Improve Proficiency Test Results and Assay Integrity with Pipette Quality Control

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Abstract

Pipette calibration frequency significantly impacts the quality of liquid delivery in the clinical laboratory. Specifically: 1) pipettes are prone to silent, random failures that may contribute to erroneous laboratory and/or proficiency test results; 2) pipette performance may not be verified frequently enough to ensure confidence in laboratory results.

Methods: Data was taken at a major biomedical research institution to illustrate “as found” performance of 53 adjustable volume pipettes that were in service.

Data was taken from independent calibration services to determine the percentages of predictable failures versus silent, random pipette failures.

Results: A high percentage of “as found” pipettes performed outside established specifications for precision and accuracy, yet the operators were unaware that silent failures were occurring and had not taken these malfunctioning pipettes out of service.

Random or unpredictable failures typically represent at least 90% of all pipette failures. Predictable failures resulting from systematic wear represent 10% or less of all pipette failures.

Conclusions: Liquid delivery results depend on pipette accuracy and precision. Quality control measures adopted for pipettes should therefore be consistent with those taken for other laboratory instruments.

Since pipettes are subject to silent and random failures and have a higher rate of failure than many other laboratory instruments, the most important aspect of pipette quality control is a calibration frequency that ensures sufficiently high reliability.

Introduction

The mechanical-action, air-displacement pipette is an indispensable device in the clinical laboratory—so much so that its proper functioning and use are often taken for granted. This mind-set presents cause for concern with respect to the quality of pipetting, especially in those assays where accuracy and precision are most critical.

Where proper pipette functioning is not adequately verified, critical test results may be compromised. Likewise, external quality assessments, such as proficiency tests, may yield incorrect results.

The data presented in this poster, taken at a major biomedical research institution and from independent pipette calibration services, illustrate that:

- Pipettes are subject to failure in ways that are undetectable even to highly skilled technologists. A high percentage of pipettes in use in clinical laboratories may therefore be performing outside of established specifications for precision and accuracy.
- Pipette failures most often result from random and unpredictable events, such as accidents or misuse, rather than from systematic, predictable wear. Such failures cannot be predicted, and can occur at any point in the service cycle.
- Many existing Quality Control programs do not adequately address the issues that impact the quality of liquid delivery.

Of the many sources of error that can occur in clinical testing, pipette functioning is relatively easy to control and cannot be overemphasized.

Silent Pipette Failures

Mechanical action pipettes, unlike the original glass pipettes, 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 user 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.

Figure 1 shows data taken at a major biomedical research institution. Fifty-three adjustable 2-20µL pipettes, then in service, were tested at 5µL. Each point on the chart represents a pipette checked by a trained operator, using ten data points.

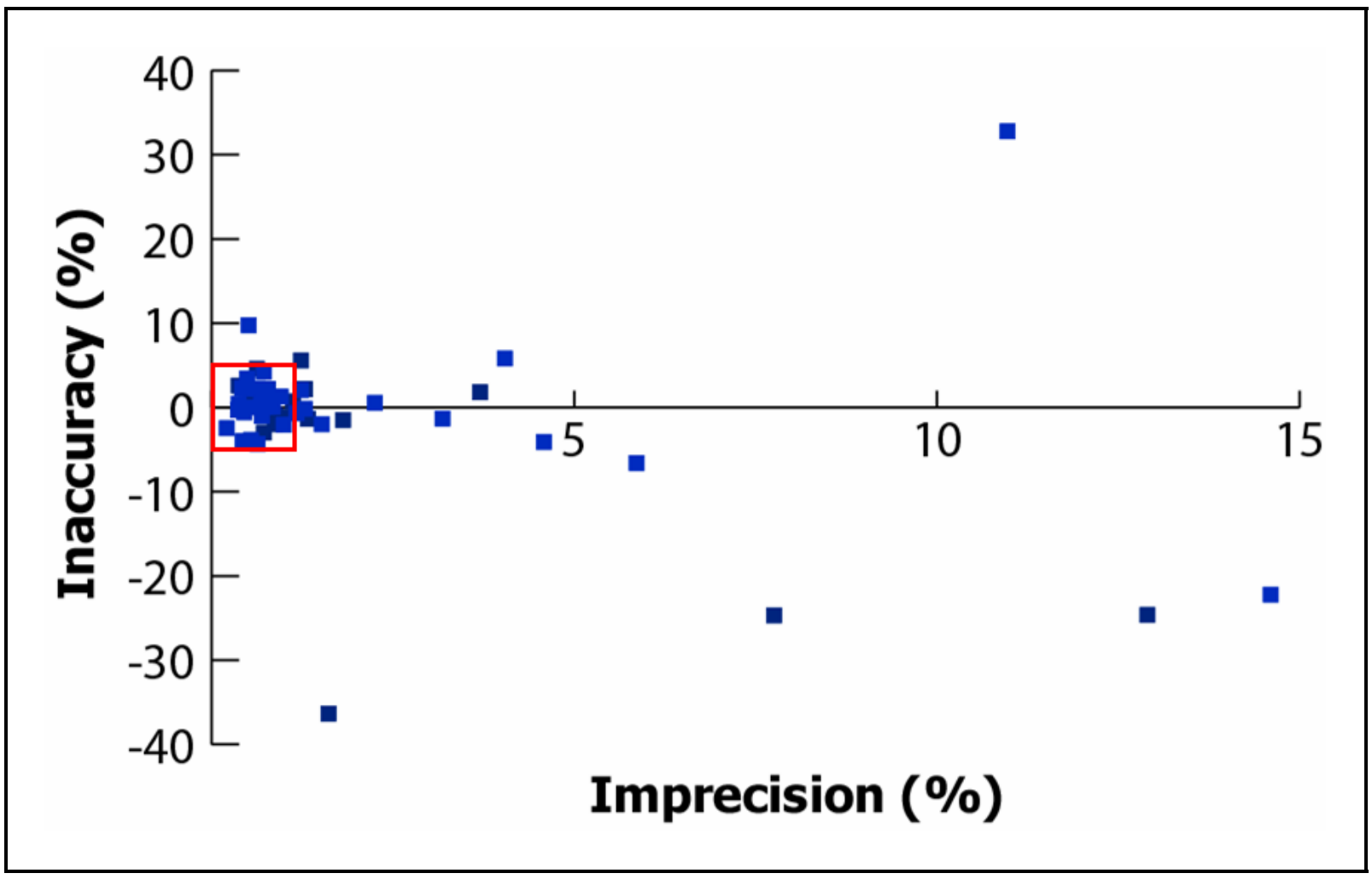

Figure 1: As-found pipette performance.

Although all of the pipettes checked were in routine daily use, a number of them (those data points not within the red box) had failed and were performing outside the laboratory's established specifications. Yet in all of these cases, the operators were unaware that silent failures were occurring, and had not taken the malfunctioning pipettes out of service.

Figure 1 also illustrates the fact that when pipettes fail, both precision and accuracy are likely to be adversely affected. This belies the common assumption that pipettes tend to "drift" out of tolerance, and will continue to deliver with precision even when improperly adjusted.

Random Pipette Failures

Pipette failure is considered *random* when it is due to accidents, misuse, or other unpredictable events. For example, a technician may accidentally draw liquid or serum 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.

In contrast, predictable (hence preventable) failures are those that arise from normal wear, and are dependent upon factors such as frequency of use and time since last maintenance.

Figure 2 illustrates failure data reported to ARTEL by independent calibration services and calibration system consultants. These data affirm that predictable failures usually comprise about 10% of all pipette failures. Thus, random or unpredictable failures typically represent about 90% of all pipette failures.

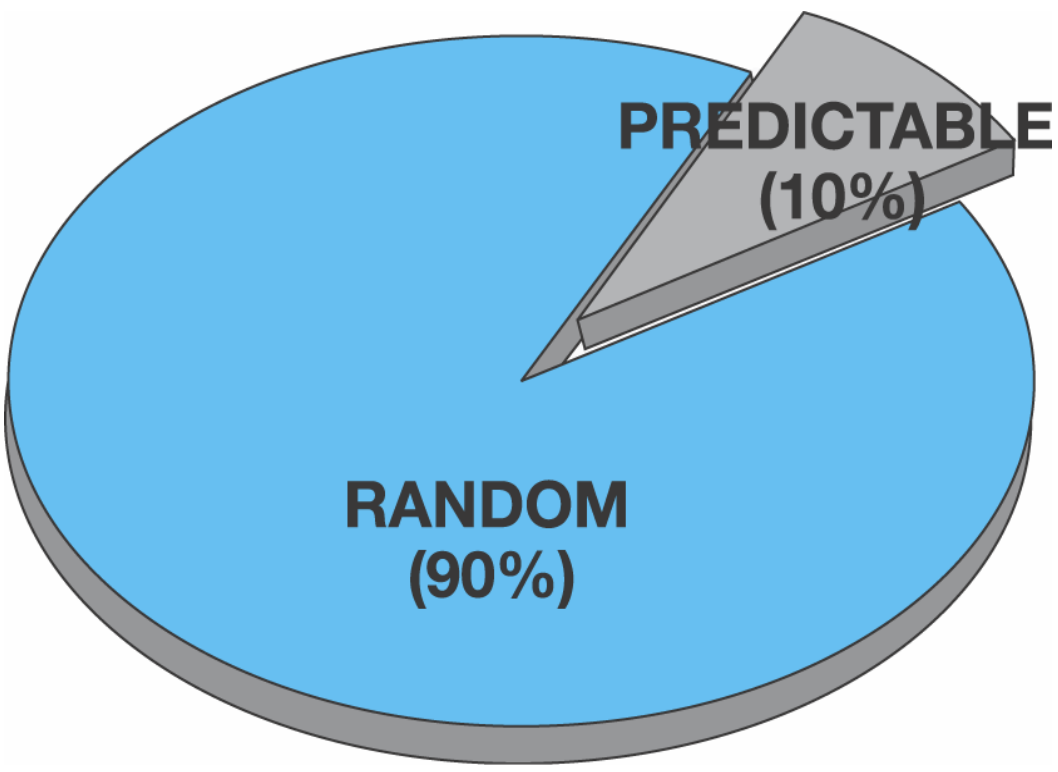

Figure 2: The nature of pipette failures in the laboratory.

Ensuring Assay Integrity

The random, silent nature of many pipette failures requires laboratories to verify the performance of their pipette populations with a frequency that is sufficient to ensure confidence in laboratory test results. The sections that follow suggest a “best practices” method for establishing an optimal pipette calibration frequency for specific laboratory environments.

Optimizing Calibration Frequency

The optimal calibration frequency for a given pipette population can be developed by examining a combination of factors:

- Mean Time Before Failure
- Target Reliability Level
- Quality Control Best Practices
- Preventive Maintenance
- Applicable Regulations

Mean Time Before Failure

The average rate at which failures occur can be expressed as Mean Time Before 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 specifications. 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. The MTBF for individual pipettes can vary significantly, depending on a number of factors, as Table 1 illustrates. Additional parameters, such as the preventive maintenance interval, may also be relevant.

Table 1: Factors Contributing to MTBF for Mechanical Action Pipettes

Material Type	Storage & Handling	Usage		Resulting MTBF
Gummy, crystalline, corrosive ↓ Low viscosity, non-corrosive	Horizontal, no rack ↓ Vertical, in rack	Daily ↓ Less than once per week	→	1 yr ↓ 4 yrs

Table 1: Factors contributing to MTBF for pipettes.

Optimizing Calibration Frequency

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, audit defensibility of clinical test results, failed proficiency tests, etc. Compliance with regulatory guidelines such as CLIA and CAP 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 3 can be used to determine the required calibration frequency.

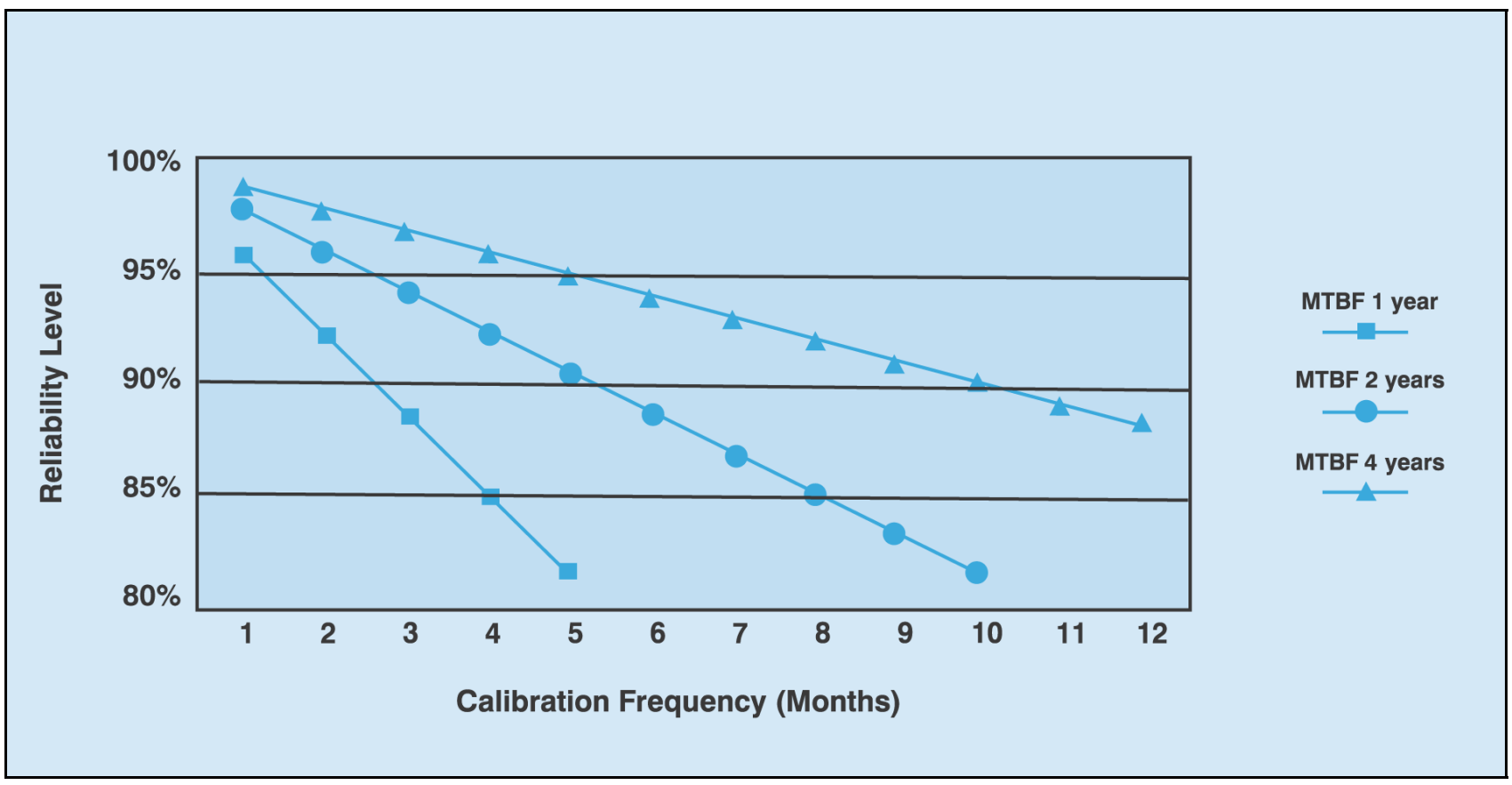

Figure 3: Calibration frequency for pipettes based on target reliability level and MTBF.

Example:

Suppose that the required target reliability level for pipettes is 95%, and the MTBF of the pipettes is two years. To determine the appropriate calibration frequency, follow the middle line of Figure 3 to where it meets the 95% level on the Y-axis. Then scan down to the X-axis to find the required calibration interval: approximately three months. Therefore, checking the pipettes at three-month intervals will ensure that pipette performance meets the established quality mandate of 95% reliability.

Optimizing Calibration Frequency

Quality Control Best Practices

Mechanical action pipettes are precision instruments, which play a critical role in a wide range of clinical assays. For that reason, they should be subject to the same quality control principles as other laboratory instruments, such as spectrophotometers and balances. Just as is required for these instruments, pipette calibration should be performed on a regular basis to verify pipette performance.

The more frequently calibration is performed, the sooner pipettes that are not operating correctly 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 particular failed pipette has not compromised clinical assays or proficiency test results. The longer a defective pipette remains in service, the greater the liability it presents in this regard.

A suggested best-practices QC program should include the following elements:

- Assign all pipettes to specific users, assays, or workstations, so that suspect results can be readily identified.
- Verify performance of pipettes often enough to ensure confidence in assay results, taking into account the MTBF for pipettes in the population. In some cases, this will entail performance verification immediately preceding and/or following a critical assay.
- Immediately verify the performance of any pipette that is “suspect;” i.e., one that has just been mishandled, or that is associated with questionable test results.
- Perform preventive maintenance (cleaning, seal replacement, re-lubrication) on a routine basis as determined by established MTBF.
- Calibrate all pipettes immediately following maintenance.

The use of an accurate, precise, and easy-to-use bench-top calibration system, such as the ARTEL PCS®, will greatly facilitate the implementation of this type of QC program.

Optimizing Calibration Frequency

Preventive Maintenance

The purpose of routine maintenance is to minimize the occurrence of predictable failures. Manufacturers recommend maintenance anywhere from annually to every four years. While these recommendations provide a starting point, maintenance schedules should be based on laboratory experience.

Pipette malfunction can occur silently, at any point during the maintenance cycle. Therefore, preventive maintenance cannot adequately protect against these random sources of failure. 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 and precise pipettes.

Pipettes that fail 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 failed pipettes do not show evidence of random failure, then one can assume such failures are due to wear, and should consider increasing the maintenance frequency.

Applicable Regulations

In order to build quality and reliability into laboratory results, the instruments used in the process must be in good condition and properly calibrated. Regulations (CLIA) and standards published by organizations like CAP and NCCLS provide minimum requirements that help ensure the quality of clinical laboratory results.

Regulations specify that all laboratory instruments—pipettes included—must be regularly calibrated. Standards also recommend a calibration frequency relating to their usage and MTBF. For example, ASTM International guidelines recommend that a comprehensive evaluation be performed quarterly, with monthly quick checks to evaluate pipette performance.

Summary

Whenever pipettes are used in the clinical laboratory, the validity of test results depends on the accuracy and precision of pipette delivery. The quality control measures adopted for pipettes should therefore be consistent with quality control measures taken for other instruments in the clinical laboratory.

Since pipettes are subject to silent and random failures, and have a higher rate of failure than many other laboratory instruments, the most important aspect of pipette quality control is a calibration frequency that ensures sufficiently high reliability.

Optimal calibration frequency is a function of:

- Mean Time Before Failure
- The laboratory's desired reliability level
- Quality Control best practices
- Preventive maintenance
- Applicable regulations and standards

References

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