# Checking Pipette Performance on the Bench Top

## A Fast and Accurate Method Using Photometry

**George Rodrigues and Barry Godowsky**

Pipetting microliter volumes of liquid is one of the most commonly performed procedures in today's pharmaceutical laboratories. The accuracy and precision of a properly functioning air-displacement pipette can be excellent — even at volumes below 0.5 µL. However, the performance of pipettes invariably degrades over time. These ubiquitous devices may fail rapidly or gradually, randomly or progressively, but they most often fail in ways that are undetectable to a technician.

A trend toward dispensing smaller volumes has made it difficult to maintain full confidence in data validity and thus regulatory compliance. So verifying timely pipette performance is all the more vital to ensuring consistent, high-quality liquid delivery. One stumbling block is the gravimetric procedure traditionally used for testing pipettes. Gravimetry becomes increasingly problematic and time-consuming at the microliter level, especially in the working laboratory environment.

Pipette calibration services or centralized metrology facilities, many of which use gravimetric methods, play an important role in maintaining and calibrating pipettes. But in many cases, the related expense and inconvenience often makes it difficult to have pipettes checked more than twice annually. Malfunctioning pipettes may therefore remain in use for extended periods, potentially compromising critical assays.

When a failed pipette is discovered, any data obtained since its last calibration can be called into question and is thus subject to reevaluation. An entire procedure or study, even a production batch could be compromised. Active identification of malfunctioning devices is the only sure way to reduce the cost of corrective actions. To achieve such a goal, analytical laboratories would require a protocol that enables interim checks on pipette performance to be made quickly and conveniently with a high degree of accuracy and precision.

The Artel Pipette Calibration System (PCS) is designed to address both economic and regulatory concerns. It is highly accurate and precise even at volumes as low as 0.1 µL and is built to check pipettes quickly and easily in working laboratories without the need for a special workstation or environmental controls. Using the PCS method, laboratories can readily increase the frequency of their pipette performance verifications — minimally affecting their operations. More frequent performance checks improve quality and ensure regulatory compliance by reducing the need for remedial follow-up resulting from "as-found" pipette failures that may have occurred several months beforehand.

**Picture 1:** The Artel PCS

**PRODUCT FOCUS:** ALL REGULATED PRODUCTS

**PROCESS FOCUS:** QUALITY CONTROL, ANALYTICAL

**WHO SHOULD READ:** LABORATORY MANAGERS AND TECHNICIANS, METROLOGISTS

**KEYWORDS:** PIPETTES, GLP, CALIBRATION, METROLOGY, PHOTOMETRY, GRAVIMETRY, LIQUID DELIVERY

**LEVEL:** BASIC

### How and Why Pipettes Fail

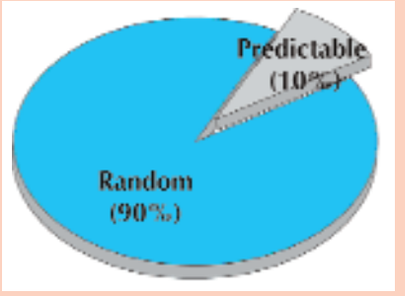
To fully understand the value and utility of the PCS technology, it is helpful to examine how and why pipettes fail. Failures arising from predictable wear —

as a direct result of how often a pipette is used and how frequently it is maintained — are termed *systematic failures*. Systematic failures in a pipette population can be reduced by fine-tuning maintenance and calibration intervals based on laboratory experience.

Systematic wear is an uncommon cause of failure in most pipette populations. Investigation of the root causes of pipette failure indicates that specific events, such as accidents or mishandling, cause most malfunctions. For example, an operator may inadvertently draw liquid into the pipette body, which can lead to premature piston corrosion or seal failure. Even storing a pipette horizontally can cause liquids to infiltrate its body. Failures that stem from such unplanned events are termed *random failures*. By definition, they cannot be predicted based on manufacturer recommendations — nor can they be effectively addressed by infrequent, scheduled maintenance.

Pipette failure data reported to Artel by independent calibration services and calibration system consultants affirm that typically about 10% of all pipette failures are systematic failures (Figure 1). Thus, random and unpredictable failures are typically about 90% of all pipette malfunctions.

**Figure 1:** The nature of pipette failures




## Silent Failures

In addition to being random, pipette failures cannot always be readily detected. Mechanical-action pipettes have numerous internal parts that can fail (Figure 2) and cause them to deliver incorrectly. Because the parts are contained within the body of the instrument, they are invisible to the user. Although some pipette failures are detectable by "look-and-feel," the vast majority are "silent" (undetectable by operators) — regardless of whether failure results from random events or predictable wear. A classic example of a silent pipette failure is a leaky internal seal, which can cause the pipette to "underdeliver," sometimes by a large margin, without an operator's knowledge.

## A Need for Interim Checks

Laboratories should verify pipette performance frequently enough to ensure acceptable data integrity and mitigate the need for remedial action. Given that most failures are random, biannual calibration and maintenance is unlikely to ensure continuous proper functioning for devices in regular use. This makes interim performance checks critical for quality assurance. Interim checks shorten the interval during which a faulty pipette may be in use and build quality into laboratory processes.

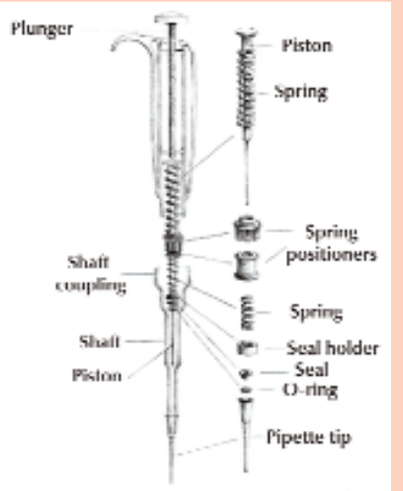
In addition to scheduled calibration and maintenance, it is often valuable to check pipette functionality as needed:

- Before and/or after procedures in which accuracy and precision are especially critical or when a high degree of confidence in the data is paramount.
- Any time damage is suspected, such as when a pipette has been dropped or used to dispense corrosive liquids.
- When problems are experienced with a particular test or procedure (to eliminate the pipette as a potential source of error).
- Whenever a pipette is associated with questionable data.
- To verify proper operation of all new pipettes as well as those that have been sent out for calibration and/or maintenance, before returning them to service.

## Transferability of Calibration

Metrologists are discussing concerns regarding the use of pipettes as transfer standards. It is widely reported — both by our customers and elsewhere — that pipettes freshly calibrated by accredited facilities, even a pipette manufacturer, may perform outside an analytical laboratory's established tolerances when checked in a less controlled environment (the conditions of use).

**Figure 2:** Parts of an air-displacement pipette (Artel illustration)




Changing environmental conditions represent a significant source of variability (**1**). When a pipette is moved from the calibration facility to a working laboratory, changes in temperature, barometric pressure, and relative humidity are likely. The effect of such changes is often reported anecdotally. However, it has also been studied systematically by several authors. In particular, the work of Karl Lochner is summarized as an informative annex (Annex B) to ISO 8655 Part 2 (**2**). Our company has also conducted its own experiments to further quantify the magnitude of errors attributable to transferability issues (**3**).

Through its impact on the rate of sample evaporation, relative humidity is by far the biggest environmental factor influencing liquid delivery (**4**). The smaller the volumes involved, the greater the variability. For example: A difference in relative humidity between the controlled conditions of a calibration laboratory (typically at or above 50%) and a working environment (probably closer to 35%) generally causes about a 3% difference in the volume of liquid dispensed when the target volume is 5 µL. When the target volume is 0.2 µL, that difference is commonly about 7%.

## Verifying Bench-Top Performance

Pipette QC programs are intended to ensure the quality of liquid delivery in working laboratories. Most such

**Table 1:** Outsourced services compared with bench-top calibration and verification

| Function | Outsourced or Centralized | Bench-Top Verification |
|---|---|---|
| Immediate performance verification as needed, such as preceding or following a critical procedure | No | Yes |
| Routine performance testing at frequent intervals[a] | No | Yes |
| Effective operator training | No | Yes |
| Calibration upon return to the laboratory | No | Yes |
| In-situ calibration of automated equipment | No | Yes |
| Uninterrupted use of pipettes | No | Yes |
| Optimum internal control of critical QC processes | No | Yes |
| Repair and preventative maintenance | Yes | No[b] |

a ASTM 1154 recommends a daily "quick check" for pipettes (**5**).

b A laboratory using bench-top verification and/or calibration may require a service provider, manufacturer, or in-house technician to perform maintenance and repair. This service provider should be fully qualified (provide a facility with appropriate environmental controls, instrumentation, supplies and parts, certified personnel, and rigorous methods traceable to national standards).

programs focus instead on how pipettes perform in metrology laboratories rather than on the results generated at the workstations where they are used. That can leave facilities open to the two concerns described above: insufficient performance verification to detect silent/random pipette failures and "calibrated" devices that do not meet specified tolerances for the facility when deployed in a working environment.

Many laboratories use an outsourced or centralized maintenance service to provide pipette quality control. Although qualified services can perform some tasks efficiently, as typically used they cannot adequately address silent and random pipette malfunctions or changes in tolerances due to environmental changes. By contrast, a pipette quality control program that includes bench-top performance verification addresses both sources of error (Table 1).

The ideal way to detect pipette failure is to perform calibrations and interim performance checks on site — in your laboratory — using the same operators working under the same conditions in which the devices are expected to function every day. This approach is optimal because it enables you to evaluate not only each pipette's state of repair, but also the effects of various environmental factors on the accuracy and precision of your calibration results.

## The Artel PCS System

The Artel PCS shown in Picture 1 provides an effective means to verify pipette performance at any time on site without taking pipettes out of service. The system can be a cost-effective adjunct to outsourced or centralized calibration services, or it can take over the calibration function completely. The PCS method is unaffected by fluctuations in temperature, humidity, or barometric pressure; it requires no special metrological skills; and it is fast and simple to perform by virtue of its high degree of automation.

Unlike the gravimetry method, our system enables personnel who are trained in proper pipetting technique to quickly and cost-effectively verify pipette performance on the bench-top where they work. The PCS is also well suited for training operators in pipetting technique because it provides immediate feedback on their results. These advantages further serve to simplify the standardization of quality practices and assay accuracy — even across multiple laboratories.

**Principles of Operation:** Our system combines a dual-dye photometric method with NIST-traceable reagents to provide an accurate and precise method for calibrating low-volume liquid delivery devices. With the PCS, performance can be verified at volumes as low as 0.1 µL and as high as 5000 µL.

The system consists of a compact and portable PCS instrument with a reagent kit made up of blanks and different dilutions of dye-containing sample solution. An easy-to-read screen-based interface guides operators through a simple protocol used to check a pipette. Volumes delivered are calculated automatically, along with other relevant statistics, and sent to the system printer and/or to a computer.

Optional Artel Pipette-Traker software allows for compliance with 21 CFR Part 11. The program provides a complete solution for the management of quality control for any pipette population and is tightly integrated with the PCS, combining ease of use with secure, retrievable data.

The PCS is fast and easy to use. In less than three minutes, an operator can calibrate a pipette using ten data points. A functional check with four data points that verifies pipette accuracy and correct operation requires about one minute. The steps involved in a pipette calibration or performance check are shown in the "PCS Procedure" box.

**Accuracy of PCS Versus Gravimetric Method:** The two most commonly used methods for verifying pipette performance are gravimetry and photometry. With gravimetry, a solution of known density (typically distilled water) is pipetted into a container, the weight of which is measured before and after pipetting. To achieve the accuracy necessary to calibrate a pipette, particularly at smaller volumes, the liquid temperature, air buoyancy, and sample evaporation must all be measured and corrected for (**6**).

With photometry, a sample aliquot is pipetted into a blank vial that contains a known volume of diluent. The sample solution contains a known concentration of a chromogen that has an absorbance peak at a convenient wavelength. After mixing the sample and diluent, the change in absorbance is measured to calculate the sample volume.

An inherent difference in the suitability of these two methods for pipette calibration becomes evident with decreasing sample volumes. At larger volumes (above 1 mL) the weight of a sample is comparatively large in relation to potential sources of uncertainty inherent in the gravimetric method (environmental conditions, vibration of

the balance, and so on), and a relatively inexpensive three- or four-place balance is adequate. At smaller volumes (e.g., 10 µL), however, the weight of each sample is so small that accurate measurement requires a five- or six-place balance and the utmost attention paid to factors such as evaporation and static electricity. Even under the most highly controlled conditions, measurement uncertainty may very well predominate and limit the accuracy and precision of results obtained.

By contrast, a photometric measurement method can simply use a more concentrated sample solution for small-volume dispenses. Sample evaporation after delivery, static electricity, and other environmental effects have no impact on the accuracy and precision of photometric results. This makes photometry inherently more accurate and precise than the gravimetric method for calibrating low-volume pipettes.

In precision and accuracy, the PCS and gravimetric methods are equivalent at larger volumes. At smaller volumes, however, the PCS provides significantly better accuracy and precision (**7**). A study at the Fraunhofer Institute for Silicate Research showed that the Artel PCS was five to seven times faster than the gravimetric method specified (**8**). The accuracy of the system was found to be as good as gravimetry for larger volumes and better than gravimetry for volumes ≤5 µL. A sevenfold reduction in the time required to verify pipette performance, with no decrease in accuracy, makes bench-top pipette checks practical.

**NIST Traceability of PCS Results:** The PCS instrument is calibrated by users in a simple process that uses NIST-traceable calibrators. They ensure the accuracy of absorbance ratios at 520 nm and 730 nm. In addition, every lot of reagents is tested for conformance at Artel's ISO 17025-accredited testing laboratory (A2LA Certification Number 2093-01). These tests and calibrations ensure that PCS results are fully traceable to NIST standards.

## PCS Procedure

**1. Insert a vial of reagent:** Operator inserts a blank vial containing an accurately prefilled volume of a known concentration of copper chloride into the instrument. The vial is locked into position and will not be moved for the duration of the procedure. The PCS reads absorbance of the vial contents at 730 nm using a photometer.

**2. Dispense the samples one at a time, using the pipette to be checked:** Operator now adds an appropriate concentration of sample solution to the blank vial. Six concentrations are available spanning the entire range of pipette volumes. The absorbance peaks of the blank and sample solutions do not overlap.

**3. Read the results of each dispense immediately on the PCS screen:** The instrument mixes the sample with the blank solution using an internal mixing mechanism. It then reads the absorbance at 520 nm with exceptionally low noise (<0.00015 A at A=1) and excellent linearity (better than 0.2% over the 0–1.5 A range). Based on this reading, the PCS calculates the volume of each aliquot of sample solution dispensed. Results depend only on the ratio of absorbances measured at 520 nm and 730 nm, so they are independent of vial imperfections and path length. The operator can now add another sample aliquot to the same vial, and the mix/read/calculate process will repeat. Absorbance is calculated automatically and the result displayed. Up to 22 samples per blank vial can be measured. In most cases, two or more pipettes of the same or different sizes can be checked using one blank vial.

**4. When all samples have been dispensed, remove the printed data record:** All results, including group statistics, are automatically printed at the conclusion of the performance verification procedure (Figure 3). These data are sufficient for quality assurance, laboratory management, and/or regulatory compliance purposes.

## Ensuring Accuracy and Precision

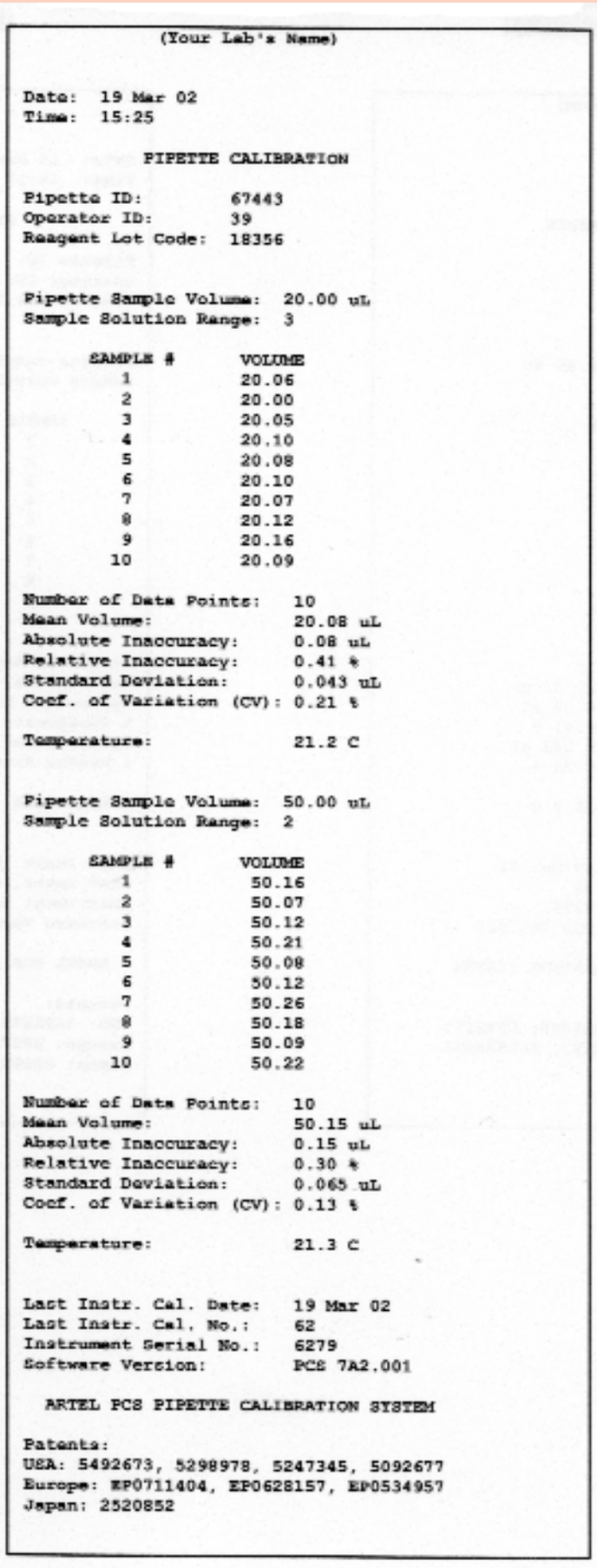
Although a small percentage of pipette malfunctions are predictable based on use, the majority of failures are unpredictable in the timing of their occurrence (random) and undetectable by operators (silent). Because pipettes tend to fail both silently and randomly, their performance should not be taken for granted.

Many pipette quality control programs do not check performance often enough relative to the high standards required in a regulated laboratory. To ensure correct operation and satisfactory accuracy and precision, pipettes should be checked regularly. Most pipettes will require repair given sufficient time and use. Regular performance verification is the best way to mitigate the risks associated with having defective pipettes in service.

The longer a defective pipette remains in use, the greater its potential to create negative consequences. Detection of a malfunctioning pipette may require your laboratory to examine data collected with that device since its last calibration. Obviously the sooner a failure is detected, the better. More frequent functional checks help minimize the need for remedial action. Robust pipette QC also helps reduce rejects, shorten approval cycles, and eliminate potential compliance problems before they happen. Equally important, a high standard for liquid delivery also ensures that a laboratory has fulfilled an ethical responsibility to its customers and to the community.

Gravimetry is the older method for pipette calibration. However, the conditions required for accurate gravimetry results at low volumes (a balance of sufficient resolution, finely controlled temperature, isolation from drafts and vibration, evaporation controls, etc.) are not generally available in working laboratories. The Artel PCS method is unaffected by environmental factors and is therefore suitable for use on the bench-top. At all

**Figure 3:** Sample printout from a pipette calibration made with the Artel PCS



(Your Lab's Name)

Date: 19 Mar 02
Time: 15:25

PIPETTE CALIBRATION

Pipette ID: 67443
Operator ID: 39
Reagent Lot Code: 18356

Pipette Sample Volume: 20.00 uL
Sample Solution Range: 3

| SAMPLE # | VOLUME |
|---|---|
| 1 | 20.06 |
| 2 | 20.00 |
| 3 | 20.05 |
| 4 | 20.10 |
| 5 | 20.08 |
| 6 | 20.10 |
| 7 | 20.07 |
| 8 | 20.12 |
| 9 | 20.16 |
| 10 | 20.09 |

Number of Data Points: 10
Mean Volume: 20.08 uL
Absolute Inaccuracy: 0.08 uL
Relative Inaccuracy: 0.41 %
Standard Deviation: 0.043 uL
Coef. of Variation (CV): 0.21 %

Temperature: 21.2 C

Pipette Sample Volume: 50.00 uL
Sample Solution Range: 2

| SAMPLE # | VOLUME |
|---|---|
| 1 | 50.16 |
| 2 | 50.07 |
| 3 | 50.12 |
| 4 | 50.21 |
| 5 | 50.08 |
| 6 | 50.12 |
| 7 | 50.26 |
| 8 | 50.18 |
| 9 | 50.09 |
| 10 | 50.22 |

Number of Data Points: 10
Mean Volume: 50.15 uL
Absolute Inaccuracy: 0.15 uL
Relative Inaccuracy: 0.30 %
Standard Deviation: 0.065 uL
Coef. of Variation (CV): 0.13 %

Temperature: 21.3 C

Last Instr. Cal. Date: 19 Mar 02
Last Instr. Cal. No.: 62
Instrument Serial No.: 6279
Software Version: PCS 7A2.001

ARTEL PCS PIPETTE CALIBRATION SYSTEM

Patents:
USA: 5492673, 5298978, 5247345, 5092677
Europe: EP0711404, EP0628157, EP0534957
Japan: 2520852

sample volumes, even as low as 0.1 µL, the system's accuracy and precision equals or exceeds the optimal results possible with gravimetry. In addition, the PCS automatically provides documentation for QC and regulatory purposes, and its results are traceable to NIST. Adoption of a pipette QC program including regular bench-top performance verification using the Artel PCS will help ensure consistent liquid delivery even in the most demanding bioanalytical environment.

## References


**1** Lochner, KH; Ballweg, T; Fahrenkrog, H-H. Factors Influencing the Measuring Accuracy of Piston Pipettes with Air Interface. *J. Lab. Med.* 1996, 20: 430–440.

**2** ISO 8655-2:2002. *Piston-Operated Volumetric Apparatus, Part 2: Piston Pipettes* (International Organization for Standardization: Geneva, Switzerland, 2002).

**3** Rodrigues, G. *Bias, Uncertainty, and Transferability in Standard Methods of Pipette Calibration* (white paper). Artel: Westbrook, ME, 2003.

**4** Clark, JP; Shull, AH. Gravimetric and Spectrophotometric Errors Impact on Pipette Calibration Certainty. *Cal. Lab.* 2003, 10(1): 36–43.

**5** *Standard Specification for Piston or Plunger Operated Volumetric Apparatus,* Specification E 1154-89 (Reapproved 1997). American Society for Testing Materials (ASTM), p. 5, paragraphs 11.2.2–11.2.3.

**6** *Laboratory Instrument Evaluation, Verification and Maintenance Manual,* 4th edition (College of American Pathologists: Northfield, IL, 1989).

**7** Curtis, RH. Performance Verification of Manual Action Pipets: Part 1. *Am. Clin. Lab.* 1994, 12(7): 8–10.

**8** Lochner, KH. Pipette Check with Gravimetric and Photometric Methods: A Comparison. *J. Lab. Med.* 1998, 22: 1–5.



*Corresponding author* ***George Rodrigues,*** *PhD, is a senior scientifist and* ***Barry Godowsky,*** *MBA, is vice president of sales at Artel, 25 Bradley Drive, Westbrook, ME 04092-2013, 1-888-406-3463; grodrigues@artel-usa.com, bgodowsky@artel-usa.com; www.artel-usa.com.*