Pipetting Handbook

RAININ
Pipetting 360°

Pipettes
Tips

Evaluation
Selection
Techniques

Get Better Results

Your Quick Guide to Good Pipetting

METTLER TOLEDO

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1. Foreword

Good Pipetting Practice is intended to help researchers make informed choices on equipment, proper pipetting and ergonomic techniques, calibration and routine operation, to get the best results possible.

Pipetting – or measuring and transferring – small volumes of liquid in the microliter and milliliter range is probably the most frequently practiced activity in research laboratories, and to be able to carry out this task quickly and precisely is an absolute prerequisite for successful laboratory work. Modern air-displacement pipettes are used for the greater part of lab work because of their numerous advantages – they are the ideal instrument for effectively dosing small quantities of liquid. A high level of productivity, with corresponding savings in man-hours, is possible through using modern high-quality pipettes and tips.

2. Project Planning, Workflow and Selection

Project Planning and Workflow

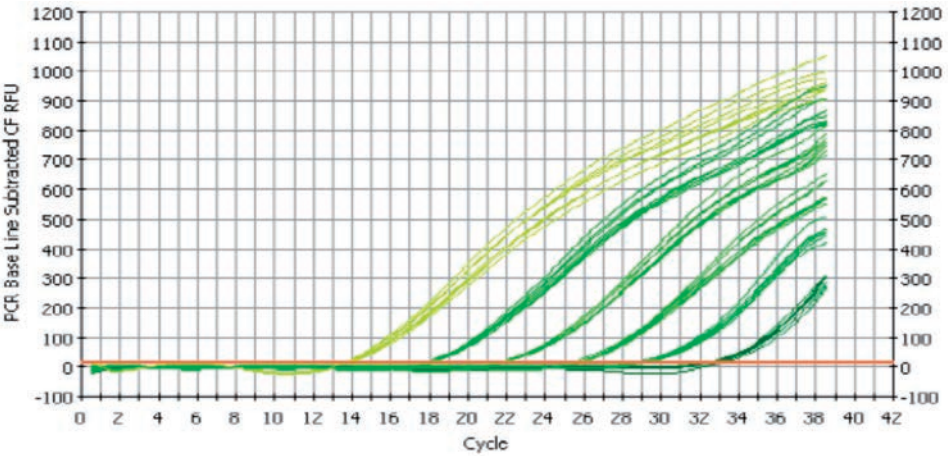
Most new projects will benefit from a full planning process where all the steps within the workflow are analyzed for maximum efficiency and data generation. From the liquid-handling perspective, this requires an understanding of the starting sample type, the end point analysis required, and sample throughput. These in turn will direct the techniques used, and the liquid-handling formats required (tubes, plates, etc.). This subsequently determines the optimal liquid-handling tools for the workflow. For any pipetting activity or action, the pipette, the associated tip and the operator technique must be considered as one system in order to deliver the accurate volume of liquid required. Choosing the correct pipette and tip and then using the most effective technique is an integral part of designing and implementing any project or experiment.

Analyzing the Workflow

The first step in the process is to identify all of the steps involved in an experimental workflow – from initial sample isolation to final data production, this will also include all of the preparation steps to support the workflow e.g. buffer or mastermix preparations. Next, identify how much variability is tolerable for the experiment to be able to produce good data. Some applications and some steps are much more sensitive to experimental variability than others, for example, any experiment involving amplification, such as qPCR, can be very sensitive to variability while a simple buffer preparation step may not. Suboptimal choice of pipette and tip as well as poor pipetting technique can be a major source of experimental variability e.g. any experiments dependent on a standard curve generated through serial dilution of standards can be severely affected by sub-optimal pipetting.

Analyzing the workflow

- Identify maximum tolerance for experimental variability
- Identify applications and steps most likely to introduce variability
  - qPCR
  - Serial dilutions



Optimizing the Workflow

Volume range and sample throughput requirements

Often a workflow will involve starting with a few liquids at relatively large volumes (e.g., preparing buffers, plating cells, etc.) where transferring 5 or 10mL with less emphasis on accuracy may be common. However, the final detection technique may use only small volumes and there may be an increased need for more accurate volume delivery.

The needs for speed and accuracy/precision must be balanced since different large volume tools have different capabilities. The recommended guideline for choosing the correct volume pipette is to estimate the working range as between 35 and 100% of the total volume indicated. For example a 1,000µL pipette has an effective working range between 350 and 1,000µL. Even though the minimum specifications may be 100µL on this volume pipette and the instrument is adjustable down to 0µL, the recommendation for using 350µL as the minimum is based upon user technique. More precise pipetting technique is required for volumes below the 35% range on pipettes. Working at the in-appropriate range of any instrument will compromise accuracy/precision.

Pipette Type	Model	Min. Capacity	Max. Capacity	Accuracy 10%	Precision 10%	Accuracy 50%	Precision 50%	Accuracy 100%	Precision 100%
Positive displacement pipette	MR-10	0.5 µL	10 µL	9%	3%	2%	0.60%	1.50%	0.60%
	MR-250	50 µL	250 µL	3%	0.60%	1.70%	0.30%	1%	0.20%
	MR-1000	100 µL	1000 µL	3%	1.60%	1%	0.50%	0.80%	0.40%
Air displacement pipette	L-10XLS	0.5 µL	10 µL	2.50%	1.20%	1.50%	0.60%	1%	0.40%
	L-200XLS	20 µL	200 µL	2.50%	1%	0.80%	0.25%	0.80%	0.15%
	L-1000XLS	100 µL	1000 µL	3%	0.60%	0.80%	0.20%	0.80%	0.15%

Accuracy and Precision

Accuracy is the ability of a pipette to provide a dispensed amount of liquid as close to the volume, as indicated by the volume setting. Typical accuracy specifications for air-displacement pipettes are approximately 1% for pipettes with nominal volume settings greater than 35%. For pipette volume settings at 10% or below, the specifications can be up to 3 times less accurate.

Precision measures the ability of the pipette to provide reproducibly similar dispenses of a liquid. A typical precision specification for air-displacement pipettes is about 1/3 to 1/4 of the accuracy specification. Precision is often referred to as repeatability or sample reproducibility, and also as standard deviation.

Certain types of pipettes are better suited than others for different sample types. For instance, viscous samples require a different technique or pipette model in order to be able to achieve good precision and accuracy in one's experiments. The following table provides some more information:

Type of sample solution		Volume range	Suggested solution	
			Manual systems	Manual systems
Viscous, organic solvent, extreme	High volume samples	20-50mL	AutoRep S	AutoRep E
Non-viscous, aqueous, ambient		20-50mL	AutoRep S	Pipet-X
Viscous, organic solvent, extreme		1-20mL	AutoRep S	AutoRep E
Non-viscous, aqueous, ambient		1-20mL	Pipet-Lite XLS, AutoRep S	Pipet-X, E4 XLS
Viscous, organic solvent, extreme	Medium volume samples	200-1000µL	Pos-D, AutoRep S	AutoRep E
Non-viscous, aqueous, ambient		200-1000µL	Pipet-Lite XLS	E4 XLS
Viscous, organic solvent, extreme	Low volume samples	10-200µL	Pos-D	
Non-viscous, aqueous, ambient		10-200µL	Pipet-Lite XLS	E4 XLS
Viscous, organic solvent, extreme	Micro volume samples	<10µL	Pos-D	
Non-viscous, aqueous, ambient		<10µL	Pipet-Lite XLS	E4 XLS

If the number of samples to be analyzed is high enough, it may make sense to switch from tube to plate format for sample preparation and/or analysis in which case using multichannel pipettes will speed up the workflow.

If multiple 96- or 384-well plates are being analyzed, it may make sense to use a 96-channel pipetting solution which will save time and reduce the chance of errors.

Sample/reagent container format requirements

Using 96-well plates may require moving multiple samples or reagents from tubes to plates or vice-versa and sometimes transfers are required between different plate formats (24-well to 96-well). Adjustable spacer multichannel pipettes can cut format change time by up to 85% because you can simultaneously move up to 8 samples at a time e.g. moving target sample from a non-formatted set of tubes into a formatted (9mm centers) 96-well plate requires only a simple aspiration of sample from the unformatted plate.

Adjustable spacer multichannel pipettes

Help move multiple samples simultaneously

- Between tubes to plates (and vice-versa)
- Between different plates (24/48/96-wells)

Sample/assay specific requirements

Complex or repeated pipetting can benefit from electronic pipettes, since they can be used for repeat dispensing and can be programmed for specific pipetting protocols. Furthermore, electronic pipettes produce more consistent data than manual pipettes, because the micro-processor eliminates human error and variability in moving the piston. This is especially noticeable with data requiring serial dilutions, where pipetting errors can be compounded, and with applications requiring amplification, such as qPCR.

Every assay and sample has unique properties that can pose challenges. For example, genomics applications should always use filter tips to minimize effects of DNA contamination of the sample or the pipette. Filters block aerosols from the sample contaminating the shaft, and subsequently contaminating later samples. Filters also help protect against microbial contamination, corrosives and salt deposits.

Electronic pipettes can benefit:

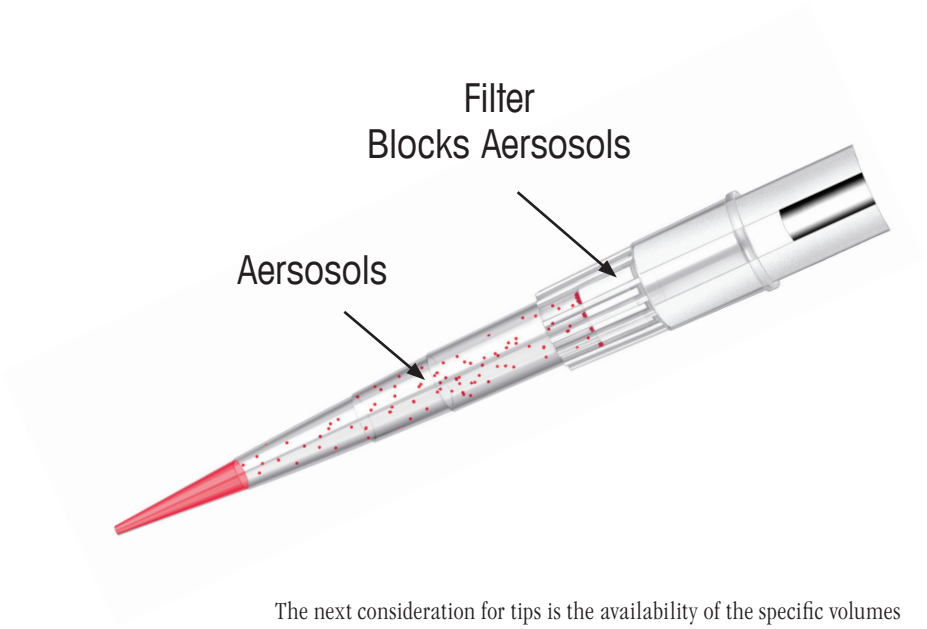
- Complex or repetitive protocols
- Applications requiring high levels of accuracy and precision (e.g. qPCR)

With the volume ranges and types of pipette(s) chosen, the next step in the process is to identify the correct tips for all of the applications that will be involved.

Tips are generally manufactured from polypropylene and although inert and capable of withstanding many solvents, hidden factors within the polypropylene should be considered. Different polypropylene resins provide key features to the manufacturer and many additives can be included in the manufacturing process that alter some of the manufacturing processes. Some of these additives may leach from the finished product and subsequently affect the experimental results. To overcome this potential issue, it is recommended to purchase tips that are qualified to be free of various leachables, making sure that the supplier provides documented evidence of the testing sensitivity for all leachables and contaminants. Examples of contaminants include DNA and Pyrogens which will be present if the manufacturing and packaging process does not take place in a clean room environment. There are many consumable molding and packaging organizations that produce products in facilities that are not fully controlled. A good production facility will ensure all workers are fully gowned and wear hats, masks and gloves and that the work environment has only filtered air in order to prevent any contamination by hair or insects. Again, certificates that support the testing of these materials should state the testing process and the sensitivity of the assay–certificates that just claim a product to be free of a specific contaminant without specifying the detection method or the sensitivity of detection do not provide any information or assurance of quality.

Unique application

- Use filter tips to minimize effects of DNA contamination or carry over
- Filter tips also protect the piston from microbial contamination, corrosives and salt deposits



The next consideration for tips is the availability of the specific volumes required for the various chosen pipettes, followed by the specialty tips that may be required for the application e.g. filtered tips for genomic applications. Finally, the packaging size will provide convenience in terms of providing sufficient quantities of tips for a given time period.

For the range of different volumes to be pipetted, a carefully planned number of manual pipettes may be needed in order to keep within the volume ranges required and to avoid repeated volume changes.

3. Selecting the Right Pipette

There are many pipetting tools available to achieve optimal results and greater productivity, at the same time providing additional benefits such as improved ergonomic features and better functionality for a given application. There are two major types of micropipettes: air-displacement and positive-displacement. Both types determine the volume of liquid dispensed by using the diameter of the piston and length of the piston stroke.

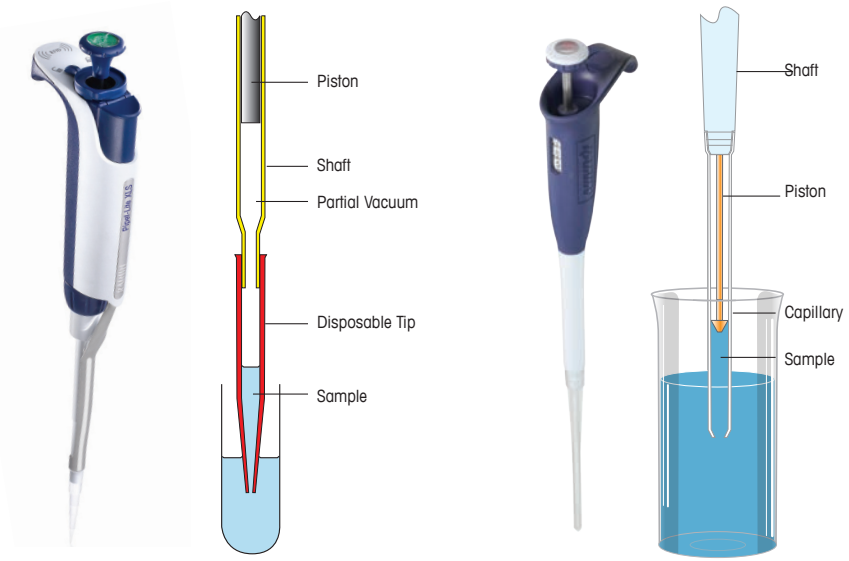


Figure 1: Air- and positive-displacement pipettes

Air-displacement pipettes

- Extremely accurate with aqueous solutions
- Economical

Air-displacement pipettes are the most common pipetting instruments found in the lab. These pipettes operate by placing the end of the tip into liquid sample, then releasing the plunger button. A partial vacuum is created when the pipette piston is moved up within the pipette body, and liquid sample moves up the tip to fill the void of the selected volume created by the partial vacuum.

Positive-displacement pipettes

- Extremely accurate with most solutions
- Recommended for viscous, dense, volatile or corrosive liquids

While not nearly as common as air-displacement pipettes, positive displacement pipettes are frequently seen in laboratory settings. These pipettes use a disposable piston and capillary system to make a physical void of the selected volume. The piston is in direct contact with the sample, and when the piston is moved upward sample is drawn into the capillary. Positive-displacement pipettes provide high accuracy and precision when pipetting aqueous solutions, but are recommended for use with viscous, dense, volatile and corrosive solutions. The disposable capillaries and pistons used with a positive-displacement pipette are more expensive compared to the disposable air-displacement pipette tips, so air-displacement pipettes are recommended when they will yield the same results.

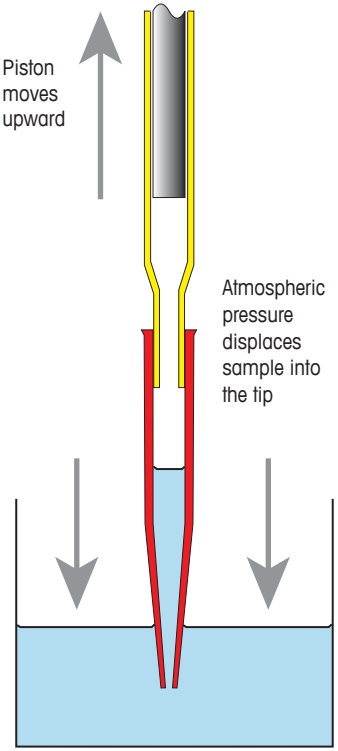


Figure 2:
Air-displacement pipette operation

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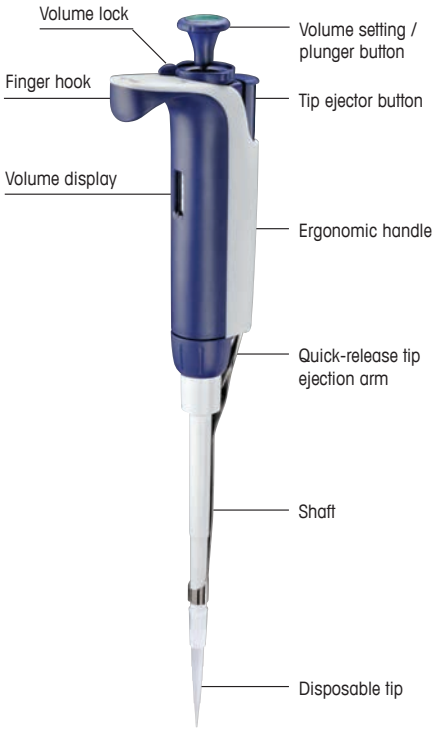


Figure 3:
Manual Pipette

Figure 4: Electronic Pipette

Electronic single-channel pipettes

Electronic pipettes have been available since the mid-1980s. In electronic air-displacement pipettes, aspiration and dispensing are controlled by a microprocessor and are initiated by pressing a trigger, rather than by using the thumb to press or release a plunger button. Using an electronic pipette most users will achieve more consistent sample pick-up and dispensing, and improved accuracy and repeatability, virtually eliminating all user-to-user variability.

Modern electronic pipettes should be simple to operate with a good user interface and large color screen. They are versatile and useful for accurately performing intricate tasks such as repeat dispensing, controlled titrations, serial dilutions, measuring unknown sample volumes and other programmable functions. With an electronic pipette it is easy to program repeated movement of the piston to mix two solutions in the tip. Electronic pipettes with aspiration and dispense speed controls can be used to pipette a wide variety of liquids. The fastest speeds are ideal for pipetting aqueous samples, slower speeds for viscous, foaming or shear-sensitive samples.

Multichannel pipettes

Multichannel pipettes are ideal for high throughput applications, including 96-well-plate ELISA work and PCR for DNA synthesis. Advanced design multi-channel pipettes, such as Rainin's lightweight 8 and 12 channel models, are ergonomically designed and load tips quickly and securely with consistent sample pickup across all channels. Adjustable spacer models allow the tip spacing to be set by the user for dispensing from 96-well plates to tube racks or to 24-well plates. Multichannel and adjustable spacer pipettes are available in manual and electronic format in a wide range of volumes.

Figure 5: Multichannel pipettes

High-throughput pipetting systems

Pipetting systems that aspirate and dispense 96 wells at once are ideal for fast efficient multi-well plate workflows. Until recently, expensive robotic systems were the only way to achieve 96-well, or whole plate pipetting. However, the Rainin Liquidator 96 – a fully manual benchtop pipetting system, requiring no electricity, no programming and no operator training – simplifies and streamlines 96-well and 384-well pipetting and can be used in the lab or in the field.

Figure 6:
Liquidator benchtop pipetting system

Specialty pipettes

Other types of pipettes (or liquid handling devices) are less common than air-displacement pipettes but are often preferred by researchers for their specific design and purpose.

Positive displacement pipettes

The Rainin Pos-D is an example of a manual positive displacement pipette.These pipettes use a disposable piston and capillary system to make a physical void of the selected volume. The piston comes into direct contact with the sample, and when the piston is moved upward sample is drawn into the capillary. These pipettes absolutely prevent cross-contamination of the pipette by the sample, as a new piston is used for each sample. This makes them ideal for PCR and other critical applications. Positive displacement pipettes are recommended for use with viscous, dense, volatile and corrosive solutions.

Figure 7:
Positive-displacement pipette

Repeater pipettes

With their syringe and built-in piston, repeater pipettes work on the positive-displacement principle. They are designed to draw in a large volume of liquid sample which is then dispensed in multiple, equal aliquots. They are available in electronic or manual versions and used disposable syringes in a wide range of volumes.

Figure 8:
Electronic (left) and manual repeater pipettes

Pipet controllers

Used primarily for large volumes (25-100 µL), pipet controllers are electronic or manual devices that provide suction for glass or plastic serological pipets. The pipet is attached to the soft "nose" and the user presses a button on the pipet controller to create a partial vacuum inside the glass or plastic pipet. The partial vacuum is displaced by the liquid under atmospheric pressure. After transferring to another vessel, the liquid is dispensed by pressing another trigger or by gravity. The simplest versions employ a soft flexible bulb that is manually squeezed and released to create and control the partial vacuum.

Figure 9:
Electronic pipet controller

Bottle-top dispensers.

Some laboratory liquids by their nature (e.g. corrosives or toxic liquids) are best left in place in fume hoods or safety cabinets, and not moved around the lab. A bottle-top dispenser is useful to safely transfer relatively small quantities of these liquids. The dispenser operates by pump action, and newer versions provide accurate and safe delivery of "hazardous" liquids in volumes up to 50mL.

Figure 10: Bottle-top dispenser

4. Choosing the Right Tip: Design, Quality and Fit

The pipette and its manufacturer-recommended tip is best viewed as a system, not two individual components. Pipette tips which are advertised for use with all pipettes often exhibit compromises in fit or design, since they are intended to fit a wide range of pipette models.
When selecting pipette tips, features to consider are design, quality and fit.

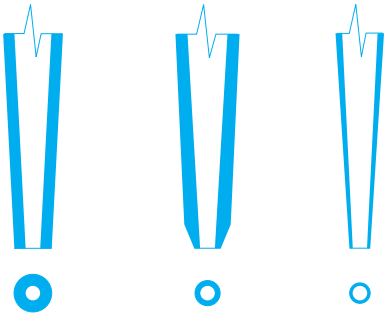


Figure 11:
Thick-wall tip (left), bevelled tip (center) and Rainin FinePoint tip (right)

Tip design

The most advanced design in pipette tips is the flexible thin walled tip with a fine point, or small tip orifice.
For small volume pipetting, less than 20 µL, Rainin FinePoint™ tips improve accuracy and precision over standard pipette tips that have thick-walls or beveled ends.

FinePoint tips are more flexible than most other standard tips, and allow the liquid sample to flow at any tip angle for complete delivery. That is, far less sample is retained on the tip as compared to thicker-walled or beveled-end tips.

Differences in tip design affect performance, accuracy and precision. However, when pipettes are used correctly they will provide guaranteed performance of specified accuracy and precision, provided that the manufacturer's recommended tips are used.

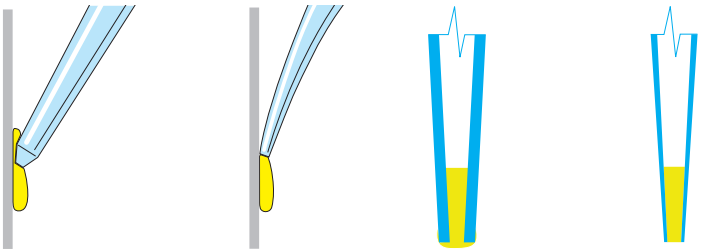


Figure 12: Dispensing (left) and sample retention (right) with beveled tip and FinePoint tip

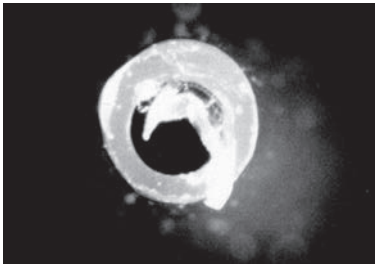
Tip quality

The most severe quality defects occur at the orifice or end-opening, where sample aspiration and dispensing is most affected. Figure 13 illustrates four tip ends in a magnified view.

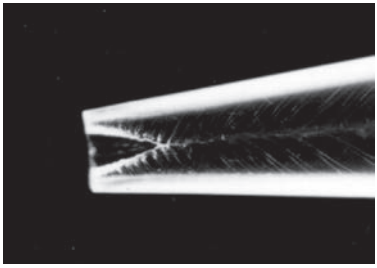
Flash is residual plastic left over from the molding process on the inside of the tip or around the aperture.

Molding defects and **coaxial defects** result from improper firing of the mold core pins after plastic has been injected. All of these defects will result in sample loss during pipetting. A high-quality manufacturing process will minimize the occurrence of tip defects and the resulting errors.

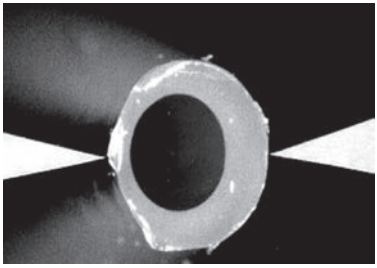
HIgh quality tip orifice



Flash on tip orifice



Molding defect



Coaxial defect

Figure 13: Tip orifices showing a good tip and three type of defect

Pipette-tip sealing

Most conical tips are built to fit any make of pipette – the seal between the interior of the tip and the exterior of the pipette shaft is large to accommodate the largest range of pipettes. This relatively large seal creates more friction between the shaft and the seal as the pipette shaft is wedged into the tip. There is no feedback mechanism to alert you when a universal-fit tip is properly sealed, which generally requires that user force the pipette shaft into the tip to assure a good seal.

Because the whole forearm is used it is relatively easy to apply too much force when loading tips, which will then generally require a correspondingly high tip ejection force. Bottom line: the force required to load and eject universal-fit tips can increase the risk of repetitive strain injuries (RSI), particularly over prolonged pipette use.

LTS™ LiteTouch™ tip ejection system

Recognizing the ergonomic and force issues of tip loading and ejection along with other tip sealing problems (especially in multichannel pipettes), Rainin developed a new tip design called LTS™ or the LiteTouch™ System to dramatically improve the fit between pipette tips and shafts. LTS significantly reduces the force required for inserting the pipette shaft and for ejecting tips.

These two characteristics of LTS work in conjunction to reduce tip ejection force

- The small seal area enables tips to seal very easily
- A positive stop is created by the shelf inside the tip that prevents the shaft being forced into the tip

The design of the seal area provides good lateral stability, which prevents the tip from falling off during use.

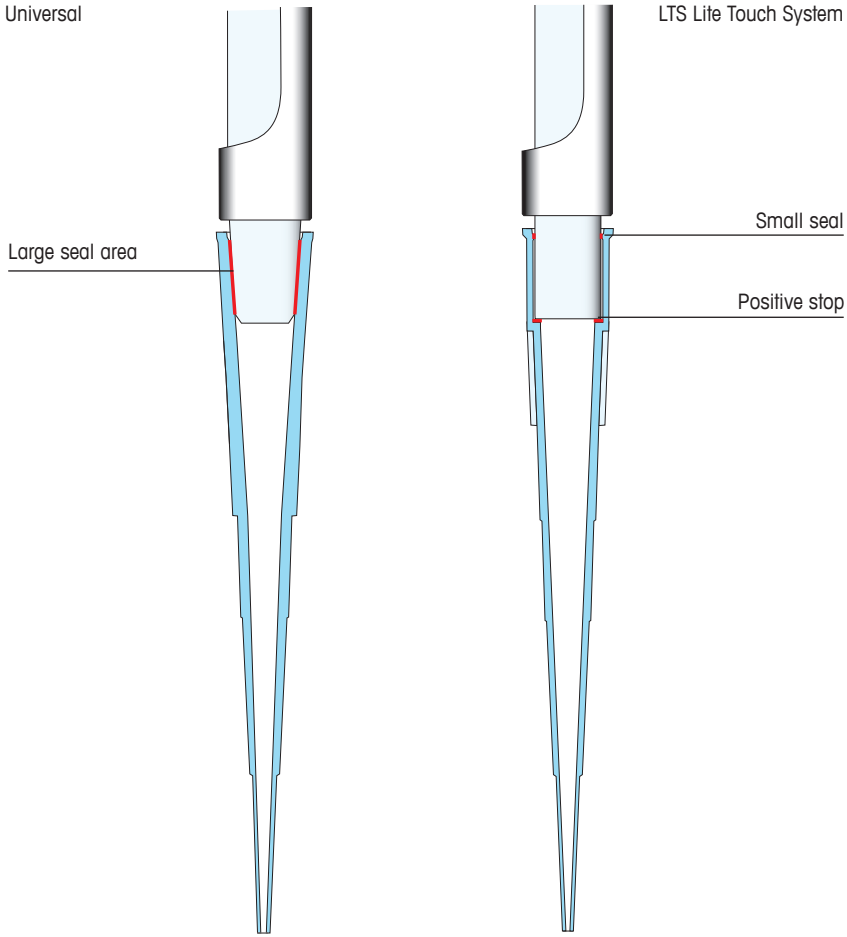


Figure 14: Universal (conical) and LTS (cylindrical) tip systems

Tip selection

In summary, to ensure smooth, constant sample flow and reduced risk of sample contamination when selecting pipette tips, it is good to consider:

Tip Material. Tips should be made of a very low retention material such as virgin polypropylene that contains no additives, dyes or recycled materials.

Tip Design. Wall thickness, flexibility,orifice size and surface finish are important factors in fitting the pipette and liquid flow in and out of the tip.

Tip Quality. Are tips manufactured in a clean room? Can individual lots be traced? Are tips free from additives or defects that can cause sample loss and error?

Specialized tips for special applications

There are several "non-standard" tip types which are useful for specialized applications or workflows.

Rainin Gel-Well™ tips are specially designed for layering gels, and are available with flat or round ends in very small orifice sizes.

Wide-orifice tips are designed to handle delicate samples such as whole cells or high molecular-weight DNA. The large orifice minimizes sample shear and prevents cell lysing. These tips are also recommended when pipetting salt solutions or cell suspensions for ease of sample collection and to prevent cell destruction.

Low-retention tips have specially-prepared super-hydrophobic polymers which enable extraordinarily "sticky" samples such as proteins to be dispensed from the tip end without any sample remaining in the tip. Such tips are not usually needed for typical liquid samples.

Rainin ShaftGard™ tips protect the pipette shaft and tip ejector from accidental contamination by enclosing these components within the tip. ShaftGard tips can be used in narrow tubes or deep wells with no risk of any part of the pipette touching the vessel walls.

Extended length tips are narrower and longer than other tips of equivalent volume. The small diameter and 102 mm length allows these tips to reach the bottom of narrow tubes and deep wells, without any part of the pipette or tip ejector touching the vessel wall.

Filtered tips, used for eliminating pipette cross-contamination or pipette contamination from aerosols without producing any discernible difference in pipette performance. Use of filter tips when pipetting volatile solutions is recommended to prevent potentially corrosive vapors from entering the pipette shaft and damaging the piston.

Capillary/pistons are designed for use with positive displacement pipettes, and are most effective with non-aqueous solutions that are dense, viscous or volatile, or for pipetting cold or warm aqueous solutions.

Sample Preparation tips. Sample preparation tips with resins embedded in the narrow end of the tip have recently become available. Rainin PureSpeed's "resin in a tip" design offers a convenient, low-cost and semi-automated method of purifying biomolecules, desalting or use in ion-exchange applications.

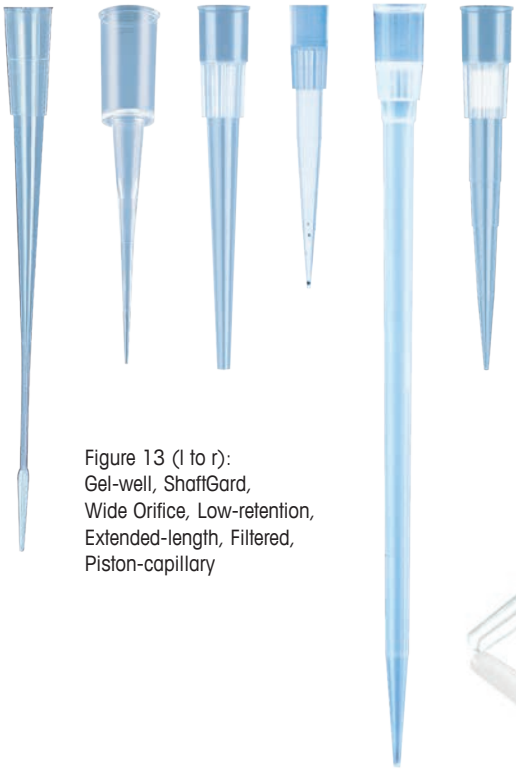


Figure 13 (l to r):
Gel-well, ShaftGard,
Wide Orifice, Low-retention,
Extended-length, Filtered,
Piston-capillary

Figure 14:
Rainin PureSpeed
Sample Preparation Tip

5. Pipetting Techniques

Correct evaluation of your application – and therefore selection of your instruments – will significantly impact the results of your research. Yet these aren't the only things researchers need to consider for optimal research results. Other influences, like correct pipetting technique and environmental conditions also impact results. Accuracy and precicion are essential to scientific research and the following pages give a brief overview into several facets of pipetting technique. Did you know for example, that by simply following these techniques, your accuracy and precision might improve by up to 5%?

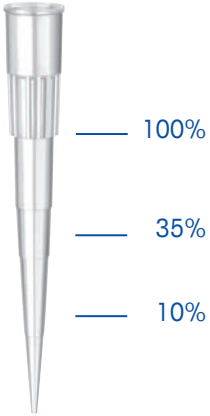
Optimal Volume Range

The normal operating range for most pipettes is 10 - 100% of nominal volume. Although this is considered to be the operating range, the performance specifications will change as the volume setting decreases.

The accuracy specifications for a 100 microliter pipette are +/- 0.8% from 50 - 100% of nominal. Yet if you were to pipette at 10 µl (or 10% of nominal), the inaccuracy specification would be more than 3 times greater, or 2.5 - 3%.

Therefore the optimal volume for the most accuracy and precision is typically 35 - 100% of nominal. Try to avoid setting a pipette's volume to less than 10% of its maximum – instead, switch to a smaller volume pipette for smaller volumes.

Volume vs. range



Pipetting down to 10% can affect accuracy by as much as 3%

Tip Immersion Depth

Particularly important for micro-volume pipettes, correct tip immersion depth can improve accuracy by up to 5%. The tip should be immersed between 1-2 mm for micro-volume pipettes and up to 6-10 mm for large-volume pipettes. If the tip is immersed too far the volume of gas in the tip is compressed, causing too much liquid to be aspirated. Liquid retained on the tip surface can also distort results. If the tip is not immersed far enough air can be drawn in, resulting in air bubbles and inaccurate volumes. Both result in inaccurate volume.

Tip immersion depth

1-10 µl: 1-2 mm

10-200 µl: 2-3 mm

200-2000 µl: 3-6 mm

Correct tip immersion depth can improve accuracy by up to 5%, so use the recommended depths as shown above (>2000 µl, use 6 - 10 mm depth).

Aspirating at the Correct Angle

The angle of your pipette tip in the sample should be as close as possible to 90° degrees and should not deviate more than 20° degrees from vertical.

For micro-volume pipettes, keeping the angle as close to vertical as possible, can improve accuracy by up to 2.5%.

Angles greater than 20° degrees can produce inaccurate measurements – too much liquid will be drawn into the tip, resulting in inaccurate aspiration.

Vertical immersion angle

Correct angle

Incorrect angle

An immersion angle of 60° degrees can cause you to aspirate up to 0.7% more liquid than intended.

Maintaining Consistency

Maintaining consistent pipetting rhythm and speed will help you produce optimal, more repeatable results. With consistent rhythm and speed you can achieve an accuracy improvement of up to 5%.

Consistent pipetting rhythm

Use a consistent pipetting rhythm from sample to sample. Avoid hurrying or rapid operation and get into a rhythm for each step in the pipetting cycle.

Large volume pipettes

For larger volumes - typically 1 ml or greater - pause about 1 second or more after the sample pick up, with the tip still in the liquid. This will allow the sample to fully aspirate.

Smooth plunger action

Maintain consistent speed and smoothness when pressing and releasing the plunger. Uncontrolled aspiration can cause bubbles, splashing, aerosols and contamination of the pipette shaft and piston and can also lead to loss of sample.

Consistent pipette rhythm and speed

Good aspiration

Tip with aspirated air

Consistent Sample Dispensing

The greatest accuracy and sample-to-sample reproducibility are achieved by ensuring that every last drop of the sample is dispensed and does not adhere to the orifice. This is especially crucial when pipetting micro-volumes, due to the small sample volumes involved.

Good dispensing technique can improve accuracy by up to 1%. When dispensing sample, make sure the end touches the vessel wall, preventing sample from remaining in the tip. After dispensing, sliding the tip end up the vessel wall to release any liquid remaining on the orifice.

Consistent sample dispensing

Dispensing against vessel wall

Dispensing into liquid

Dispensing onto liquid surface

Dispense into the liquid or onto the liquid surface.
When dispensing directly into or onto liquid, use reverse-mode pipetting to avoid picking up sample after dispensing.

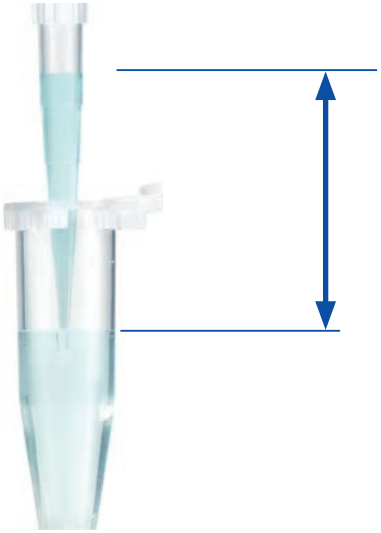
Pre-rinsing Tips

Pre-rinsing the tip two or three times forms a liquid film in the tip than can increase the accuracy by up to 0.2%. Pre-rinsing helps neutralize capillary effects in micro-volume pipettes and, for large-volume tips, equalizes the air temperature inside the tip with the temperature of the sample.

Exceptions to pre-rinsing

Pre-rinsing can adversely affect results when pipetting very warm and cold solutions, such as from an ice bath, or solutions above 37°C, as it may result in up to 5% error.

Pre-rinse the tip



Avoiding Temperature Variation

Constant room temperature

An ideal temperature for pipetting is 21.5°C ± 1°C, the same as used for calibration. Avoid drafty or sunlit areas with large or sudden temperature changes that could compromise aspiration accuracy. Pipetting at a constant temperature can improve results by as much as 5%.

Allow time for equilibration

Another important aspect of temperature variation is the equilibration time. Pipette accuracy is affected by temperature variation of hot or cold samples – cold liquids tend to deliver in excess, while warm liquids may deliver smaller volumes than expected. Unless otherwise specified, allow sufficient time for your pipettes and liquids to reach equilibrium temperature.

Hand-warming effects

Over long periods of pipetting, heat from hand can warm the pipette, causing the air space inside to expand and produce inaccurate results.

Avoid the effects of hand-warming by using high-quality pipettes made from PVDF-polymers. In addition, between pipetting cycles, replace the pipette on its stand instead of holding it in your hand.

Consistent Micrometer Settings

When changing the volume from a higher to a lower setting, dial down to the desired volume setting. However, when changing the volume from a lower to a higher setting, first turn the selector wheel about 1/3 turn above the desired volume setting then slowly down to the setting. This avoids mechanical backlash and results in greater accuracy.

Good Pipetting Practice

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