Artel Lab Report

6

This publication provides technical information regarding the use, application, and metrology related to liquid handling instrumentation.

> **"** *...the key point is that the calibration schedule should be frequent enough to assure data validity...* **"**

FDA, *Guidance for Industry Good Laboratory Practices Questions & Answers* [1]

Calibration Frequency
for Pipettes

Understanding how pipettes fail

Silent Failures

Mechanical action pipettes, unlike the original glass pipette, contain many internal parts. Some pipette failures are evident, either to the eye or by the feel of the pipette action. In these instances, the operator is aware that the pipette is not operating correctly. However, when the internal mechanism of a pipette fails, and it is not obvious to the operator, a silent failure has occurred. For example, a corroded piston or a leaking seal could cause the pipette to deliver incorrectly—sometimes by a wide margin—undetected by the operator.

Silent Failure Data

Figure 1 shows data taken at a major biomedical research institution. Fifty-three adjustable 2-20 µL pipettes, then in service, were tested at 5 µL. Each point on the chart represents a pipette checked by a trained operator, using ten data points. Although all of the pipettes were in routine daily use, a number of them had failed and were performing outside the laboratory's established tolerances[2]. In all these cases, the operators were unaware that silent failures had occurred, and had not taken the pipettes out of service.

Random Failures

Pipette failure is considered random when it is due to accidents, misuse, or other unpredictable events. For example, an operator may accidentally draw liquid into the body of the pipette, causing piston corrosion or premature seal wear. In the real world of laboratory use, random failures cannot be prevented by infrequent scheduled maintenance.

As illustrated in Figure 2, random or unpredictable failures typically represent at least 90% of all pipette failures. In contrast, predict-able (hence preventable) failures are those that arise from normal wear, which are dependent on factors such as frequency of use and time since last maintenance. Predictable failures represent 10% or less of all pipette failures.
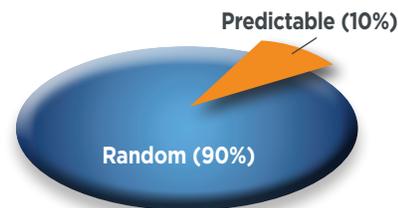
Figure 2. Pipette Failures in the Laboratory

Determining Calibration Frequency

Mean Time Between Failure
The average rate at which failures occur can be expressed as Mean Time Between Failure (MTBF). To determine MTBF, a group of pipettes is tracked to determine how long it takes each one to fail. A failure is defined as performance that falls outside the laboratory's established tolerances. The mean of all the failure times is the MTBF for that specific group of pipettes.

Once MTBF is determined, one can predict how long a pipette can be expected to maintain accuracy and precision.

MTBF, along with reliability level, QC principles, and regulations, combine to influence the development of a suitable calibration frequency. The MTBF for individual pipettes can vary significantly, depending on a number of factors, as shown in Figure 3.

Figure 1: As-found pipette performance
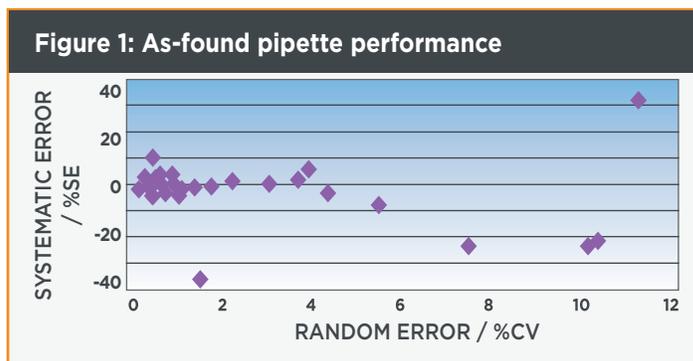
Target Reliability Level

Another essential element in the determination of calibration frequency involves establishing a level of target reliability for liquid delivery, based on the quality mandate of the laboratory. Reliability level is expressed as a percent: 95% reliability means that, at any given time, 95% of the pipettes in a population are working correctly, while 5% are generating incorrect results.

Factors to consider when establishing a target reliability level include assay precision, the potential impacts of failed pipettes on patient outcomes, legal defensibility of results, production batch release decisions, and so forth. Compliance with regulatory guidelines may also be an important factor.
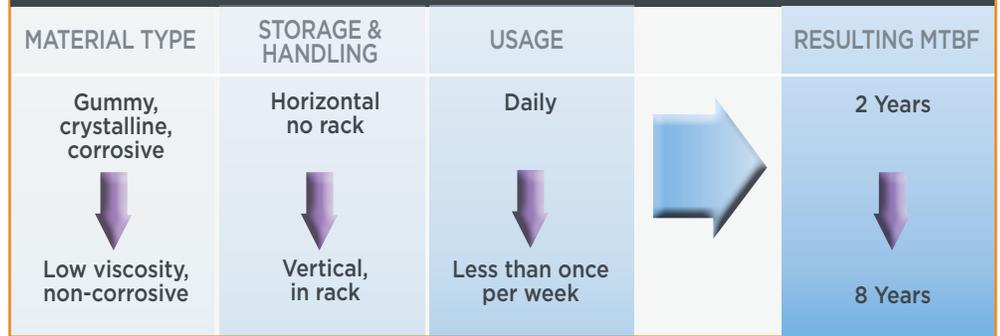
Given the established target reliability level for a laboratory and the MTBF for the pipettes, the graph in Figure 4 can be used to determine the required calibration frequency.

Example:

Suppose that the required end of period reliability level for pipettes is 95% and the MTBF of the pipettes is four years.

To determine the appropriate calibration frequency, follow the green line of Figure 4 until it meets the 95% level on the Y-axis. Then read down to the X-axis to find the required calibration interval: slightly more than two months. Therefore, checking the pipettes at two-month intervals will provide assurance that pipette performance meets the established quality mandate.

Figure 3. Factors contributing to Mean Time Between Failures (MTBF) for mechanical action pipettes

MATERIAL TYPE	STORAGE & HANDLING	USAGE		RESULTING MTBF
Gummy, crystalline, corrosive	Horizontal no rack	Daily		2 Years
↓	↓	↓	⇒	↓
Low viscosity, non-corrosive	Vertical, in rack	Less than once per week		8 Years

QC Principle

Mechanical action pipettes are precision laboratory instruments. For that reason, they should be subject to the same quality control principles as other sensitive instruments, such as spectrophotometers and balances. Just as is required for these instruments, calibration should be performed on a regular basis to verify pipette performance.

The more frequently calibration is performed, the sooner malfunctioning pipettes will be detected and taken out of service. In addition, more frequent calibration can help eliminate the need to review laboratory data to ensure that incorrect liquid delivery by a failed pipette has not compromised laboratory results. The longer a defective pipette remains in service, the greater the liability it presents in this regard.

Regulations

In order to build quality into laboratory results, the instruments used in the process must be in good condition and properly calibrated. Regulations and standards published by organizations such as the U.S. Food and Drug Administration (FDA)[1], and ASTM[6] International provide minimum requirements that help ensure the quality of laboratory results. These form the groundwork upon which a laboratory should establish its frequency of pipette calibration, as part of good quality control practices.

Regulations specify that all laboratory instruments used in production—pipettes included—must be routinely calibrated at suitable intervals. In particular, FDA GLP[3], GMP[4], and QSR[5] requires that control of measurement test equipment include procedures for establishing calibration intervals.

The Clinical and Laboratory Standards Institute (CSLI) recommends that pipettes (single and multi-channel) and automated liquid handlers be calibrated every 3 to 6 months. A minimum of two volumes must be tested (nominal and lowest setting) with ten replicates at each volume. An additional test at 50% of nominal volume is recommended[7]. Therefore, both the MTBF described in this article and applicable regulations and guidelines should be considered.

Figure 4. Calibration frequency for pipettes, based on target reliability level and estimated Mean Time Between Failures (MTBF)
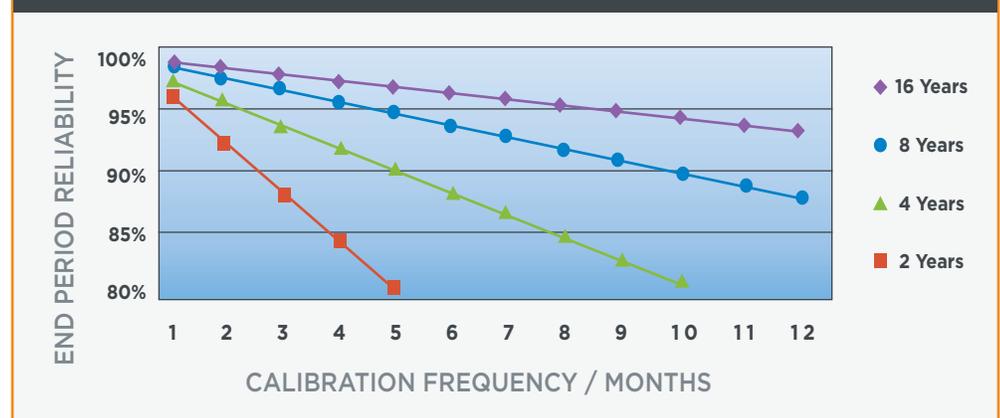
Conclusion

Whenever pipettes are used in a procedure, the corresponding laboratory results depend on the accuracy of pipette delivery. The quality control measures adopted for pipettes should therefore be consistent with quality control measures taken for other instruments in the laboratory.

Since pipettes are subject to silent and random failures, and have a higher rate of failure than most other laboratory equipment, the most important aspect of pipette quality control is a calibration frequency that achieves sufficiently high reliability. Calibration frequency is a function of the MTBF for the devices used in the lab, the lab's desired reliability level, and its established QC principles. Keep in mind also the important regulatory guidelines that pertain to your laboratory, to use as a foundation for establishing an appropriate calibration frequency.

> Establishing an appropriate calibration frequency will minimize the chances that laboratory results are comprised by incorrect liquid delivery, helping to ensure traceability, accountability and confidence in the results.

Q&A

Q: If I am running controls, why do I need to be concerned about checking my pipettes?

A: Controls provide an important check on laboratory results. However, a control that falls within established limits does not provide a guarantee that all sample results are correct. For instance, if a pipette is failing intermittently due to leaking seals, then the controls may pass, yet some of the sample results would be incorrect due to pipette imprecision. A failed control tells you, at the end of the testing process, that something was wrong with the process, materials, or equipment. And, indeed, this was the way "quality" was achieved in many laboratories in past years. More recently, however, most laboratories have become convinced that it is both less expensive and more reliable to build quality into a laboratory result up-front than it is to discover the problems at the end of the process.

Q: How often do we need to perform preventive maintenance (cleaning, lubrication, seal replacement, etc.) on our pipettes?

A: Manufacturers recommend maintenance anywhere from annually to every four years. While these recommendations provide a good starting point, maintenance schedules should be based on laboratory experience. The purpose of routine maintenance is to minimize the occurrence of predictable failures. A failed pipette should be examined to determine whether or not the failure was random (due to an accident or misuse), or predictable (the result of simple wear). Events that result in random failure will usually leave evidence; such as material aspirated into the pipette body, or damage to the shaft. Failures resulting from accumulated wear generally do not show these types of evidence. If a significant number of your failed pipettes do not show evidence of random failure, then you can assume such failures are due to wear, and you should consider increasing the maintenance frequency.

Q: If I perform regular preventive maintenance on my pipettes, do I need to worry about calibration?

A: Yes. Pipette failures can happen silently at any time, at any point during your maintenance interval. Because failure can occur immediately after accidents or misuse, preventive maintenance cannot adequately protect against these random sources of failure. Note also that the random nature of most pipette failure in the everyday laboratory environment is not reflected in data from some pipette manufacturers. To obtain their data, these manufacturers subject their pipettes to a series of repetitive stress tests, carried out by laboratory robots under ideal conditions, resulting in predictable wear and gradual failures. Preventive maintenance can only prevent predictable failures. However, random (i.e., unpredictable) failures are best detected by the laboratory's established pipette calibration protocols. Effective calibration protocols, combined with appropriate preventive maintenance, comprise the best way to ensure accurate pipettes.

Q&A

Q: I use the "tip drip test," aspirating liquid into my pipette tip and observing whether any liquid drips out. Is this as sufficient a check as calibration?

A: In a high-volume pipette, the "tip drip test" will sometimes indicate a seriously leaking seal. Unfortunately, with low-volume pipettes, surface forces prevent liquid from dripping out of the pipette tip, even when seal leakage is very severe. And even with high-volume pipettes, a tip drip test may not uncover other problems, such as intermittent leakage, or leakage during only one part of the pipetting cycle. These types of failures are best detected during calibration.

Q: Do the same checking guidelines apply for multi-channel pipettes as for air displacement, single-channel pipettes?

A: The same guidelines do apply. For a multi-channel pipette, it is important to check the function of each channel, since they can develop problems independently. A practical procedure would be to verify one channel, using ten data points, at each of three different volume settings. Then verify that the other channels are performing properly, by using fewer data points at two volume settings.

Q: Our pipettes undergo a vacuum test after maintenance or repair. Does that mean we don't need to calibrate them?

A: No. Calibration is still essential to ensure correct pipette operation. A vacuum test can only detect air leaks. While it can usually determine whether new seals and o-rings have been installed correctly, a vacuum test tells you nothing about whether the pipette is correctly adjusted to deliver the proper volume. A further concern is that vacuum testing frequently cannot detect small leaks; it is therefore not suitable even as a leak test for low-volume pipettes. Additionally, in regulated environments, guidelines mandate full performance verification before reintroducing a device into service. This explicitly renders vacuum testing an inadequate substitute for pipette calibration. In short, vacuum testing is no bill of good pipette health where accuracy and precision are concerned.

References:

1. Guidance for Industry. Center for Drug Evaluation and Research, Food and Drug Administration, July, 2007

2. Artel Lab Report 5: "Setting Tolerances for Pipette Performance"Available on the web: **www.artel-usa.com/Lab_Reports.aspx**

3. Code of Federal Regulations (CFR), Title 21, Part 58.63 **www.ecfr.gov**

4. Code of Federal Regulations (CFR), Title 21, Part 211.160 **www.ecfr.gov**

5. Code of Federal Regulations (CFR), Title 21, Part 820.72 **www.ecfr.gov**

6. Standard Specification for Piston or Plunger Operated Volumetric Apparatus, ASTM E 1154-14.

7. Clinical and Laboratory Standards Institute, General Laboratory Equipment Performance Qualification, Use a nd Maintenance, 2019 : clsi.org/standards/products/quality-management-systems/documents/qms23/

8. Performance Verification of Manual Action Pipettes, Part I and Part II. Richard H. Curtis, American Clinical Laboratory, October and December, 1994.

9. General requirements for the competence of testing and calibration laboratories. ISO/IEC 17025:2005.