the push-button. With the push-button, volume can be easily adjusted with one hand. Push-button volume adjustment is available on all Microman pipettes and on Pipetman pipettes manufactured after April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

A helpful hint for improving reproducibility

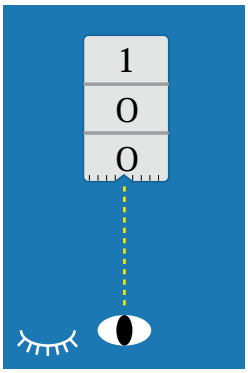
Always finish setting clockwise. In this way, if any mechanical backlash should occur, it will always be in the same position. Here's how to obtain a clockwise volume setting:

- when decreasing the volume setting, turn the thumbwheel slowly until the desired setting is displayed. Do not overshoot the required position,
- when increasing the volume setting, rotate the thumbwheel approximately 1/3 of a turn above the desired setting, and then slowly turn back to decrease the volume until you reach the desired setting.

A helpful hint for improving accuracy

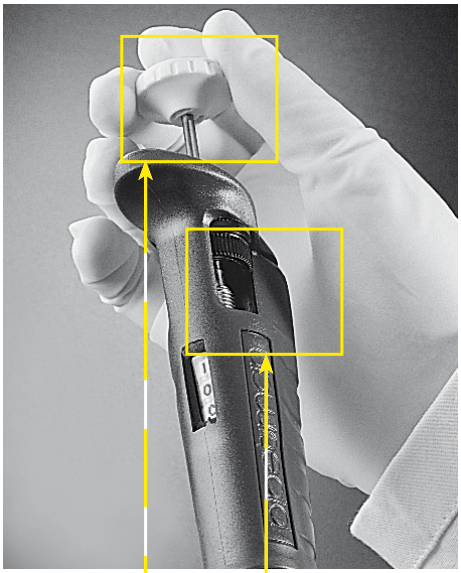


Incorrect alignment : error



Correct alignment : accurate reading

To avoid parallax errors, make sure that the volume indicator and the volume marking selected are in your direct line of vision. At close range, you may find it helpful to close one eye.



The electronic patented multifunctional LCD of the Pipetman Ultra offers greater readability and zero risk of parallax errors.

Important

To avoid internal damage to your pipette, never attempt to force the volume setting beyond the limits shown in the tables on *page 14.*

3.4 Follow the instructions for forward or reverse mode pipetting

The choice of operating mode can significantly affect the results of an analysis.

Gilson pipettes are calibrated for forward mode pipetting. Manufacturers should list all pertinent modes of operation and specify for which mode the instrument is calibrated.

The forward mode is the usual way of pipetting with an air-displacement pipette like Pipetman.
See page 26.

The forward mode for positive-displacement pipettes like Microman eliminates the purge stroke.
See page 30.

The reverse mode is only possible with air-displacement pipettes. It is used for solvents or slightly viscous liquids.
See page 28.

Wiping

If necessary (viscous liquids such as cream), wipe the outside of the tip or the capillary with a clean medical wipe. Do not touch the orifice. Choose a tissue which is resistant, lint-free, and inert to acids and solvents. Dispose of the tissue in a safe, hygienic manner.

Important

When working with high risk specimens, do not wipe the disposable part. Make sure fluid depth penetration does not exceed 3 mm. Then touch-off from the sidewall of the vessel.

Learning more

Pre-rinsing

Greater uniformity and precision of dispensing are usually obtained by providing identical contact surfaces for all aliquots. This is achieved by pre-rinsing with the same liquid that is being dispensed. To pre-rinse, aspirate with the tip and then dispense back into the original reservoir or to waste.

Q

When should I pre-rinse the tip?

A

Pre-rinsing should be performed
- **every time you change a tip**
- **every time you increase the volume setting**

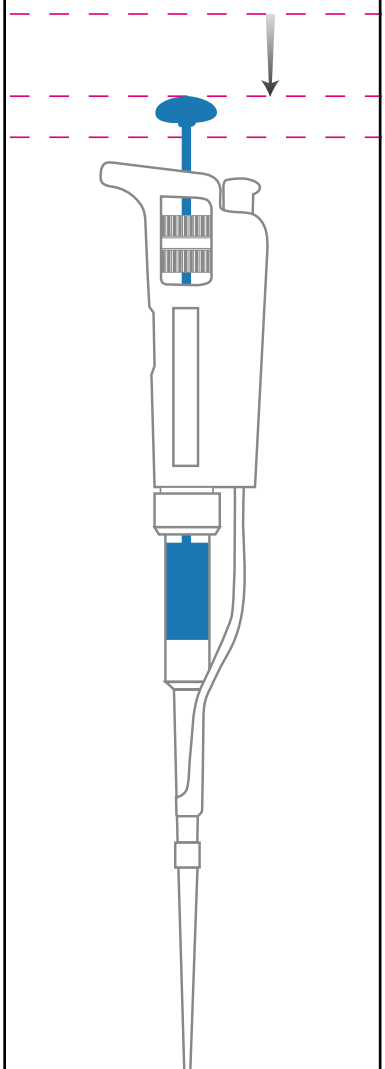
Don't forget to pre-rinse
- **capillaries and pistons for Microman**
- **DistriTips for Distriman**

Air-displacement / Forward mode

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

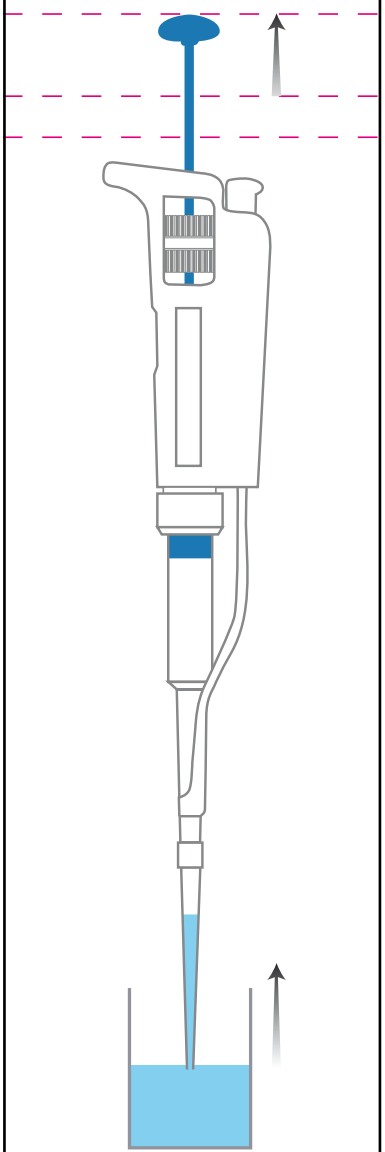
1 Preparation

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



2 Aspiration

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.

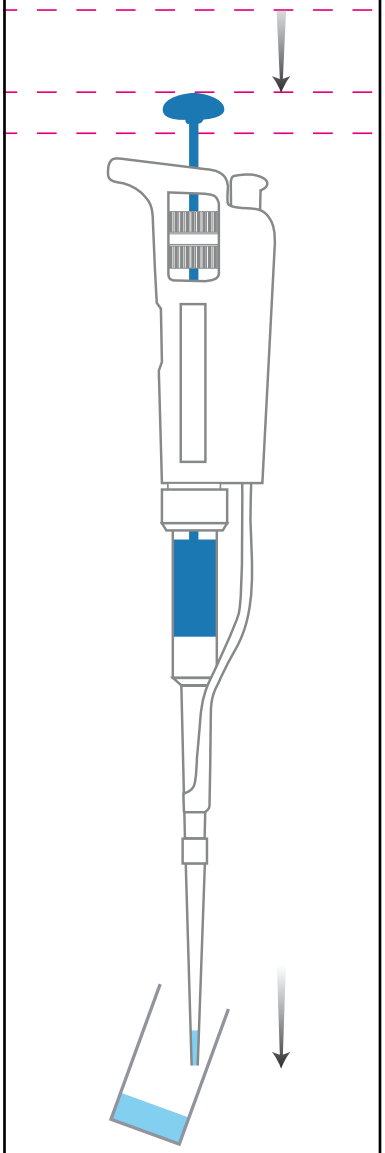


** The immersion depth of your tip can have a significant effect on your results. If the tip is immersed too deeply, droplets will form on the outside of the tip and they will be deposited along with your sample. If the tip is not immersed deeply enough, vortexing will occur and your pipette will not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

volume *µl*	**immersion depth** *mm*
0.1 - 1	1
1 - 100	2-3
101 - 1000	2-4
1001 µl -10 ml	3-6

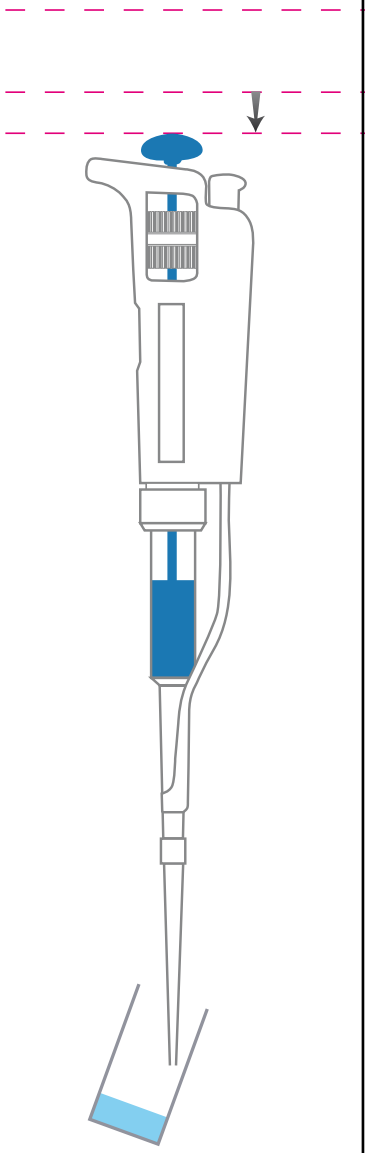
3 Distribution

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.



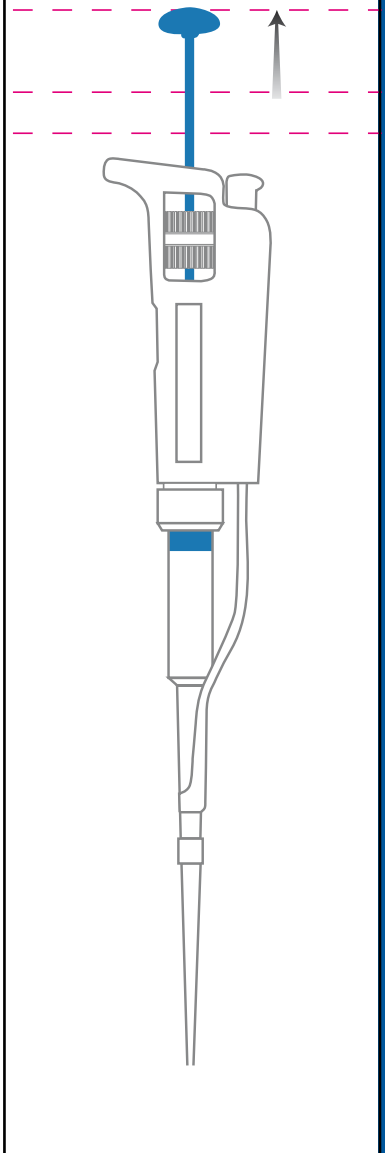
4 Purge

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



5 Home

Allow the plunger to move up to the rest position.



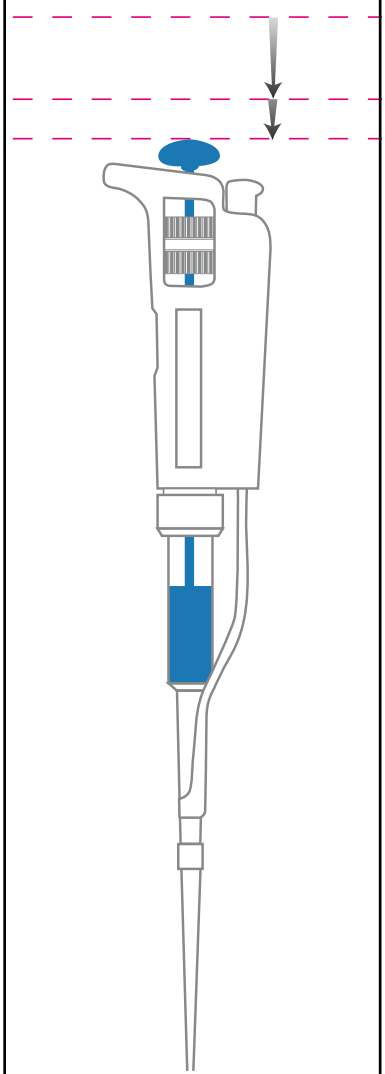
Air-displacement / Reverse mode

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 as film inside the tip during dispensing.

1

Preparation

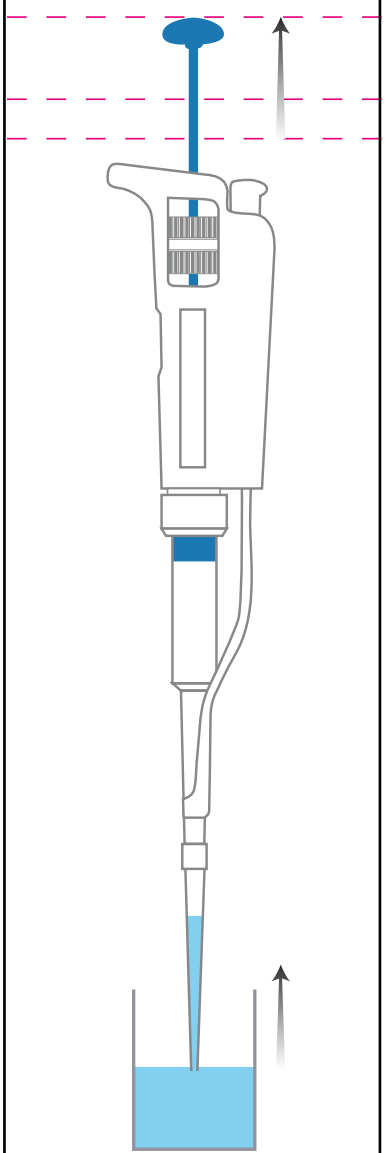
Hold the instrument in a nearly vertical position. Depress the plunger smoothly to the second stop position.



2

Aspiration

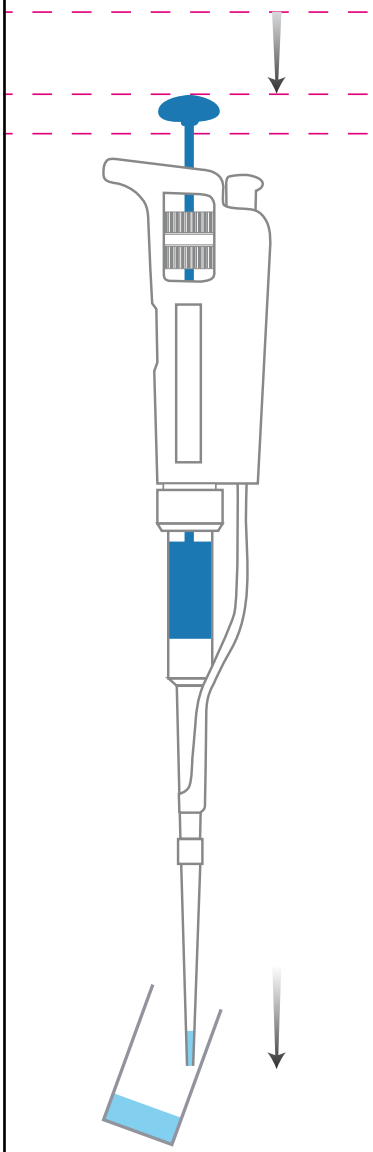
Immerse the pipette tip in the liquid*. Allow the plunger to move up smoothly to the rest position. Wait one second so that all the liquid has time to move up into the tip.



** See page 26.*

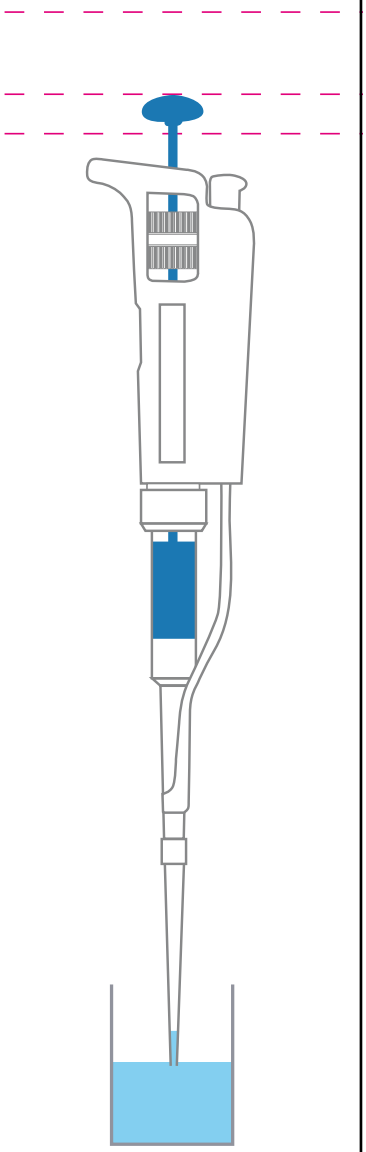
3 Distribution

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.



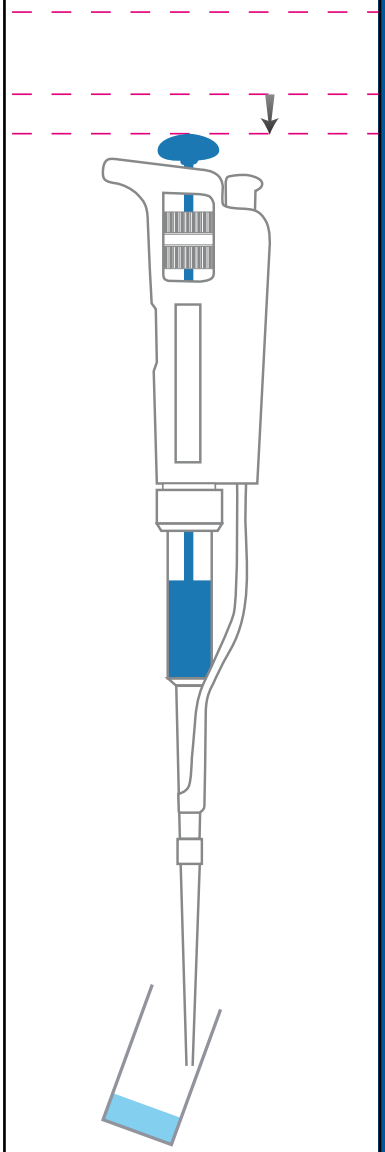
4 Re-aspiration

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 operation 2.



5 Complete purge

Wait one second and 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.



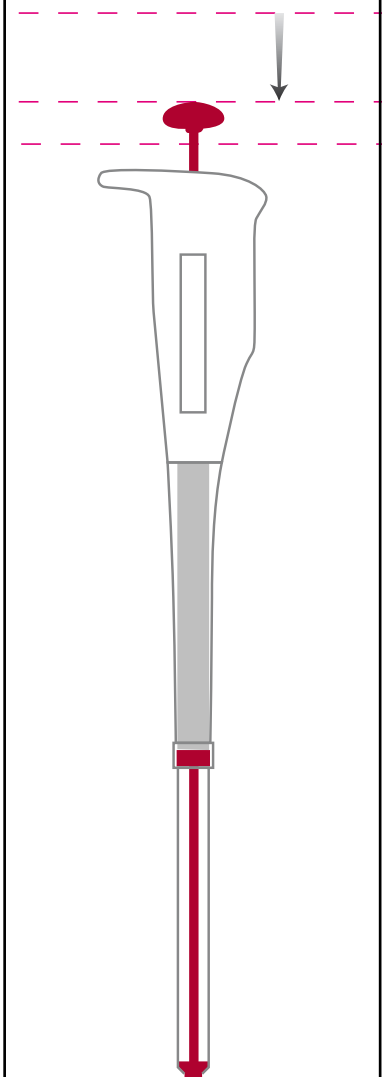
Positive-displacement

In positive displacement pipettes, the piston enters into direct contact with the liquid; there is no air interface.

Direct contact enhances accuracy and precision for liquids which are too heavy or too viscous to be displaced by air. Direct contact allows aspiration of volatile liquids without evaporation. In addition, the absence of air permits rapid pipetting without cavitation.

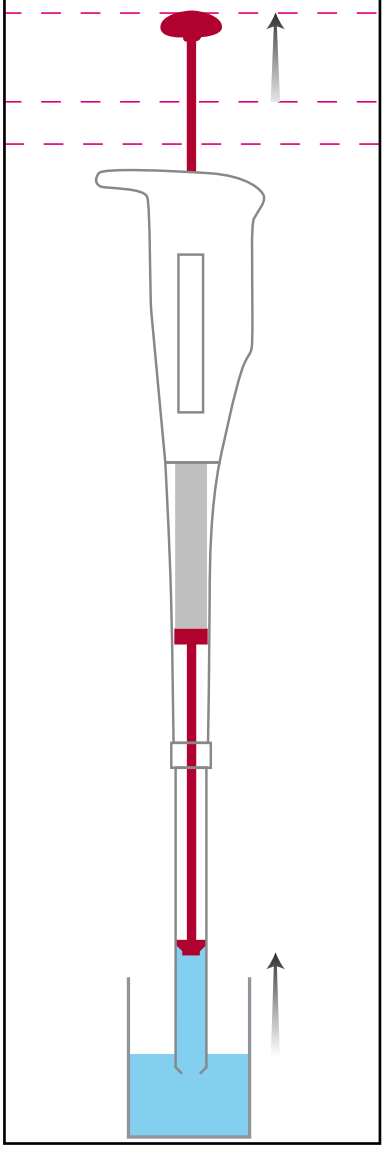
1 Preparation

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



2 Aspiration

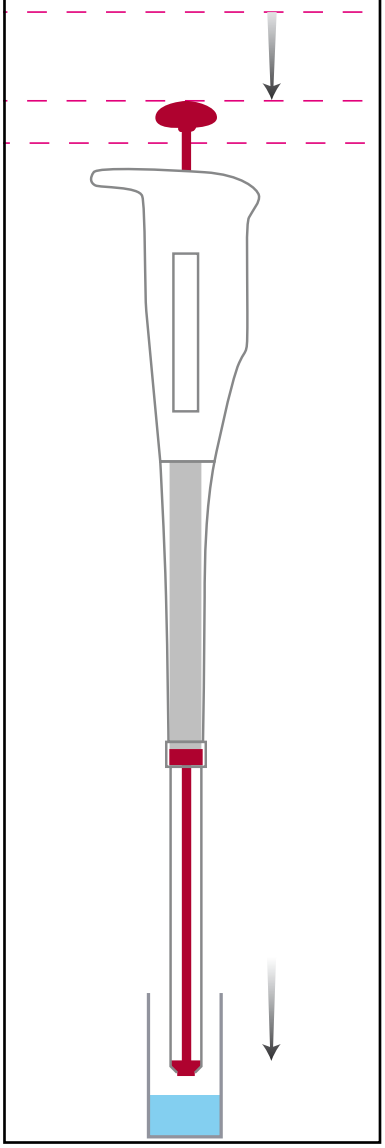
Immerse the capillary/piston in the liquid*. Release the plunger letting it move up to the home position. The piston moves up and the ambient pressure forces the desired volume of liquid through the orifice into the capillary.



* See page 26.

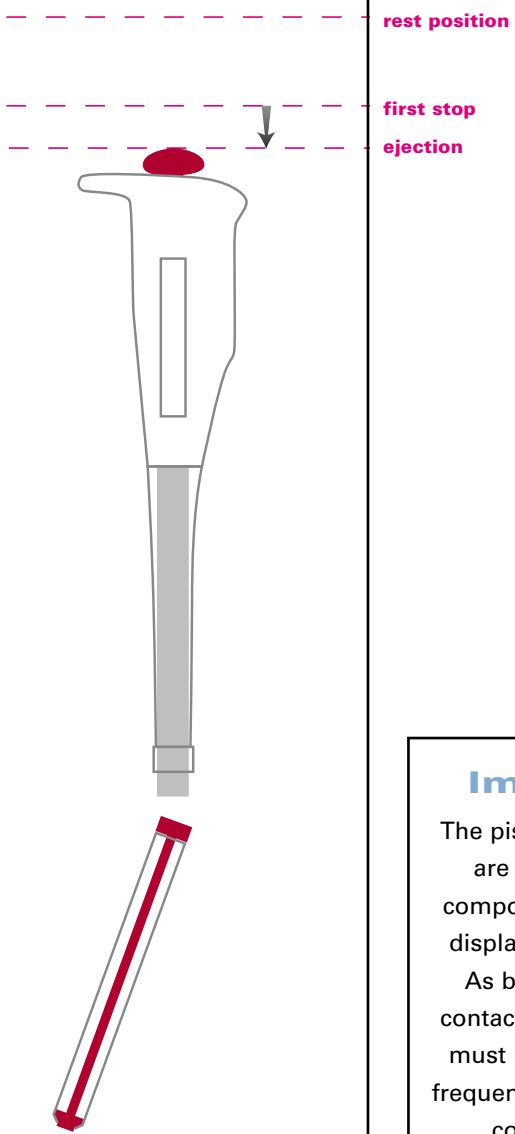
3 Distribution

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



4 Ejection

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

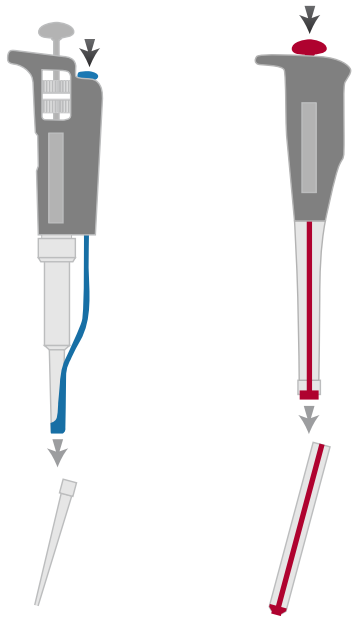


Important

The piston and capillary are the volumetric components of positive displacement pipettes. As both parts are in contact with liquid, they must both be replaced frequently to avoid cross-contamination.

3.5 Eject the used tip and store the pipette in an upright position

To avoid touching contaminated tips, hold the pipette over the trash can and press the tip ejector push-button.



PIPETMAN
no-touch
disposal of tip

MICROMAN
no-touch disposal of
capillary and piston

When to change a tip

Repetitive dispensing of samples.

For repetitive dispensing of the same liquid (diluent, buffer or reagent), use the same pipette tip.
This method is economical and efficient.
It is advisable to pre-rinse the tip at the beginning of the test series.

Transferring single samples of different liquids.

Select a new pipette tip for each new liquid. It is recommended to pre-rinse every new pipette tip.

Important

Discarded tips contain liquid residues, particularly when a pipette is used in the reverse mode. Take suitable precautions when discarding disposables.

Autoclavable bags are available for collecting, autoclaving and/or eliminating biohazardous waste. Special bags and anti-beta containers are also available for solid and liquid radioactive waste.

Always eject used tips. The residual liquid can damage your pipette.

Q

Why do I need a pipette holder?

A

To prevent corrosion, contamination and breakage

Do not leave your pipette lying on the workbench where it can come into contact with chemicals or fall off and break.

Always store your pipette vertically to prevent liquids from running inside the shaft of the pipette.

Preventing contamination

Chapter 4

Avoidance of contamination in the laboratory requires the use of strict precautions. Among these are decontamination of pipettors, wearing gloves and choosing an appropriate pipette tip.

The importance of these precautions is evident when we consider the extreme sensitivity of modern techniques such as PCR, which allows detection of a single molecule. We must also bear in mind the dangers of radioactivity and the risk of personal contamination from a pathogenic micro-organism.

Personal exposure

Prevention

- Wear a lab coat.
- Wear gloves.
- Wear protective glasses.
- Wear a mask.
- Wipe work bench before and after with an appropriate cleaner for your application (cell culture, radio-active components, pathogenic samples...).
- Work under hood.
- Work behind a radioactivity shield.
- Avoid touching used tips.
- Use unbreakable capillaries and pistons.

4.1 Types of contamination and how to prevent them

Pipette-to-sample

Contaminated tips or a contaminated pipette will, in turn, contaminate samples.

Prevention

- **Use sterilized tips and clean or autoclave the parts of your pipette which are in contact with the sample (see pages 36-37).**
- **Change the tip after each sample.**

Sample-to-pipette

Contamination can occur if the sample or aerosols from the sample are allowed to enter the body of the pipette.

Prevention

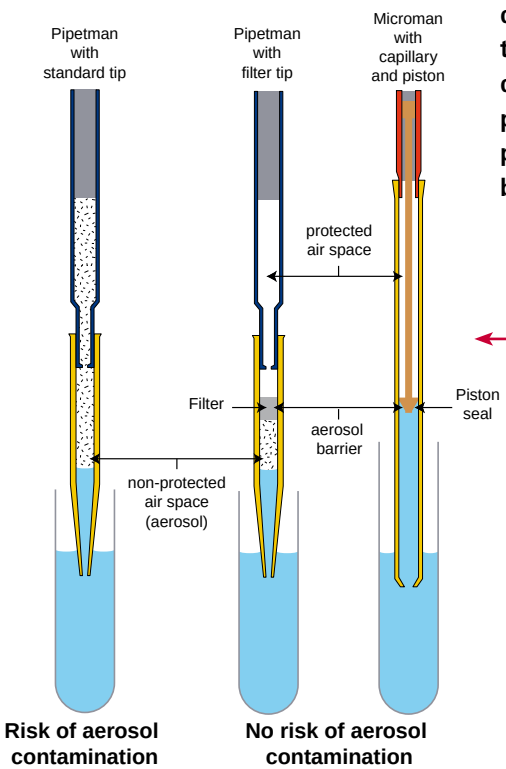
- **To prevent liquids from running into the pipette body, avoid inclining your pipette excessively and always store the instrument vertically.**
- **Release the push-button slowly.**
- **To prevent aerosol contamination, use filter tips with Pipetman or choose a Micropman positive-displacement pipette with built-in aerosol barrier.**

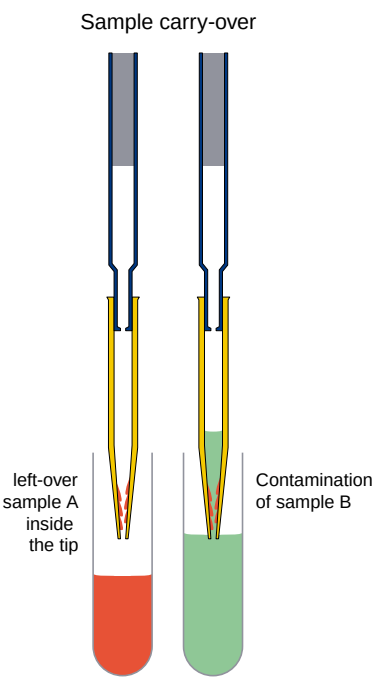
Sample-to-sample (also known as sample carry-over)

A portion of sample A can adhere to the inside wall of the tip after sample delivery. The left-over portion of sample A can mix with the next sample (B) and may cause a false test result.

Prevention

Change the tip after each sample.





4.2
Decontaminating your Pipetman

If your pipette is potentially contaminated, one of the following procedures should be carried out before further use or maintenance.

Autoclaving

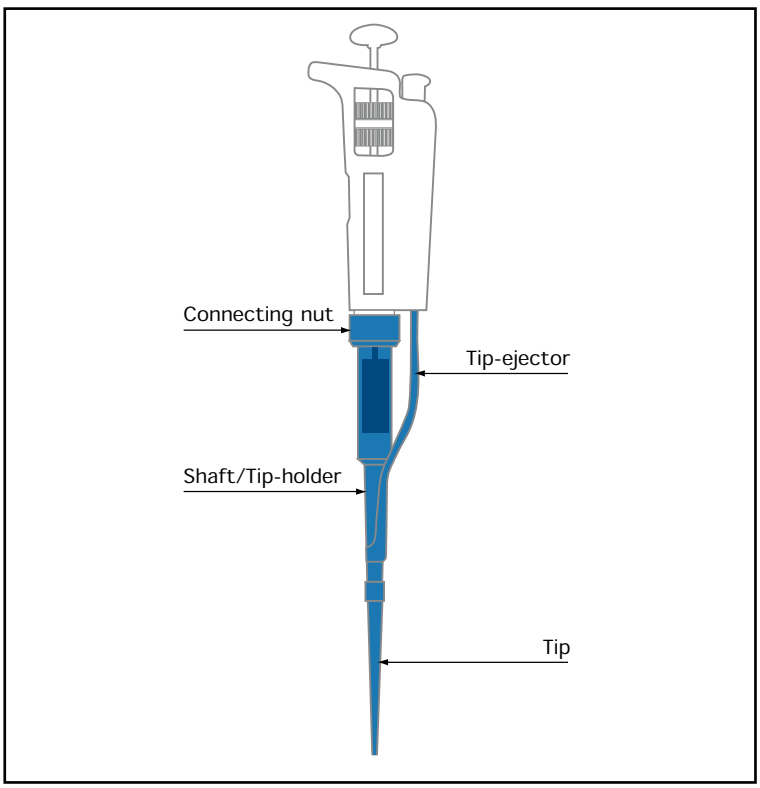
This is the most usual means of sterilization. Gilson Diamond tips and certain parts of Pipetman pipettes may be sterilized in the laboratory under the following conditions : moist heat/121°C/20 minutes/1 bar.

Note : Autoclaving has a limited spectrum of action and will not destroy RNase for example.

Autoclavable parts of Pipetman and Pipetman Ultra

The Pipetman and Pipetman Ultra lower parts (light blue) can be autoclaved.

IMPORTANT: The piston assembly (dark blue) of Pipetman cannot be autoclaved.



Chemical cleaning

This technique is used by Gilson for decontaminating pipettes returned for service. The Pipetman is dismantled and fully immersed in an ultrasonic bath with a detergent recommended for laboratory instruments. Next, the parts are immersed in a virucidal, bactericidal and fungicidal solution. It is strongly recommended to rinse the pipette with water and to dry thoroughly.

Note : A 2% formaldehyde solution is a common choice (CIDEX® from Johnson and Johnson). 10% Clorox® has been recommended as a decontaminant for elimination of DNA templates in the PCR laboratory. However, while it is always possible to find a liquid with the right properties to counter a given risk, no single liquid can counter every risk.*

UV radiation

Work surfaces may be decontaminated by exposure to 300nm UV light for 15 minutes. This method is not recommended for pipettes because, unlike contaminating liquids or vapors, UV rays cannot penetrate inside the pipette.

Beta (β) or gamma (γ) radiation

This method is used by manufacturers for products sold under the label "sterilized". The penetrating rays are highly effective for the relatively inert plastics used to manufacture pipette disposables. Costly and requiring special installations, irradiation is reserved for producers of large quantities of disposables.

Note : The choice of gamma or beta rays is determined by the type of plastic used to manufacture the pipette or the disposable.

Ethylene oxide

Ethylene oxide and its reaction product ethylene chlorohydrin are highly toxic and mutagenic. Therefore it is reserved for the sterilization of plastic materials which may be altered by irradiation.

* A. Prince and L. Andrus, PCR : How to kill unwanted DNA, BioTechniques **1992**, *12*, 359-360.

PCR is a trademark of Hoffman LaRoche

Caring for your pipette

Chapter 5

For long life and optimum performance, pipettes should be returned for complete service once a year.

If you do encounter a minor technical difficulty, chances are very good that the malfunction can be repaired right in your own laboratory.

Warning: use only genuine Gilson replacement parts available exclusively from your authorized Gilson representative.

The Pipetman Two-minute Inspection will help you diagnose faults and decide whether the pipette should be repaired in your lab or returned to your representative for service.

Step 1
Check the records

Step 2
Assess pipetting functions

Step 3
Leak test

Step 4
Disassembly

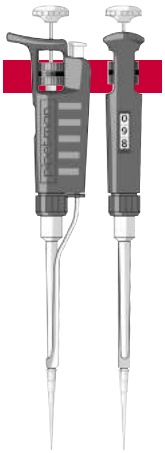
Step 5
Reassembly

Important

Never handle an unknown pipette without wearing gloves. It could be contaminated.

5.1 Quick Diagnosis: The Pipetman Two-minute Inspection

Step 1- Check the records



- **Use the serial number to identify the pipette and to determine its age.**
- **Check laboratory records for the date of last servicing.**

Record Keeping

GLP (Good Laboratory Practice) laboratories must keep detailed records of every step of the analytical procedure. When a pipette is used, the laboratory must be able to produce information such as:

- *Identity of the pipette (identification number)*
- *History of the pipette (dates of servicing, repairs in the lab, calibration checks, operator's name, etc.)*
- *Specifications*
- *Control method*
- *Environmental conditions*

This information may be classified manually, but more and more laboratories are turning to computerized Quality management systems.

Learning more

Q

How old is my pipette?

A

All Gilson pipettes carry a serial number which identifies the pipette and the date of manufacture

After January 2006

A A 50001

Year Month Production Number

Jan. 1984 - Dec. 2005

A 10369 H

Year Production Number Month

Before 1984

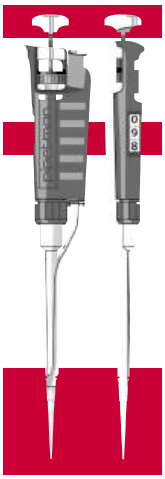
G 80 12345

Month Year Production Number

Letter	Month
A	January
B	February
C	March
D	April
E	May
G	June
H	July
J	August
K	September
L	October
M	November
N	December

Letter	Year	
A	1984	2006
B	1985	2007
C	1986	2008
D	1987	2009
E	1988	2010
G	1989	2011
H	1990	2012
J	1991	2013
K	1992	2014
L	1993	2015
M	1994	2016
N	1995	2017
P	1996	2018
Q	1997	2019
R	1998	2020
S	1999	2021
T	2000	2022
U	2001	2023
W	2002	2024
X	2003	2025
Y	2004	2026
Z	2005	2027

Step 2-
Assess pipetting functions



- **Go through the entire volume range using the push-button adjusting knob.**

(Use the thumbwheel on pipettes dated before May 1995. See Learning More, page 24).

- The thumbwheel should move smoothly.
- The minimum and maximum settings should correspond to the pipette's normal volume range as indicated in the instruction manual (or see page 14).
- Check the alignment and the movement of the volumeter display.

- **Set the volumeter at maximum value and depress the push-button slowly.** The movement should be smooth. "Hitches" in the motion or variations in the friction may be due to a scratch or corrosion on the piston, or to a bent operating rod. Listen for a spring noise which would indicate incorrect positioning of the spring inside the Pipetman.

- **Fit a Gilson tip and depress the tip-ejector** to verify the proper operation of the ejector.

Learning more

Q

How can I prevent piston corrosion?

A

After contact with corrosive liquids, a piston should be cleaned with alcohol and a soft tissue.
Take care to avoid shocks or scratches.

For more information about pistons, see page 8.

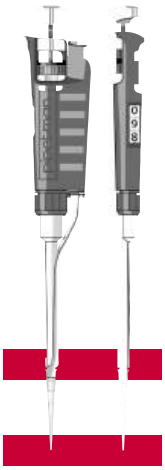
Q

Tips do not fit well. What should I do?

A

- **Always use Gilson tips for Gilson pipettes.**
- **Push upward on the tip-ejector to make sure it is positioned properly.**
- **Clean the tip holder with alcohol. If it is worn or has been chemically attacked, order a new part from your Gilson representative.**

Step 3-
Leak test



For Pipetman pipettes P1000 to P10ml

- Select the maximum volume setting and fill the tip with water.
- Stabilize for 20 seconds.

If a drop appears at the end of the tip, there is a leak.

For Pipetman pipettes P2 to P200

- Select the maximum volume setting and fill the tip.
- Stabilize for 20 seconds.

If a drop appears at the end of the tip, there is a leak.

If no drop appears, re-immerse the tip in the test liquid.

- While immersed, the level of the liquid in the tip should remain constant.

If the level in the tip goes down, there is a leak.

Q

What are the main causes of leaks?

A

- **Tip holder scratched or damaged**
- **Use of non-Gilson tips**
- **Use of non-Gilson seals**
- **Vapor pressure from organic solvents.**

Q

How do organic solvents cause leaks?

A

When an organic solvent is used with an air-displacement pipette, leaks may occur. These leaks are caused by the difference between the vapor pressure of the solvent and pressure of the air cushion between the piston and the sample (see page 9).

Solution

1 *Use a positive-displacement pipette because it has no air cushion. (See page 6.)*
2 *If you are using an air-displacement pipette, saturate the air cushion of your pipette with solvent vapor by aspirating and distributing solvent repeatedly. The leak will stop when pressure equilibrium is reached.*

Step 4-
Disassembly

Important

Pipetman ultra micro pipettes, Models P2 and P10, include miniaturized parts. It is best to avoid dismounting these pipettes in the laboratory.

■ **Disassemble the bottom part of the pipette to confirm your diagnosis.**

■ **Disassembly protocol:**

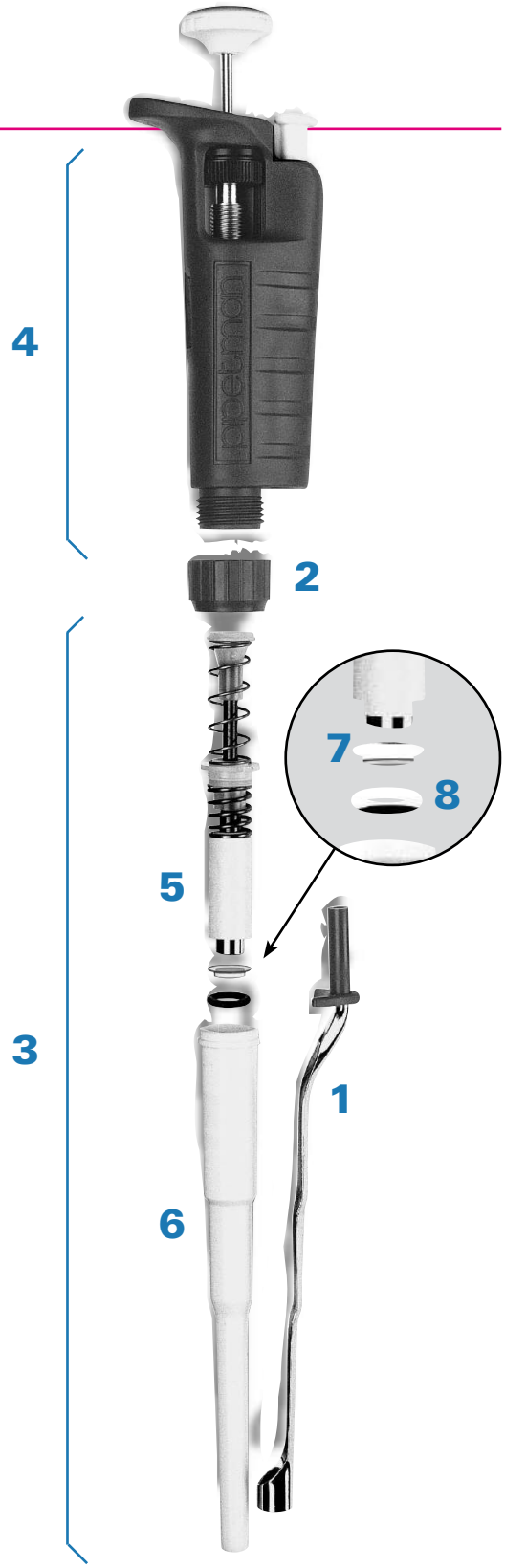
- Eject the tip.
- Pull the tip-ejector **1** down.
- Unscrew the connecting nut **2**.
- Separate the handle **4** from the bottom **3** part of the pipette.
- Remove the piston **5** from the tip holder **6**.
- Check the piston **5** surface, the seal **7** and the O'ring **8**.

Never disassemble the upper part of your pipette

Step 5-
Reassembly

■ **To avoid losing or damaging fragile parts, reassemble the pipette immediately.**

Make sure to respect the correct order of parts: the piston seal **7** should always be positioned before the O'ring **8**.



5.2 Repair in the lab or return for service?

Problem

The pipette is more than one year old and records show that it has not been serviced within the past 12 months

Solution

Perform a Two-minute Inspection (see page 38). If you cannot service the pipette yourself, return it to your Gilson representative for service.

Problem

For models other than P2, U2, P10 and U10, you have identified damage to the push-button, connecting nut, piston seal, O'ring, tip holder or tip ejector

Solution

Spare parts may be ordered from your Gilson representative. These parts may be changed in the laboratory with no effect on the performance of your pipette.

Problem

For all other damage, and for Models P2 and P10

Solution

Return the pipette for service.

Good routine maintenance helps prevent costly repairs.

Control and calibration

Chapter 6

A pipette is to liquids what a laboratory balance is to solids. Like balances and all other precision instruments, pipettes should be periodically inspected, cleaned, maintained and calibrated so that they will perform within the specifications established by the manufacturer.

In chapter five, we learned about inspecting, cleaning, and maintaining pipettes. In this chapter, we will learn about specifications and find out how to determine whether or not your pipette needs to be adjusted.

When Big Ben chimes...

Every day at five 'o'clock I synchronize my watch with Big Ben's chimes.

If my watch sometimes shows 4:59, sometimes 5:01, it is said to be fairly accurate, but not very precise.

If my watch systematically shows 5:02, it is precise, but not very accurate.

If it always shows 5:00, my watch is both accurate and precise.

6.1 What are published specifications?

Specifications are established by the manufacturer. They guarantee, in terms of accuracy and precision, the performance of all pipettes of a given brand and a given model at a certain volume setting.
The table below shows the performance which you could obtain from any Pipetman Model P1000 set at 200, 500 or 1000µl, provided that the pipette has been properly maintained and is used under the same operating conditions as those indicated.

Pipetman P 1000

		Gilson Maximum Permissible Errors		ISO 8655 Maximum Permissible Errors	
Model	Volume (µL)	Systematic error (µL)	Random error (µL)	Systematic error (µL)	Random error (µL)
P1000	200	± 3	≤ 0.6	± 8	≤ 3
	500	± 4	≤ 1	± 8	≤ 3
	1000	± 8	≤ 1.5	± 8	≤ 3

These specifications are defined for pipette used in the forward mode. The gravimetric method is used with the temperature of the distilled water and all other conditions stabilized between 15 and 30 °C. The values given include all components of error due to both normal handwarming and the changing of the tip.

IMPORTANT: To be in acordance with the ISO 8655 standard, the specifications of the pipette must be within the maximimum permissible errors established by the ISO 8655 committee.

Learning more

Q What is accuracy?

A Accuracy is the ability of a measuring instrument to give responses close to a true value.*

Q What is precision?

A Precision is the ability of an instrument to provide closely similar responses (measurements). Precision is also referred to as repeatability and/or reproducibility.**

* AFNOR : NF X 07-001, 1994 (F), P. 41.
** For complete definitions, see glossary P.50.

accurate but not precise | precise but not accurate | accurate and precise

6.2
How to calculate volumetric accuracy and precision

"Accuracy" and "precision" are qualitative terms. The corresponding quantitative terms are "systematic error" and "random error".

• Evaluation of accuracy

The specified accuracy is the limit to the systematic error, which is the difference between the mean volume of actual measurements and the true value of the volume set on the instrument.

The systematic error (E) can be estimated as follows :

$$E = \bar{V} - V_0$$

E systematic error

V_0 nominal volume

$\bar{V}$ mean volume

$$\bar{V} = \frac{1}{n} \sum_{i=1}^{n} V_i$$

V_i individually measured volume

n number of measurements

The accuracy of a pipette can be expressed as a percentage of the nominal volume:

$$E\% = \frac{\bar{V} - V_0}{V_0} \times 100$$

Important

The mean value and number of replicates must be stated, and the experimental procedure used must be described in such a way that other workers can repeat it. See example of specifications section 6.1.

• Evaluation of precision

The specified precision is the limit to the random error, which is the distribution of the measured values around a mean value. For pipettes, precision refers to a within-series group of data, and therefore to repeatability.

The random error is then quantified by the standard deviation of measurements performed at a given volume setting under the same measuring conditions.

The standard deviation (SD or "s") can be estimated as follows :

$$SD = \sqrt{\sum_{i=1}^{n} \frac{(\bar{V} - V_i)^2}{n - 1}}$$

$\bar{V}$ mean volume

$$\bar{V} = \frac{1}{n} \sum_{i=1}^{n} V_i$$

V_i individually measured volume

n number of measurements (minimum 10)

The precision of a pipette can also be expressed as a percentage of the mean volume. This is known as relative standard deviation (RSD) or coefficient of variation (CV), and is estimated as follows :

$$RSD = \frac{SD}{\bar{V}} \times 100$$

6.3 Using the gravimetric method

The gravimetric method is recommended by pipette manufacturers and international standard organizations (ISO 8655). It is based on the determination of the weight of water samples delivered by the pipette.
Implementation of this method requires the strict monitoring of environmental conditions and the systematic use of adequate and controlled equipment.

General considerations

Gilson pipettes are designed to compensate for the effects of normal handwarming during the test series. However, the instrument being evaluated must not be over-warmed by extensive handling.

21.5 °C ±1.5 — The **temperature** should be 21.5 ± 1.5°C (293-296 K) — **70.7 °F** ±2.7

1013 hPa ±25 — **Average barometric pressure** in the test lab should be 1013 ± 25hPa (mbar) — **14.5 psi** ±0.36

50-75% — **Relative humidity** should be maintained at 50 - 75% in order to reduce the evaporation rate and to control the build-up of electrostatic charges. — **50-75%**

Note : *If the pipettes are used and therefore checked outside these conditions, the weight of the setting volume of water aspirated will have to be corrected according to the conversion table (µl/mg). See appendix II.*

Required equipment

• *Calibrated thermometer with a standard uncertainty of ⩽ 0.2* °C
A calibrated thermometer readable to 0.1°C to measure both ambient and water temperatures at the beginning and at the end of the test series.

• *Hygrometer with a standard uncertainty of ⩽ 10 %*
A calibrated hygrometer to check the constant of humidity in the air during the test.

• *Barometer with a standard uncertainty of 0.5 KPa*
A calibrated barometer to check the atmospheric pressure.

• *Distilled water*
Use distilled or deionized water conforming grade 3 as specified in ISO 3696, degassed or air-equilibrated. The water shall be at room temperature;

• *Balances*
Laboratory balances required for the test should meet or exceed the following performances:

Selected volume (V) of apparatus under test	Balance Resolution mg	Repeatability and linearity mg	Standard uncertainty of measurement mg
1 µl ⩽ V ⩽ 10 µl	0.001	0.002	0.002
10 µl < V ⩽ 100 µl	0.01	0.02	0.02
100 µl < V ⩽ 1000 µl	0.1	0.2	0.2
1 ml < V ⩽ 10 ml	0.1	0.2	0.2

• *Vessels*
Test equipment should correspond to the following indications:

Instruments	Volumes	Sample Reservoir	Weighing Vessel	Balance Resolution	Other equip.
U2 - U20 P2 - P20 PUM x20 F2 - F10 M10 to M25 M100	0.1 to 20 µl	Ø 35 mm H 50 mm	Ø 10.5 mm H 13 mm	0.001 mg	Lid Tweezers Filters
U100 - U200 P100 - P200 F25 - F200 PUM x300 M50 - M250	> 20 to 200 µl	Ø 35 mm H 50 mm	Ø 21 mm H 50 mm	0.01 mg	Lid
U1000 - U5000 P1000 - P5000 F250 - F5000 M1000	> 200 to 5000 µl	Ø 50 mm H 70 mm	Ø 35 mm H 50 mm	0.1 mg	Lid
U10 ml P10ml	> 5 to 10 ml	250 ml beaker	Ø 40 mm H 100 mm	0.1 mg	Lid

Some remarks about balances

With modern analytical balances, a laboratory needs only two balances to check an entire stock of pipettes ranging from 0.1µl to 10ml. A good combination would be one six-digit balance and another one that works on two scales, for example 50g with sensitivity 0.01mg and 200g with 0.1mg sensitivity.

The test balances should be calibrated, maintained and recognized by the national department of weighing and measurements.

To minimize vibration, the balances should be on a marble table or the equivalent. Keep the balance area free of draughts and the ambient area free of dust.

From weight to volume

Conversion to volume must take into account the density of the liquid as well as evaporation during the cycle time. For each measurement, the corresponding volume (Vi) can be calculated as follows :

$$V_i = (W_i + \bar{e}) Z$$

W_i is the weight as read on the balance

$\bar{e}$ is the mean evaporation loss during the cycle time

Z expressed in l/mg, is a conversion factor incorporating density of water buoyed in air, at test temperature and barometric pressure

NB : *For measurements higher than 20 µl, the evaporation factor can be disregarded.*

For your reference, a complete example is given in Appendix I.

Estimation of the Z factor (conversion factor)

The Z factor is not just equal to the density of water adjusted to the local temperature and pressure parameters. It must also take into account the air density and the density of weights used to calibrate the balance.

For very low volumes, application of the Z factor may not affect the final result.

The detailed formula as well as the table indicating the factor Z to take into account are given in Appendix II.

Estimation of the e factor (evaporation loss)

Evaporation that occurs during the gravimetric test depends mainly on temperature, humidity and cycle time of work. It may have a noticeable effect on small volume measurements (particularly for Pipetman P models P2, P10 and P20, Pipetman F models F2, F5, F10 and F20 and Microman models M10 and M25). Evaporation loss is estimated by running a series of four simulated weighing cycles and calculating the mean weight loss per weighing cycle in mg. Perform each weighing cycle without adding the aspirated liquid to the vessel. Dispense the liquid into a dummy vessel. The mean evaporation e, is calculated as follows :

$$\bar{e} = \frac{1}{4} (e_1 + e_2 + e_3 + e_4)$$

A procedure for the determination of e is given in Appendix III.

6.4 Performance check procedure

1. Place distilled or deionized water from the container in the weighing vessel to a depth of at least 3 mm.
2. Record the test conditions (ambient and water temperature, relative humidity, barometric pressure).
3. Select the test volume of your variable-volume piston pipette.
4. Fit the tip or capillary/piston assembly to the pipette (the manufacturer's specifications are valid only when test is performed with the manufacturer's tips).
5. Pre-wet pipette tip five times to reach humidity equilibrium in the dead volume of the pipette, but do not take into account for calculations.
6. Change tip.
7. Pre-wet the tip once.
8. Pipette the test volume.
9. Determine tare mass (reset balance).
10. Open balance door, retrieve weighing container, deliver sample, replace on the balance and close the door.
11. After allowing display to stabilize, record the weight.
12. Repeat the test cycle until ten measurements have been recorded as a series of weights W1 to W10.
13. For sample below or equal to 50 µl, estimate evaporation loss by repeating steps 8 to 10 exactly as a normal sample weighing but without actually adding any sample to the weighing container. Record absolute value (ei) and repeat several (m) times.
14. Record test conditions.Check that values are still within recommended limits.
15. Use the average of the first and the second values of temperature and barometric pressure to determine the correction needed (Z). See appendix II.
16. Calculate the accuracy and the precision and compare with manufacturer's specifications. (To calculate accuracy and precision, see page 46.)

6.5 When to perform the test

Since accuracy and precision have a direct influence on the quality of analytical results, it is imperative that the performance of individual pipettes be compared regularly with manufacturer's specifications.
Gravimetric analysis is a practical, widely used method for testing the performance (accuracy and precision) of a pipette.

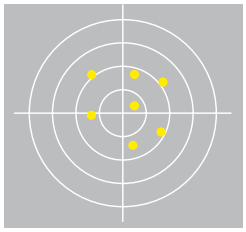
Frequency*	Control	Action	Who
Daily	Preventive maintenance	Leak test (See the "Two-minute inspection")	End-users
Weekly	Preventive maintenance	Accuracy check	End-users
Every three months	Preventive maintenance	Cleaning & Diagnosis: - Visual inspection - Check functions	End-users
Annually	Complete maintenance	Replacement spare parts of first level (seals/ O-rings, tip holder...) Adjustment - Calibration	Service Center & End-users
		Replacement spare parts of second level (Piston, volumeter, operating rod) Adjustment - Calibration	Gilson Authorized Service Center

* Frequency should be adapted to the type of sample, the number of the pipetting tasks and the environmental conditions of the laboratory.

Important

Always check your pipette for mechanical faults (see Two-minute Inspection, page 38) before performing a gravimetric test.

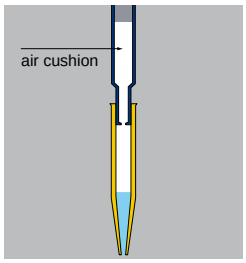
Accuracy*



Accuracy is the ability of a measuring instrument to give responses close to a true value.

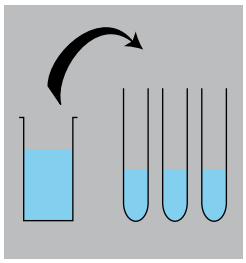
Note: "accuracy" is a qualitative concept.

Air cushion



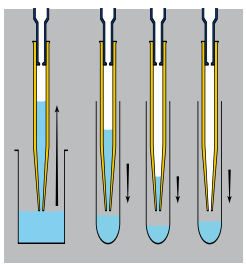
Also called "dead volume", the air cushion is the volume of air located between the lower part of the pipette piston and the surface level of the sample.

Aliquot



Measured portion of a homogeneous entity. A general term referring to multiple samples of any solution, mixture, etc.

Dispenser



An instrument for de-livering predetermined volumes of liquid from a reservoir. The reservoir may be integrated into the instrument or connected externally.

Error (of measurement)*

The result of a measurement minus a true value of the measurand.

Commentary : this difference or deviation (positive or negative) may be expressed either in the units in which the quantity is measured (absolute error), or as a percentage of the true value (relative error).

Error (random)*

The result of a measurement minus the mean that would result from an infinite number of measurements of the same measurand carried out under repeatability conditions.

Notes :

1 Random error is equal to error minus systematic error,

2 Because only a finite number of measurements can be made, it is possible to determine only an estimate of random error.

Error (systematic)*

The mean that would result from an infinite number of measurements of the same measurand carried out under repeatability conditions minus a true value of the measurand.

Notes :

1 Systematic error is equal to error minus random error,

2 Like true value, systematic error and its causes cannot be completely known.

Commentary : systematic error quantifies the error of accuracy of a pipette.

Good Laboratory Practice

Good Laboratory Pratice (GLP) is concerned with the organizational process and the conditions under which laboratory studies are planned, performed, monitored, recorded and reported.

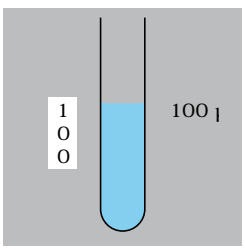
Measurand*

Particular quantity subject to measurement.

Example : vapor pressure of a given sample of water at 20°C.

Note : the specification of a measurand may require statements about quantities such as time, temperature and pressure.

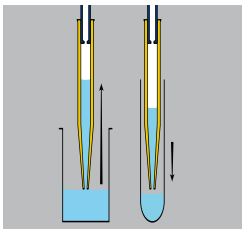
Nominal volume*



The rounded or approximate value of a characteristic of a measuring instrument that provides a guide to its use.

Examples : **a)** *1 L as the value marked on a single-mark volumetric flask,* **b)** *100 µl as the setting appearing on the volumeter of a pipette.*

Pipette/Pipetter



An instrument for transferring a predetermined volume of liquid from one vessel to another. A pipetter is not connected to a reservoir.

Precision

Precision is a qualitative term. It is the ability of the instrument to provide closely similar responses (measurements). Precision is often referred to as repeatability and/or reproducibility.

Repeatability* (of results of measurements)

Repeatability is the closeness of agreement between the results of successive measurements of the same measurand carried out *under the same conditions* of measurement. Repeatability conditions include:

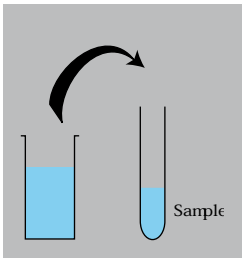
- the same measurement procedure,
- the same operator/observer,
- the same measuring instrument, used under the same conditions,
- the same location,
- repetition over a short period of time.

Commentary : For pipetting, it is necessary to minimize variations due to the operator (example: cycle time) to a minimum.

Reproducibility* (of results of measurements)

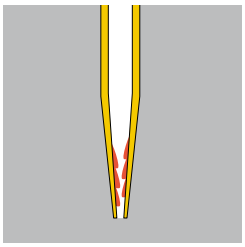
Reproducibility is the closeness of the agreement between the results of measurements of the same measurand carried out *under changed conditions* of measurement (ex: different operator).

Sample



The appropriate representative part of a liquid wich is to be analyzed. The term "test sample" is used when necessary to avoid confusion with the statistical term "random sample from population".

Sample carry-over



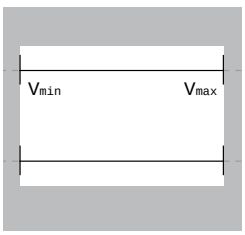
The portion of the sample that is retained in the instrument after sample delivery and that may affect subsequent samples.
Note : Carry-over from a positive-displacement pipette is less than from air-displacement pipette.

True value

True value is a value that would be obtained by a perfect measurement.

Working range

Total volume and temperature range, as well as ambient conditions, for which instrument performance is specified.



Note: Do not select volumes outside recommended limits.

**Definitions abstracted from AFNOR: NF X 07-001, 1994 (F), pp. 20, 24, 26-28, 38.*

APPENDIX I - Example of a performance check

Below is an example of how to evaluate the performance of Pipetman P10 at 1 µl.

1. Determine the mean value $\bar{e}$ of the evaporation loss e_i that occurs during your pipetting cycles. Proceed as described in appendix III to determine e_i

$$\bar{e} = \frac{1}{m} \sum_{i=1}^{m} e_i$$

m : number of weighings

e_1 = 0.016 mg e_3 = 0.021 mg
e_2 = 0.018 mg e_4 = 0.017 mg

$\bar{e}$ = $(e_1+e_2+e_3+e_4) / 4$

$\bar{e}$ = (0.016+0.018+0.021+0.017) / 4

$\bar{e}$ = 0.018 mg/per cycle

2. Change the pipette tip and perform the first weighing. Then, keep a regular cycle and perform the 10 following measurements.

W_r = 0.957 mg
W_1 = 0.968 mg W_6 = 0.966 mg
W_2 = 0.960 mg W_7 = 0.955 mg
W_3 = 0.984 mg W_8 = 0.972 mg
W_4 = 0.942 mg W_9 = 0.958 mg
W_5 = 0.969 mg W_{10} = 0.967 mg

W_r rinsing measurement which is disregarded for the calculation

3. Calculate the mean weight

$$\bar{W} = \frac{1}{n} \sum_{i=1}^{n} W_i$$

n number of weighings

W_i weighing results

$\bar{W}$ = (0.968+0.960+0.984+0.942 + 0.969+0.966+0.955+0.972 + 0.958+0.967) / 10

$\bar{W}$ = 0.964 mg

4. Calculate the mean volume

$$\bar{V} = (\bar{W}+\bar{e}) \times Z$$

For a temperature of 21.5°C and an air pressure of 1013 hPa, the Z factor is equal to 1.0032 µl/mg (see table in Appendix II).

$\bar{V}$ = (0.964+0.018) x 1.0032

$\bar{V}$ = 0.985 µl

5. Evaluate accuracy

Systematic error (**E**) :

$$E = \bar{V} - V_0$$

V_0 true value set on the instrument

E = 0.985 - 1 = **- 0.015 µl**

Relative error (**E%**) :

$$E\% = (\bar{V} - V_0) \times 100 / V_0$$

E% = (-0.015 x 100) / 1 = **- 1.50 %**

6. Evaluate precision (repeatability)

Standard Deviation (SD_W)

$$SD_W = \sqrt{\sum_{i=1}^{n} \frac{(W_i - \bar{W})^2}{n - 1}}$$

$$SD_W^2 = \frac{1}{n - 1} \sum_{i=1}^{n} (W_i - \bar{W})^2$$

$$SD_W^2 = \frac{1}{9} \left[\begin{array}{l} (0.968-0.964)^2 + (0.960-0.964)^2 + (0.984-0.964)^2 + \\ (0.942-0.964)^2 + (0.969-0.964)^2 + (0.966-0.964)^2 + \\ (0.955-0.964)^2 + (0.972-0.964)^2 + (0.958-0.964)^2 + \\ (0.967-0.964)^2 \end{array} \right]$$

SD_W = 0.011 mg

Random error (**SD_V**) :

SD_V = SD_W x Z

SD_V = 0.011 x 1.0032 = **0.011 µl**

APPENDIX II - Z Factor

The reference calculation equation is : $Z = [1/(P_W-P_A)]\ [1-(P_A/P_B)]$

Where : P_A = density of air at t°C.
P_W = density of the test liquid at t°C.
P_B = density of the balance weights. Use 8 g/cc for P_B

Note : Weights conforming to International Recommendation No. 33 of OIML have been adjusted to give results when weighing in air as if the density of the weights were 8.0 g/ml.

Values of the conversion factor Z (µl/mg) as a function of temperature and pressure for distilled water

Temperature °C	Air pressure hPa					
	800	**853**	**907**	**960**	**1013**	**1067**
15	1.0018	1.0018	1.0019	1.0019	1.0020	1.0020
15.5	1.0018	1.0019	1.0019	1.0020	1.0020	1.0021
16	1.0019	1.0020	1.0020	1.0021	1.0021	1.0022
16.5	1.0020	1.0020	1.0021	1.0022	1.0022	1.0023
17	1.0021	1.0021	1.0022	1.0022	1.0023	1.0023
17.5	1.0022	1.0022	1.0023	1.0023	1.0024	1.0024
18	1.0022	1.0023	1.0024	1.0024	1.0025	1.0025
18.5	1.0023	1.0024	1.0025	1.0025	1.0026	1.0026
19	1.0024	1.0025	1.0025	1.0026	1.0027	1.0027
19.5	1.0025	1.0026	1.0026	1.0027	1.0028	1.0028
20	1.0026	1.0027	1.0027	1.0028	1.0029	1.0029
20.5	1.0027	1.0028	1.0028	1.0029	1.0030	1.0030
21	1.0028	1.0029	1.0030	1.0030	1.0031	1.0031
21.5	1.0030	1.0030	1.0031	1.0031	1.0032	1.0032
22	1.0031	1.0031	1.0032	1.0032	1.0033	1.0033
22.5	1.0032	1.0032	1.0033	1.0033	1.0034	1.0035
23	1.0033	1.0033	1.0034	1.0035	1.0035	1.0036
23.5	1.0034	1.0035	1.0035	1.0036	1.0036	1.0037
24	1.0035	1.0036	1.0036	1.0037	1.0038	1.0038
24.5	1.0037	1.0037	1.0038	1.0038	1.0039	1.0039
25	1.0038	1.0038	1.0039	1.0039	1.0040	1.0041
25.5	1.0039	1.0040	1.0040	1.0041	1.0041	1.0042
26	1.0040	1.0041	1.0042	1.0042	1.0043	1.0043
26.5	1.0042	1.0042	1.0043	1.0043	1.0044	1.0045
27	1.0043	1.0044	1.0044	1.0045	1.0045	1.0046
27.5	1.0044	1.0045	1.0046	1.0046	1.0047	1.0047
28	1.0046	1.0046	1.0047	1.0048	1.0048	1.0049
28.5	1.0047	1.0048	1.0048	1.0049	1.0050	1.0050
29	1.0049	1.0049	1.0050	1.0050	1.0051	1.0052
29.5	1.0050	1.0051	1.0051	1.0052	1.0052	1.0053
30	1.0052	1.0052	1.0053	1.0053	1.0054	1.0055

APPENDIX III - Evaporation loss

Procedure for the determination of evaporation loss

Use the same distilled water, weighing vessel and balance as you will be using for the gravimetric check.

1. Half fill the weighing vessel with distilled water.
2. Cover the weighing vessel with its lid and place it on the balance using a pair of tweezers.
3. Aspirate a sample.
4. Tare the balance and take the weighing vessel out of the balance.
5. Take off the lid with tweezers.
6. Dispense the sample into a dummy vessel.
7. Replace the lid on the weighing vessel and, using tweezers, replace the vessel on the balance.
8. Read the negative result e_1 (record the absolute value).
9. Repeat steps 3 to 8, three times to obtain e_2, e_3, and e_4.
10. Calculate the evaporation loss $\bar{e}$ using the formula :

$$\bar{e} = \frac{1}{4}\,(e_1 + e_2 + e_3 + e_4)$$

In normal conditions, this value is usually between 0.01 mg and 0.03 mg.

NOTES

NOTES

Order Online at:

www.pipetman.com

Or Contact Gilson at:

1-800-GILSON1

Gilson, Inc. World Headquarters
P.O. Box 628098
Middleton, WI 53562-8098 USA
Telephone: 608-836-1551 or 800-445-7661
Fax: 608-831-4451

GILSON

$20 US

ISO9001 Certified