

## Choosing and Maintaining Your Pipettes Why Best Practice Matters



**In any laboratory, the correct use and maintenance of pipettes is essential to ensure precise, accurate results. Scientific staff has a broad range of pipettes to choose from – fixed or variable volume, air or positive displacement, single channel or multichannel, manual or electronic – and selection of the most appropriate type and volume range is crucial. Equally important is establishing a regular pipette testing program to confirm that performance remains within the specified limits, supported by preventive maintenance and calibration as necessary.**

This White Paper is an introduction and practical guide to the proper use, testing, maintenance and calibration of piston pipettes for scientists, technicians and other laboratory workers, covering single channel and multichannel, manual and automatic pipettes with dispensing volumes ranging from microliters to milliliters. The different types of pipette and their operating principles are described,

including recommendations for selecting the most appropriate pipette according to your application. Guidance is provided for routine pipette performance testing, as well as general daily pipette care, maintenance and calibration. Finally, the importance of operator training to ensure compliance with best pipetting practice is discussed.

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## 1. Types of Pipette

Pipettes can be divided into two main groups, fixed and variable volume, which are further sub-divided into air and positive displacement pipettes (Figure 1). The operating principle is essentially the same for all types of pipette; a predetermined volume of liquid is forced out of the pipette tip by the application of mechanical pressure to a piston or plunger working over a fixed length in a cylinder. The volume of liquid dispensed is determined by the diameter of the piston and the length of the piston stroke.

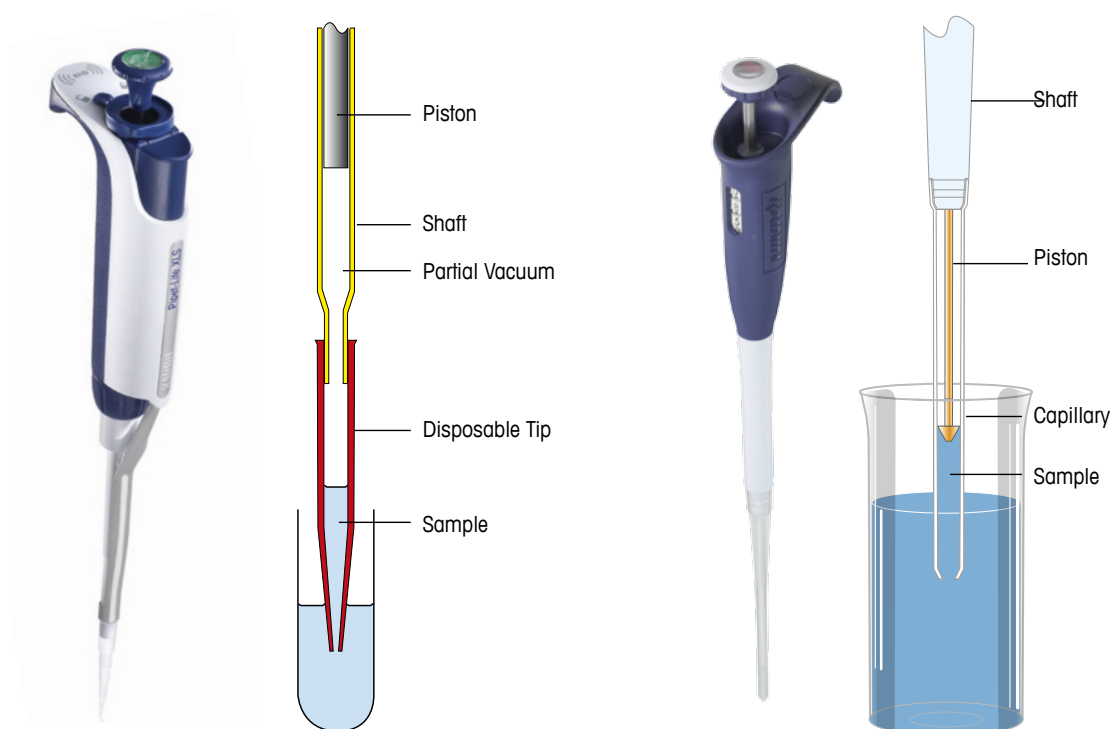


Figure 1: Air and positive displacement pipettes.

### 1.1 Fixed Volume Pipettes

Fixed volume pipettes are designed to dispense a specific, set volume of liquid – the nominal volume – which cannot normally be altered. However, some fixed volume pipettes are designed to allow minor adjustments to be made within set narrow limits, enabling users to compensate for errors found during performance testing or when using liquids with differing physical properties from water.

### 1.2 Variable Volume Pipettes

Adjustable volume pipettes have been available since the early 1970s, and allow the user to vary the dispensing volume over a range specified by the manufacturer. For these pipettes, the nominal volume is defined as the upper limit of the manufacturer's designated volume range.

### 1.3 Air Displacement Pipettes

Air displacement pipettes offer economical and extremely accurate pipetting of aqueous solutions, and are the most common type of laboratory pipette. The pipettes operate by depressing the plunger button down to the stop and placing the end of the tip into the liquid sample. When the plunger is released, the attached piston is returned to its original position by the piston spring – or by the motor in an electronic pipette – generating a partial vacuum and creating a void as it moves up within the pipette body. Atmospheric pressure forces liquid into the pipette tip, completely filling this void (Figure 2). In principle, the volume of liquid in the tip is the same as the volume of air that was displaced from the cylinder by the piston.

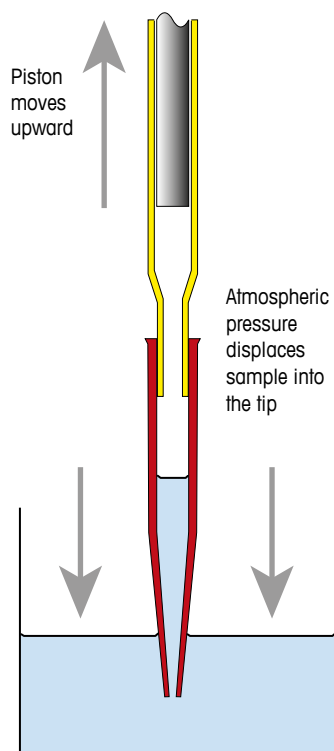


Figure 2: Air displacement pipette operation.

### 1.4 Positive Displacement Pipettes

Although not as ubiquitous as air displacement pipettes, positive displacement pipettes are frequently used in the laboratory as they offer precise pipetting of special solutions/liquids such as viscous, dense, volatile and corrosive liquids. Positive displacement pipettes use a disposable piston and capillary system to create a void of the selected volume. The piston is in direct contact with the sample and, as it moves upward, the 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.

## 1.5 Manual Single Channel Pipettes

Manual single channel pipettes with variable volume settings ranging from 1  $\mu\text{l}$  to 20 ml are by far the most commonly found laboratory pipettes. Pipette design has advanced considerably over time to incorporate features such as a large ergonomic plunger button for aspirating and dispensing liquid, single-handed volume adjustment, a mechanical volume display, a finger-hook to allow the hand to rest between pipetting cycles and an ejector button with a shock absorber for easy tip ejection (Figure 3).

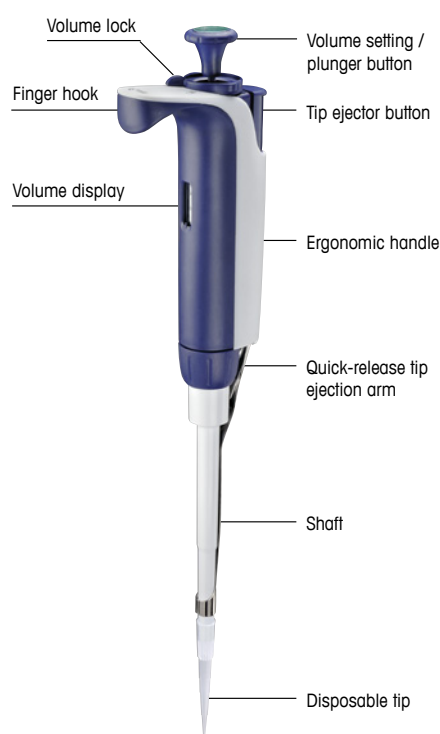


Figure 3: A manual single channel pipette.

## 1.6 Electronic Single Channel Pipettes

Electronic pipettes (Figure 4) using microprocessor-controlled aspiration and dispensing – initiated by pressing a trigger, rather than by using the thumb to press or release a plunger button – have been available since the mid-1980s. Most users will achieve more consistent sample pick-up and dispensing with an easy-to-operate electronic pipette incorporating a good user interface and large color screen, improving accuracy and repeatability and virtually eliminating user-to-user variability.

Electronic pipettes are versatile, enabling intricate tasks such as repeat dispensing, controlled titrations, serial dilutions and measuring unknown sample volumes – plus a range of other programmable functions – to be accurately performed. Repeat movement of the piston to mix two solutions in the tip is easily programmed, and controlled aspiration and dispense speed allows a wide variety of liquids to be accommodated; fast speeds are ideal for pipetting aqueous samples, slower speeds are more suited to viscous, foaming or shear-sensitive samples.



Figure 4: An electronic single channel pipette.

## 1.7 Multichannel Pipettes

Lightweight, ergonomically-designed multichannel pipettes (Figure 5) offering rapid, secure tip loading and consistent sample pick-up across all channels are ideal for high throughput applications such as 96-well plate ELISAs and PCR for DNA synthesis. Standard multichannel pipettes and adjustable spacer models – allowing tip spacing to be set up to accommodate 24- or 96-well plates, as well as tube racks – are available in both manual and electronic formats, covering a wide range of dispensing volumes.



Figure 5: Manual and electronic multichannel pipettes.

## 2 Pipette Performance Management

### 2.1 Best Practices for Pipette Quality Control

As precision laboratory instruments, pipettes are subject to stringent quality control regulations; noncompliance causes major administration headaches for metrology and the enduser, and correcting the problem is often labor intensive and costly.

Pipette failures have many consequences. Manufactured products may not meet specifications and have to be recalled, and incorrect conclusions may be drawn from experimental results, with the analysis having to be repeated. Not only is this costly and time consuming, but there is a risk of failing to publish or get to market in time, losing market share, and a loss of credibility. The effectiveness of a regulated laboratory's GMP/GLP program becomes questionable, making more intensive audits likely, and validation costs increase. The more frequent the calibration, the earlier defective pipettes will be detected and taken out of service, decreasing the potential for incorrect results and helping to minimize the need for remedial action.

High quality pipettes, professionally serviced and maintained, are crucial to scientific success and to comply with regulatory requirements. Pipetting performance is influenced by many factors, most notably regular routine testing and maintenance, and correct pipetting technique. As precision instruments, pipettes are subject to the same quality regulations – for example GLP and cGMP – regarding calibration and maintenance as other critical laboratory instruments to ensure that the desired performance specifications are met. This means that pipettes must:

- have regular functional checks to verify performance and be periodically calibrated according to a documented procedure
- undergo periodic maintenance and be properly handled
- be operated by trained individuals with demonstrated competence

This ensures continual correct performance, helping to reduce the risk of out of tolerance results and incorrect data.

### 2.2 Sources of Liquid Delivery Variability

Pipetting variability has a number of different causes, most commonly:

- Systematic failures; foreseeable failures due to predictable wear, based on factors such as frequency of use and maintenance intervals
- Random failures; unexpected failures due to arbitrary events such as accidents or mishandling. These failures occur randomly with respect to the pipette service cycle, and cannot be accurately predicted
- Operator technique; inconsistent or incorrect pipetting technique is probably the largest single source of liquid delivery variability, and is frequently due to lack of training in the correct use of pipettes
- Environmental factors; pipette performance varies under different environmental conditions, for example, changes in temperature and humidity
- Device tolerance limits; inaccuracy and imprecision inherent in the pipette itself can also give rise to a small amount of variability in liquid delivery. Typically, these limitations will be specified by the manufacturer, based on best case performance.

### 2.2.1 Systematic Versus Random Failures

Systematic pipette failures are those that arise from simple wear, generally reflecting the extent to which a pipette is used and its frequency of maintenance. Adjustment of the service cycle and calibration interval, based on a review of the 'as found' performance history of the pipette, may help prevent these failures. In contrast, random failures due to accidents, mishandling or other unplanned events can occur at any point in the service cycle and, owing to their unpredictable nature, are harder to eliminate. For example, an operator may inadvertently draw liquid into the pipette body, causing the piston to corrode, or simply drop the pipette. Such failures cannot be prevented by scheduled maintenance cycles, but regular pipette performance testing will help to detect them earlier.

## 3 Assuring Pipette Performance

Assuring pipette performance is vital for accurate, precise pipetting. This requires a dedicated care and maintenance plan to be established, ensuring pipettes are regularly cleaned and decontaminated, and that performance testing, calibration and preventive maintenance is carried out. While some of these tasks can be performed by the pipette user in house, others require the use of a specialized service provider (Table 1).

	Content	Responsibility
Cleaning and decontamination	External cleaning and decontamination of the pipette at regular intervals	Pipette user (day to day) / Service provider (with PM service)
Inspection	Inspection of the pipette to check for any damage that may have occurred	Pipette user(day to day) / Service provider (with PM service)
Pipette performance testing	Pipette performance check, scheduled based on process risk (daily or at least weekly)	Pipette user
Calibration	Pipettes need to be calibrated according to application requirements. Calibration intervals are typically set according to quality standards and requirements.	Service provider / ISO 17025 accredited laboratory
Preventive maintenance	Pipette functionality check, including replacement of wear parts, done at least once per year or more frequently for high usage or when pipetting potentially ruinous liquids.	Service provider / ISO 17025 accredited laboratory

Table 1: The essential requirements of a pipette care and maintenance plan.

### 3.1 Daily Care of Pipettes

#### 3.1.1 Cleaning and Decontamination

The solvents chosen for cleaning and decontamination should enable the removal of all liquids the pipette has had contact with, and the manufacturer's recommended cleaning protocol should be carefully followed as some solvents may adversely affect the materials a pipette is constructed of. With electronic pipettes, extra care must be taken to ensure that cleaning fluids do not come into contact with the pipette mechanism. Many pipettes can withstand autoclaving – although partial dismantling may be necessary – providing the manufacturer's instructions regarding the suitability of the sterilization media used and the maximum temperatures and pressures allowed are adhered to.



### 3.1.2 Inspection

All pipettes should be inspected at regular intervals to ensure correct functioning and to check for any wear or damage that could affect accuracy and precision. The pipette mechanism must be tested to confirm correct operation and smooth piston movement. The measurement accuracy of a pipette is dependent on achieving a good seal between the tip and the tip holder so that no leakage occurs, and so it is important to inspect the tip holder, looking carefully for any marks or distortion. It is also essential to check for any sign of leakage during pipetting, as this could indicate leaking seals or O-rings, or the use of an ill-fitting or inappropriate tip. However, users should be aware that with fluids such as high vapor pressure liquids, leakage may be caused by a small amount of the liquid changing to a gaseous state, resulting in an increase in pressure in the dead volume of air.

## 4 Testing, Calibration and Preventive Maintenance

To assure reliable pipette performance, a scheduled testing and maintenance program should be established, including the following best practices:

- verification of pipette performance, at a frequency based on the mean time between failures (MTBF), to ensure data validity
- immediate verification of the performance of any pipette that has been dropped or otherwise mishandled, or which is associated with questionable data
- planned comprehensive preventive maintenance with thorough cleaning, seal/O-ring, shaft, piston replacement when needed, and re-greasing per manufacturer specifications
- calibration of pipettes with appropriate balances; micro and analytical balances, special balance for multichannel pipette calibration
- operator training in the correct operation and storage of pipettes, with periodic verification of pipetting competence under everyday working conditions

What differs significantly between laboratories is the frequency of pipette performance testing, preventive maintenance and calibration. In quality control, diagnostic and other laboratories that routinely audit equipment to comply with stringent regulatory guidelines, pipette servicing is performed far more frequently than, for example, in academic research departments. In general, the frequency depends largely on the significance of a pipette failure, as illustrated in Figure 6.

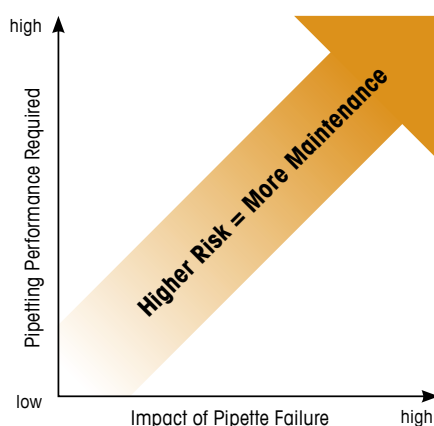


Figure 6: A risk-based approach to pipette maintenance.

The relationship is simple; if your application depends on rare samples and/or costly procedures or reagents, or the accuracy of the results is critical, then pipettes should be checked and serviced frequently.

## 4.1 Testing and Calibration

Although the methods used overlap to a considerable extent, a distinction must be made between testing and calibration. Testing is a routine operation, normally performed by the user, which allows the customer to test against process tolerances, ensuring that pipette performance remains within pre-established acceptable limits. Calibration must be performed by a specialized service provider, and compares the expected volume with the actual volume delivered by a pipette together with the associated measurement uncertainty.

### 4.1.1 Pipette Performance Testing

Routine performance checks are essential to ensure correct functioning, accuracy and precision of pipetting, and to maintain data integrity. Performance should be compared against the manufacturer's specifications and process requirements on a regular basis, helping to identify any maintenance and (re)calibration needs. For piston pipettes, ISO 8655-1:2002, 7.3, recommends establishing a regular testing routine using either ISO 8655-6 or alternative test methods taking into account:

- the required accuracy of liquid delivery
- the frequency of use
- the number of operators using the pipette
- the number of dispense cycles performed on each occasion of use
- the nature of the liquid dispensed (corrosiveness, solvent strength, etc.)
- supplier recommendations

Pipette accuracy is normally determined gravimetrically, and a quick check to verify the entire pipetting system – user, pipette, tip and environment – requires a calibrated laboratory balance, a thermometer, deionized and degassed water, and a suitable weighing vessel (Figure 7); an evaporation trap is recommended. Software packages are also available to guide the process, analyze data and record pipette performance. Pure water is dispensed in a single pipetting operation, weighed, and the mass recorded. The mean value of the weighing series is multiplied by the "Z-factor"<sup>1</sup> to convert the mean value of the mass to a volume result.

Replicate measurements are made, and corrections applied to compensate for any variation from standard temperature and atmospheric conditions, as well as any significant evaporation of the water during the test period. Variable volume pipettes should be tested at two or three volume settings, at the maximum (nominal) volume, 50% of the maximum volume and at the lower limit of their range as specified by the manufacturer, or 10% (of the nominal volume) as described by ISO 8655 as the useful minimum volume (ISO 8655-1:2002, section 3.1.7, Note 1). The results are compared to the appropriate specifications to determine the accuracy and precision of the pipette. In order to be considered within specification, results must fall within an accuracy range, and not exceed a precision (standard deviation) value.

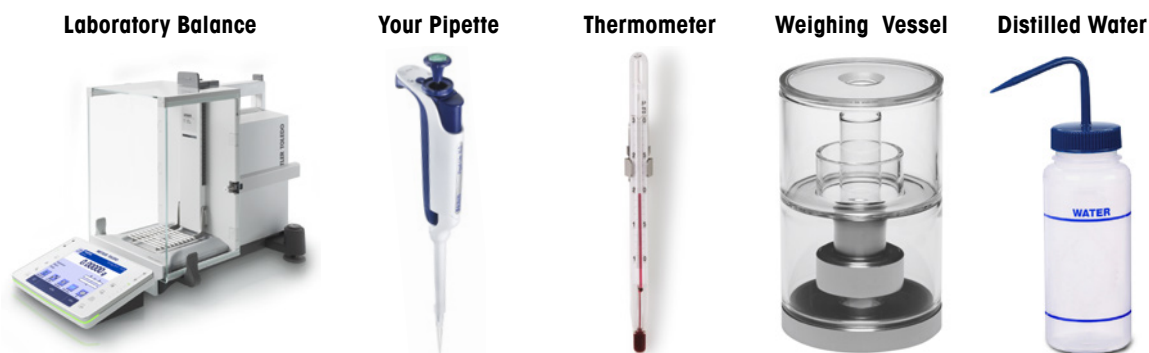


Figure 7: A quick check requires minimal equipment.

<sup>1</sup> The Z-factor is used to convert mass into volume according to temperature and pressure. The Z-factor is specified in Annex A of the EN ISO 8655-6:2002 standard.

A pipette performance test of accuracy and an estimation of precision should be performed at least monthly, even daily or weekly, or before a major experiment, and more frequently if indicated by the pipette's physical condition or extended use. It is important to use an analytical balance with a resolution appropriate to the selected volume of the pipette (Table 2), and sample size must also be considered for a quick check, four replicate measurements are sufficient.

Selected volume of apparatus under test V*	Readability
$1 \mu\text{l} \leq V \leq 10 \mu\text{l}$	0.001 mg
$10 \mu\text{l} < V \leq 100 \mu\text{l}$	0.01 mg
$100 \mu\text{l} < V \leq 1000 \mu\text{l}$	0.1 mg
$1 \text{ ml} < V \leq 10 \text{ ml}$	0.1 mg
$10 \text{ ml} < V \leq 200 \text{ ml}$	1 mg

\* For practical purposes, the nominal volume may be used to choose the balance.

Table 2: Minimum balance requirements for pipette performance testing.

## 4.2 Preventive Maintenance and Calibration

Preventive maintenance and calibration have equally important roles to play, and both are included in a pipette service cycle (Figure 8). Typically, preventive maintenance can eliminate 97% of errors, while 3% of errors are corrected with calibration services. To guarantee proper pipette performance therefore preventive maintenance and calibration need to be combined.

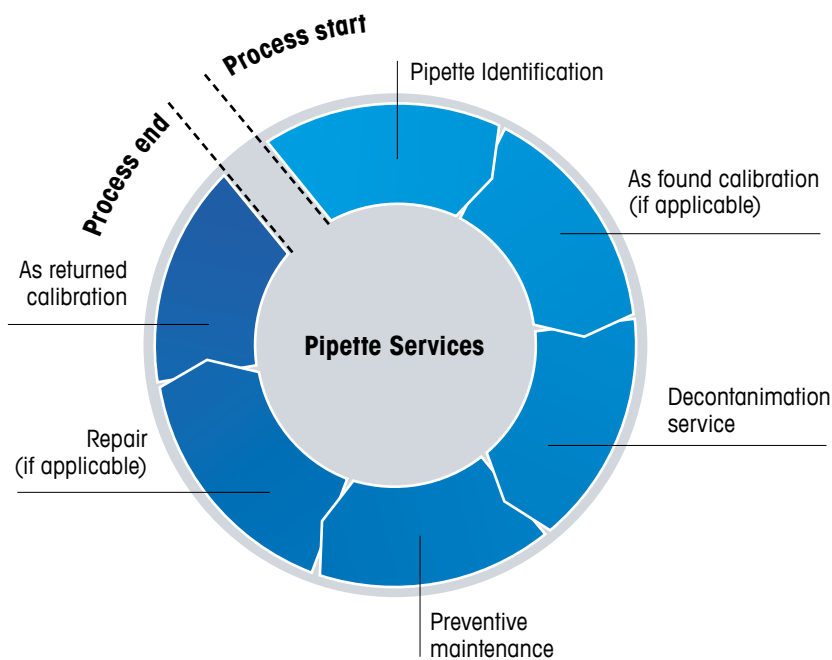


Figure 8: A typical pipette service cycle.

Preventive maintenance is performed at least annually, and focuses on long-term performance (Table 3). While this may be carried out on-site by trained laboratory personnel who check pipette function, perform leak tests and replace any parts necessary, the use of a specialized service provider is highly recommended. In contrast, calibration establishes current performance gravimetrically (Table 3), and is always performed in a controlled environment by certified calibration personnel. The pipette's 'as found' and 'as returned' status is determined and a certificate of calibration issued, reporting the accuracy and precision of the pipetting results.

	<b>Preventive Maintenance</b>	<b>Calibration</b>
Primary objective	<ul style="list-style-type: none"> <li>Ensures long-term pipette performance</li> </ul>	<ul style="list-style-type: none"> <li>Checks current pipette performance</li> </ul>
Service(s) performed	<ul style="list-style-type: none"> <li>Parts replacement</li> <li>Function check</li> <li>Leak test</li> <li>Cleaning</li> </ul>	<ul style="list-style-type: none"> <li>Gravimetric measurements               <ul style="list-style-type: none"> <li>- As found</li> <li>- As returned</li> </ul> </li> <li>Calibration certificate</li> </ul>
Environment	<ul style="list-style-type: none"> <li>Professional lab or on-site</li> </ul>	<ul style="list-style-type: none"> <li>Controlled lab environment</li> </ul>
Staff, equipment & system requirement	<ul style="list-style-type: none"> <li>Access to manufacturer parts</li> <li>Highly trained personnel</li> <li>Inventory control process</li> </ul>	<ul style="list-style-type: none"> <li>High precision micro balances</li> <li>Certified calibration personnel</li> <li>Calibration software</li> </ul>
Recommended Frequency	<ul style="list-style-type: none"> <li>Minimum once per year</li> </ul>	<ul style="list-style-type: none"> <li>- As mandated by quality requirements</li> </ul>

Table 3: The differences between calibration and preventive maintenance.

#### 4.2.1 Preventive Maintenance

Preventive maintenance is the key to peak pipetting performance and reducing, or even preventing, out-of-tolerance failure measurements between calibrations. Over time, piston surfaces can deteriorate and become rough, causing premature seal failure, pipette inaccuracy or sample contamination, and shaft ends can become worn, especially if force is applied when mounting tips. Leakage may gradually develop and, if not discovered early, can adversely affect data integrity.

As part of a comprehensive preventive maintenance, function and leak tests are performed and, where necessary, repairs are made and parts are replaced, ideally with original manufacturer spares (Table 4).

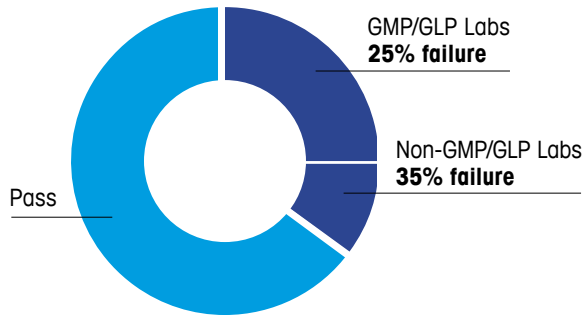
<b>Preventive maintenance</b>	<b>Repair</b>
Inspection and function check	Mechanical failures – plunger, micrometer, tip ejector. etc.
Cleaning	Electronic failures – PCBs, LCD screens, etc.
Pressurized leak test	
Replacement of parts such as seals, O rings, shafts and pistons, ideally with original manufacturer spares	

Table 4: Experienced service providers offer comprehensive preventive maintenance and repair of pipettes.

It is crucial that pipette performance is 'as new' after servicing, ensuring peak performance and trouble-free pipetting. A US East Coast pharma study from 1998 to 2003 found that pipettes that were 1) not serviced or 2) subject to calibration and repairs alone had 'as found' failure rates between 25 and 35% (Figure 9). With regular servicing by a certified provider using manufacturer approved spare parts, as found failure rates decreased to below 5% (Figure 10), clearly demonstrating the benefit of professional preventive maintenance.

For example, experienced service providers recommend that under normal use, pipette seals should be replaced at least once per year, and shafts and pistons every three to five years. A recent study that analyzed data from over 120,000 pipettes serviced in a year indicated that an estimated 95% of all pipette failures can be attributed to one or more components of the sealing system (Figure 11).

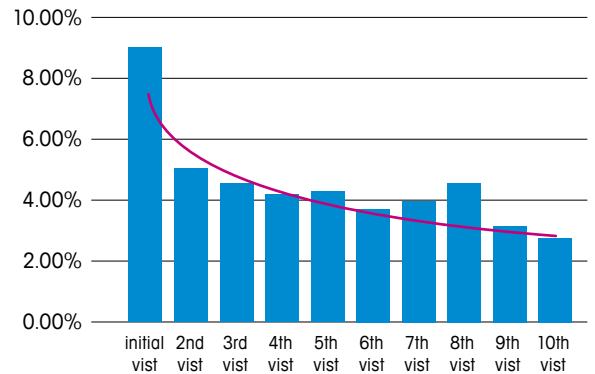
**Pipette failure rates without preventive maintenance**



Based on East Coast Pharma study – 1998-2003

Figure 9: Pipette failure rates tend to be between 25 and 35% without preventive maintenance.

**Pipette as found failure rate when continuously serviced by Rainin**



Based on Rainin service center 2011 data

Figure 10: The benefit of regular pipette servicing.

**Sealing System Failures**

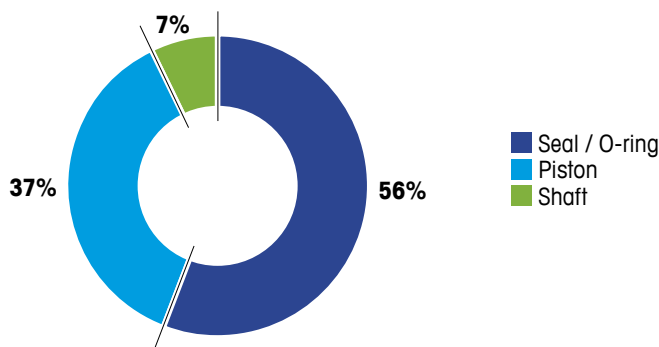


Figure 11: Reported causes of pipette failure.

#### 4.2.2 Calibration

Calibration is defined as testing of the pipette against the manufacturer’s specification by a certified service provider, calculating the measurement uncertainty of the result. However, calibration is frequently confused with other procedures, including checking a pipette using a laboratory balance, adjusting the calibration mechanism to comply with a given set of specifications, or a wide range of compliance and regulatory activities which, performed properly, help ensure trouble-free regulatory audits. A comprehensive pipette service provides:

- ‘as found’ performance data
- preventive maintenance
- ‘as returned’ performance data
- calibration certificate
- and service label

#### 4.2.2.1 Professional Calibration Set-up

Professional calibration service providers are ISO 17025 accredited and can meet or exceed ISO 8655 guidelines. Calibration service performed according to ISO 17025 are carried out in a controlled environment, using marble weighing tables in a room with carefully regulated temperature and humidity, free from vibration and drafts. Professional calibration service facilities provide:

- Skilled, qualified staff to perform calibration in a controlled environment – a vibration and draft-free room on the ground level with carefully controlled relative humidity, atmospheric pressure and temperature
- Appropriate gravimetric balances (Table 2) which are regularly calibrated using weights traceable to global standards, placed on 600 pound (~ 300Kg) marble tables away from walkways and windows
- Pipettes are calibrated per the manufacturer published specifications at appropriate volume settings (typically 10%, 50% and 100%)
- Pipettes and the deionized water are allowed to equilibrate to room temperature prior to calibration
- To minimize errors due to evaporation, the test cycle time is kept to a minimum. For very small volumes, mathematical compensation for evaporation is applied

#### 4.2.2.2 Testing Method

The general procedure is based on the gravimetric method described in section 5.1.1. Pipette Performance Testing. Values are adjusted to compensate for evaporation, and used to calculate the true mass and volume dispensed, based on the density of water at specific temperatures and corrections for air buoyancy (see ISO 4787). It is important to note that, in contrast to performance testing which follows process tolerances; calibration includes comparison of the pipette performance with the manufacturer's specifications, as well as calculation of the measurement uncertainty.

For fixed volume pipettes, the test volume is the nominal volume. Variable volume pipettes are tested at:

- the nominal volume (100%)
- 50% of the nominal volume
- the lower limit of the volume range as defined by the manufacturer, or 10% as described by ISO 8655 as the useful minimum volume (ISO 8655-1:2002, section 3.1.7, Note 1)

## 5 Finding a Good Service Provider to Maintain Your Pipettes

Laboratories often face a wide choice of preventive maintenance and calibration service offerings from many companies. Careful consideration of a great deal of information is necessary to find the right service provider, in particular:

- Are they ISO/IEC 17025 accredited?
- Are genuine manufacturer's spare parts used, and labor and parts backed by a service warranty?
- Is calibration carried out at least at the 10% and 100% of nominal pipette volume?
- Are they using 6 or 7 digit balances for small volume pipettes and multichannel pipette calibration workstation for multichannel pipettes?
- Are the reported 'as found' failure rates<sup>2</sup> greater than 0%?
- Is past report history available, and are customer audits encouraged?
- Is the service subcontracted, and does it have the flexibility to meet your needs?
- Are there any hidden charges?
- Are pipette technicians certified and subject to periodic proficiency testing?
- Are the calibration certificates generated electronically generated via a pipette calibration software?
- Is your calibration data backed up in a secured server?

Choosing a service provider that can answer yes to all of the questions above will assure pipette accuracy and precision, giving complete confidence in experimental results.

## 6 Operator Training

Inconsistent or incorrect pipetting techniques are probably the largest single source of liquid delivery variability in the laboratory, and are generally due to a lack of operator training in the correct use of pipettes.

Key points for an operator to understand are:

- optimizing the volume range
- setting the volume correctly
- the effect of tip immersion angle, depth and time
- aspiration rates
- dispensing technique
- pre-rinsing pipette tips
- hand-warming effects
- the correct pipette technology for challenging liquids

Correct training improves pipetting technique, reduces errors and ensures data quality.

<sup>2</sup> 'As found' status refers to pre-service calibration to establish how a pipette has performed since its last service and calibration. While these should, ideally, be very low, 0% failure rates are questionable since it is unlikely that there would be no failures at all. Pipette as found failures are most commonly due to poor treatment, inappropriate use and lack of day-to-day maintenance by users.

## 7 Summary

The correct use and maintenance of pipettes is essential to achieve precise, accurate results. Selection of the most appropriate pipette for the task in hand is crucial, as is the implementation of a regular testing program, supported by preventive maintenance and calibration, and operator training. By following these guidelines, laboratories will achieve maximum accuracy and reproducibility of pipetting.

## 7 References

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