Pipet Quality Control: A Microliter of Prevention...

by Ralph Bertermann

In the day-to-day operation of a chemical, analytical, quality, or testing laboratory, significant attention is often focused on key analytical instruments, such as spectrophotometers and HPLC systems. These devices cost many thousands of dollars to purchase, and are also expensive to calibrate and maintain. There is no question that their proper calibration and maintenance is essential to the laboratory's operations, as well as to ensure compliance with regulations and to produce valid data.

There is, however, one critically important component of virtually every laboratory's operations that is used in a wide range of methods, but that is often overlooked when it comes to calibration and maintenance: the ubiquitous air displacement pipet. After all, what is there to worry about where pipets are concerned? Are they not simple, reliable devices that are easy to use with minimal training? Isn't annual or semi-annual pipet calibration enough to ensure data validity and compliance with regulations? In a word, no.

Taking pipets for granted

Contrary to widely held beliefs, the air displacement pipet is a precision instrument, capable of consistent volume deliveries in the range below 1 mL. Even so, pipets can introduce significant data variability if not used and maintained properly.

Since pipets are precision instruments, they are subject to the same regulations that apply to the calibration and maintenance of other critical laboratory instruments. Pipets therefore require:

- Periodic calibration according to a documented procedure, along with periodic functional checks to ensure proper ongoing performance
- Periodic maintenance and proper handling
- Operation by persons who have demonstrated competence in using the instrument.

The GLP and cGMP regulations,[1] in line with recommended practices and various international standards, are very clear as to what is expected in order to ensure the proper operation of an instrument in a regulated environment. These sources of guidance all echo the same requirements for calibration, a sampling of which is shown in Table 1.

From a regulatory standpoint, one key reason why it is important to ensure a high standard of pipet performance is that, in the event of an as-found failure to meet specifications, the laboratory is required to investigate whether or not the data obtained with that failed pipet could have adversely affected measurements associated with research, quality, or product safety. More frequent verification of pipet performance, in the form of periodic functional checks, can help to reduce this risk, and greatly simplify the time and expense associated with this needed remedial action.

Table 1 Risk of undetected pipet failure for different performance verification intervals

Regulation	Relevant excerpt
21 CFR Part 58:63[2]	"Equipment used for the generation, measurement, or assessment of data shall be adequately tested, calibrated, and/or standardized."
21 CFR Part 211:68	"If such equipment is so used, it shall be routinely calibrated, inspected, or checked according to a written program designed to ensure proper performance."
21 CFR Part 211.164[3]	"The calibration of instruments, apparatus, gauges, and recording devices at suitable intervals in accordance with an established written program containing specific directions, schedules, limits for accuracy and precision, and provisions for remedial action in the event accuracy and/or precision are not met. Instruments not meeting established specifications shall not be used."
21 CFR Part 606.60[4]	"The equipment shall be observed, standardized and calibrated on a regularly scheduled basis as prescribed in the Standard Operating Procedures Manual and shall perform in the manner for which it was designed so as to assure compliance with the official requirements prescribed in this chapter for blood and blood products."
21 CFR Part 820:72[5]	"Each manufacturer shall ensure that all inspection, measuring, and test equipment … is suitable for its intended purpose and is capable of producing valid results."
ANSI/NCSL Z540-1-1994[6]	Calibration Laboratories and Measuring and Test Equipment—General Requirements (9.1): "All measuring and testing equipment having an effect on the accuracy or validity of calibrations shall be calibrated and/or verified before being put into service."
ISO 9001:2000[7]	Quality Management Systems—Requirements (7.6): "Where necessary to ensure valid results, measuring equipment shall: a) be calibrated or verified at specified intervals, or prior to use, against measurement standards traceable to international or national measurement standards; where no such standards exist, the basis used for calibration or verification shall be recorded."
ISO/IEC 17025:2000[8]	General Requirements for the Competence of Testing and Calibration Laboratories, (5.6.1): "All equipment used for tests and/or calibrations, including equipment for subsidiary measurements (e.g. for environmental conditions) having a significant effect on the accuracy or validity of the result of the test, calibration or sampling shall be calibrated before being put into service."

As-found failures: A case in point

The concerns expressed below (paraphrased) by a manager whose job responsibilities include pipet quality control at a pharmaceutical API QC laboratory are echoed by many laboratory supervisors and managers regardless of the class or type of instrument being discussed, including pipets.

> Under our formal calibration program, we send pipets out for calibration every six months. Often several pipets are found to be out of tolerance. Our calibration department then sends us a form so we can document whether our data results were impacted. By the time we know about the problem we've already shipped product, and it may have already been consumed by patients. What can we do at this point? We have no choice but to conclude that there were no significant impacts to the

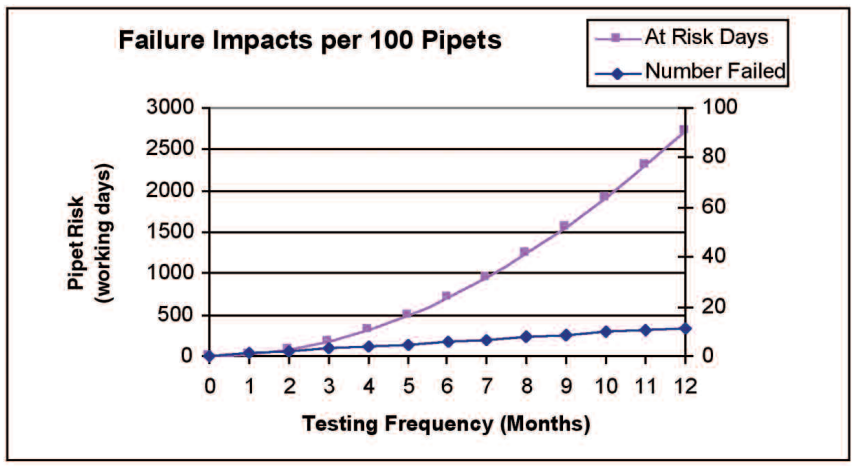

Figure 1 Risk of undetected pipet failure for different performance verification intervals.

data—that's the reality. We'd all feel better if we could know for sure that our pipets are working properly before we release the product.

The key to peace of mind in such situations is to verify pipet performance more frequently. As Figure 1 shows, the number of "at risk" days during which a laboratory is exposed to the consequences of undetected pipet failure rises, and falls, sharply relative to the frequency of performance checks.

Take, for example, a laboratory that strives to maintain a target reliability level for pipets of 95%, and which verifies the performance of its population of 100 pipets every six months. If five pipets test outside of established tolerance limits, following is the number of working days of data that have been placed at risk and must be re-evaluated for mitigation purposes: five devices × six months × 30 days/month = 640 days.

If that same laboratory verifies pipet performance every four months, following is the risk in working days: three failed devices × four months × 30 days/month = approx. 360 days of data at risk. If pipets are tested every three months, the risk drops to approx. 180 days.

Sources of liquid delivery variability

As with all precision instruments, it is vitally important to understand how pipets function, and how best to handle and operate them to ensure valid and repeatable performance and results. It is also important to understand what factors influence the accuracy and precision of pipets, and what operator training is required to use them effectively. Documented schedules and procedures for calibration (with traceability of measurements to national standards), along with periodic maintenance, are required to ensure that each pipet is functioning within the laboratory's established tolerances.

Pipetting variability arises from these principal sources:

- Systematic failure. Some pipet failures occur as a result of predictable wear, based on factors such as frequency of usage or time since last maintenance.
- Random failure. Pipet failure commonly results from random events, such as accidents or mishandling, as opposed to systematic wear. Such failures cannot be accurately predicted, and they occur randomly with respect to the service cycle.
- Operator technique. Inconsistent or incorrect pipetting technique is probably the largest single source of liquid delivery variability in most laboratories. Operators frequently have not been trained in the correct use of pipets.
- Environmental factors. Pipets perform somewhat differently under different environmental conditions, relative to changes in temperature and humidity.
- Device tolerance limits. A small amount of variability in liquid delivery is due to inaccuracy and imprecision inherent in the pipet itself. These values are typically known based on the manufacturer's specifications for the device, which usually reflect best-case performance.

For the purposes of this article, discussion is confined to the first three sources of variability, since these are by far the most significant.

Systematic versus random failures

Systematic pipet failures are those that arise from simple wear, as a function of how often the pipet is used and how frequently it is maintained. Systematic failures can often be predicted and prevented by adjusting the service cycle and calibration interval based on a

review of the as-found performance history of the device.[9,10]

Random pipet failures are those due to accidents, mishandling, or other unplanned events. An operator may inadvertently draw liquid into the pipet body, for instance, leading to piston corrosion. The simple act of storing the pipet in a horizontal position may lead to the migration of liquids into the body of the pipet. In the laboratory environment, random failures cannot be prevented by infrequent, scheduled maintenance. This is simply because these failures are not dependent on predictable factors such as usage frequency or time since last maintenance. Random failures can (and do) occur at any point in the maintenance cycle.

Silent failures

An important aspect of the majority of pipet malfunctions, whether due to unplanned events or predictable wear, is that they are silent in nature. Mechanical action pipets have a number of hidden internal parts (seals, piston, etc.) that can fail, thereby causing the pipet to deliver incorrectly. While some pipet failures are detectable by "look and feel," the majority of failures are silent, that is, they are undetectable by the operator. A typical example of a silent pipet failure is a leaking internal seal, which can cause the pipet to underdeliver, sometimes by a large margin, without the operator's knowledge.

Operator technique

In order to help ensure that products meet quality and safety requirements, all personnel must be qualified for their particular assignments. This is spelled out in the regulations, per 21 CFR Part 211.25 (a), which states, "Each person engaged in the manufacture, processing, packing, or holding of a drug product shall have education, training, and experience, or any combination thereof, to enable that person to perform assigned functions."[3]

As regards pipets, this means that 1) operators who have assigned pipetting duties as part of laboratory methods must demonstrate competency in pipetting technique. 2) An ongoing investment must be made in operator training. In particular, pipet technique training must be conducted on a regular basis. 3) Operator competence to perform assigned duties must be documented via training records.

Based on published studies,[11] as well as the author's personal observations, it is clear that many experienced laboratory technicians do not employ proper or consistent pipetting technique. Moreover, even properly trained technicians exhibit person-to-person variability in technique that can affect results. Simple steps such as prewetting the pipet tip, immersing the tip to the proper depth, and allowing liquids and equipment to equilibrate to ambient temperature can significantly improve accuracy and precision. (A concise list of "10 Tips" for proper pipetting technique is available on-line at www.artel-usa.com/tips.htm.)

The good news is that adequate training is simple and inexpensive to provide, and can greatly improve pipetting technique.

Compliance requirements for pipets

A quality control program for liquid delivery should start with an understanding of what regulations require in the way of records and operational practices. As with other precision instruments, audit risks for pipets include:

- Undetected pipet failures or improper operation of devices in use
- Inadequate calibration/maintenance data
- Inadequate training data
- Inadequate QC (no established tolerances, no established calibration frequency, no provision for on-site performance verification, etc.).

In order to ensure compliance with regulations, the following should be established for pipets:

- Appropriate and realistic tolerance limits for accuracy and precision based on the laboratory's process requirements and historical experience. Manufacturers' specifications are based on ideal conditions and are typically too stringent for real-world applications; hence, reliance on them can result in false failures, requiring a costly, documented follow-up process. On the other hand, setting tolerances too low can negatively affect process quality.
- A realistic target reliability goal based on the laboratory's quality mandate. Too low a level may reduce quality and increase noncompliance risks. Too high a level may result in higher cost to customers. In a benchmarking

survey conducted by NCSL International (formerly the National Conference of Standards Laboratories) in Boulder, CO, the majority of respondents set reliability goals for general-purpose measurement and test equipment at 90% or greater.
- An optimal calibration frequency, based on the established target reliability level and the mean time before failure (MTBF) for the pipet population.
- A program to train operators in proper pipetting technique.
- A program for pipet calibration and interim performance checks on the benchtop. Infrequent, off-site calibration does not adequately address the leading sources of liquid delivery error: silent and random malfunction and improper technique. Benchtop calibration not only facilitates more timely detection of nonsystematic pipet failures, thereby reducing the need for remedial action, but also provides a means to evaluate and train operators in proper technique.

Best practices for pipet quality control

Pipets are precision laboratory instruments, and are therefore subject to the same quality control regulations as other sensitive instruments such as spectrophotometers and analytical balances. This includes regular calibration.

The longer a defective pipet remains in service, the greater the potential for negative consequences. The more often calibration is performed, the sooner defective pipets will be detected and taken out of service. More frequent functional checks can also help to minimize the need for remedial action.

A QC program for pipets should include the following best practices:

- Verify performance of pipets often enough to ensure data validity, based on the laboratory's experience with MTBF for the pipet population.[12]* Performance verification protocols should include provisions for on-site performance verification immediately preceding and/or following a critical procedure, in order to mitigate the potential impact of a malfunction. These intermediate checks should be carried out according to defined procedures (see ISO 17025, 5.5.10).
- Assign all pipets to specific operators, assay methods, or workstations to facilitate the identification of problematic results.
- Train all operators in proper pipet operation and pipet storage practices, and verify their capability periodically on the benchtop, under every-day working conditions.
- Immediately verify the performance of any pipet that has just been dropped or otherwise mishandled, or that is associated with questionable data.
- Verify proper operation of all new pipets received from a manufacturer, as well as pipets that have been sent out for calibration, before placing them in service. This quality concern is specifically addressed by ISO 17025, 5.5.9, which states: "When, for whatever reason, equipment goes outside the direct control of the laboratory, the laboratory shall ensure that the function and calibration status of the equipment are checked and shown to be satisfactory before the equipment is returned to service." This point is important because pipets calibrated at an environmentally controlled facility often deliver incorrectly when returned to the working environment. The ISO 8655:2 standard, for example, estimates the magnitude of imprecision in this context due to typical differences in humidity, temperature, and other factors to be on the order of 5%.[13]
- Perform preventive maintenance (cleaning, seal replacement, relubrication) on a routine basis as determined by the established MTBF. Calibrate all pipets immediately following maintenance.

*Ten data points are adequate to verify both accuracy and precision. Four data points are sufficient as a "quick" check" (per ASTM 1154) or other form of benchtop verification process to assess correct and/or accuracy alone.

Conclusion

A program to ensure accurate and precise pipet performance is not only a good idea; it is a legal requirement. The consequences of noncompliance, such as remedial follow-up and/or the invalidation of critical data, can be more time-consuming and expensive than implementing a proper quality assurance program in the first place. In short, taking pipets for granted can be a painful and costly mistake.

Why aren't laboratories more diligent about the quality of liquid delivery? It usually comes down to the issue of cost relative to perceived benefit. Many times, the belief that pipets are beneath concern is rooted in a lack of understanding of the nature of pipet failure, coupled with an attitude of complacency.

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The reality is that cGMP-compliant pipet QC makes solid competitive sense. It pays for itself many times over in cost and time saved through averting the need for remedial action and assay repeats. Consider the many hours of paperwork required just to check assay results, methods, etc., when a pipet is finally found to be operating incorrectly. Add to that the additional resources wasted if the condition was significant enough to impact results. These expenses, even if they are incurred infrequently, far outweigh the cost of maintaining a high standard of pipet performance in the first place. A microliter of prevention up front can result in kilograms of savings later.

Robust pipet QC can also reduce rework and rejects, compress approval cycles, and eliminate potential compliance problems before they happen. Equally important, a standard for liquid delivery that ensures compliance with regulations also ensures that a laboratory has fulfilled an ethical responsibility to its customers and the community. For all these reasons, building quality into results up front should not be on the wish list in today's business and regulatory environment; it should be standard operating procedure.

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