Improving Method Validation Results by Optimizing Pipette Calibration Frequency

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Abstract

Analytical method validation is predicated on the assumption that all equipment employed in the process under consideration is functioning within acceptable tolerance limits. The air displacement laboratory pipette is a precision instrument utilized in a wide range of laboratory processes. Yet, pipettes are frequently overlooked as a source of inaccuracy and imprecision in method development and validation results.

This poster reports on the relationship between the frequency of pipette calibration and the variability of liquid delivery. Specifically:

- Pipette malfunction represents a significant and insidious problem, since failures introduced by wear and mishandling are typically both random in their occurrence and undetectable by the operators.
- Many laboratories verify pipette performance infrequently; perhaps only once or twice annually. Many silent, random pipette failures are thus not rectified in a timely manner. The resulting variability in assay results can negatively affect both method development and method validation.
- An adequate program of pipette performance verification—above and beyond infrequent, scheduled maintenance—provides the means to minimize the impact of these unpredictable and undetectable failures.

Guidelines will be presented for establishing an optimal pipette calibration frequency for specific laboratory environments.

Introduction

In order to achieve valid results, analytical method development and validation practices depend on the assumption that the equipment involved is functioning correctly, and within established tolerances. For the majority of devices used in regulated laboratories, the high degree of attention devoted to these issues ensures that all equipment works as expected.

In the case of the air displacement pipette, however, proper functioning is frequently not adequately validated. Consequently, critical results can be compromised. The data presented in this poster illustrate that:

- Pipettes are subject to failure in ways that are undetectable even to highly skilled analysts.
- Pipette failure most often results from random events, such as accidents or misuse, rather than from 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, even where method validation results are at stake.

Silent Pipette 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.

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.

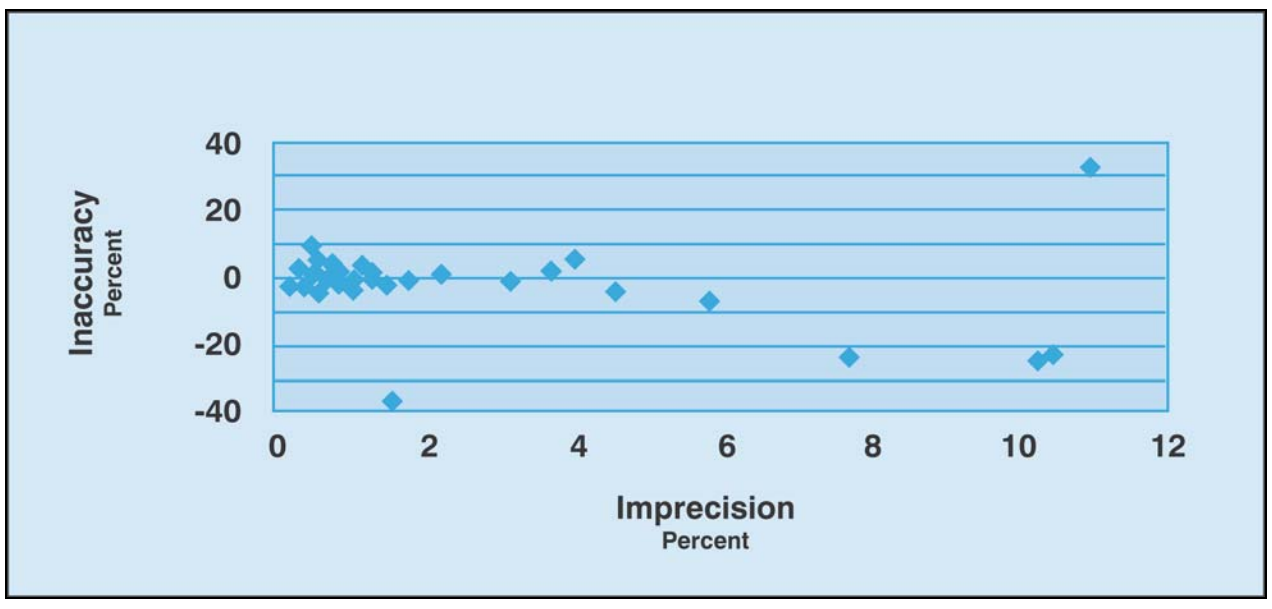

Figure 1: As-found pipette performance.

Although all of the pipettes checked were in routine daily use, a number of them 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, 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.

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

Figure 2 illustrates failure data from independent calibration services. These data show that predictable failures represent 10% or less of all pipette failures. Random or unpredictable failures typically represent at least 90% of all pipette failures.

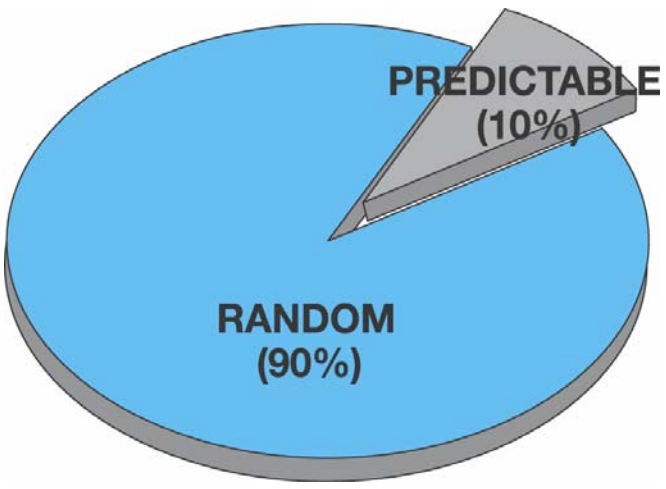

Figure 2: The nature of pipette failures in the laboratory.

Ensuring Data 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 acceptable data integrity. The sections that follow outline a methodology for establishing an optimal 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 shows. 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	Horizontal, no rack	Daily	→	1 yr
↓	↓	↓		↓
Low viscosity, non-corrosive	Vertical, in rack	Less than once per week		4 yrs

Table 1: Factors contributing to MTBF for mechanical action 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 performing outside of established tolerance limits.

Factors to consider when establishing a target reliability level include assay precision, the potential impacts of failed pipettes on research outcomes, audit defensibility of results, production batch release decisions, and so forth. Compliance with regulatory guidelines for remedial follow-up based on error tracking requirements 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.

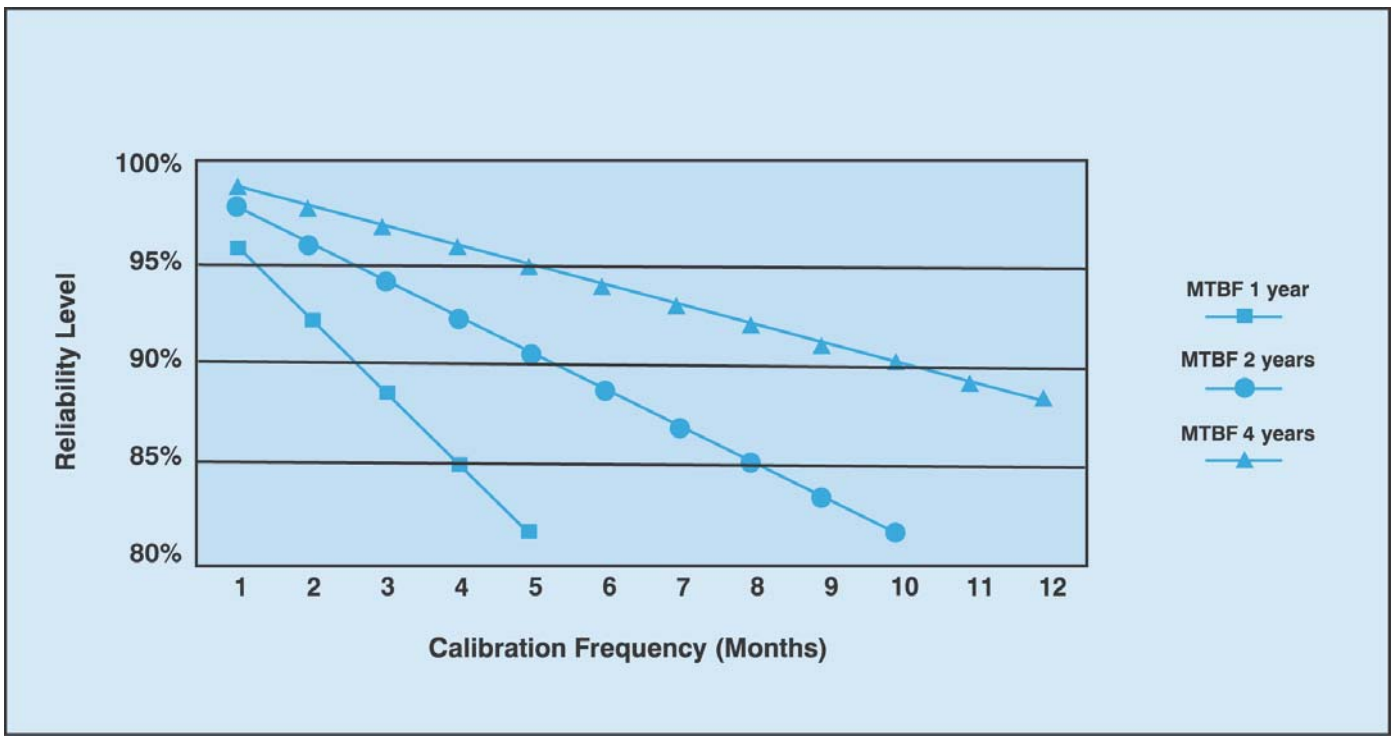

Figure 3: Calibration frequency for pipettes based on target reliability level and MTBF.

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 provide assurance that pipette performance meets the established quality mandate of 95% reliability.

Optimizing Calibration Frequency

Quality Control Best Practices

Mechanical action pipettes are precision laboratory instruments, which play a critical role in method validation. 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, 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 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:

- Assignment of all pipettes to specific operators, assay methods, or workstations, so that suspect results can be readily identified.
- Verify performance of pipettes often enough to ensure data validity, 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 procedure.
- Immediately verify the performance of any pipette that is “suspect;” i.e., one that has just been mishandled, or that is associated with questionable data.
- 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. 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 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 method validation results, the instruments used in the process must be in good condition and properly calibrated. Regulations and standards published by organizations like ISO and ASTM International provide minimum requirements that help ensure the quality of laboratory results.

Regulations specify that all laboratory instruments—pipettes included—must be regularly calibrated. Regulations also state that all such instruments should be checked with a frequency relating to their usage and MTBF. To summarize FDA guidelines: "…the key point is that the calibration schedule should be frequent enough to assure data validity…"

Summary

Whenever pipettes are used, whether in method validation or in any other laboratory procedure, the results depend 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 laboratory.

Since pipettes are subject to silent and random failures, and have a higher rate of failure rate 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

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