by Wendy Vaccaro

Handling liquid is one of the most common processes in life science laboratories, whether they're drug-discovery and compound-management laboratories or analytical chemistry, genomics, or proteomics facilities. Processes in which liquids are handled include sample preparation, dilution, standards preparation, and reagent addition.

The means for delivering liquid samples have advanced dramatically over time, from the traditional glass micropipette to today's electronic, variable-volume pipettes and automated


Artel's extreme pipetting expedition in Death Valley National Park

The Laboratory Environment as a Source of Error

liquid handlers. Liquid-handling processes have become further complicated by a radical reduction in the average volumes handled. Combined with the potentially significant consequences of liquid- handling error, such as noncompliance, wasted time and money, inefficient use of scarce samples and compounds, and false data, it's clear that liquid handling can be a major source of risk. Processes must be put in place to monitor, manage, and minimize this risk. Liquid-handling quality assurance (LHQA) is necessary in all laboratories.

For laboratory quality assurance, all critical tasks must be standardized, including liquid handling. Laboratory LHQA programs commonly focus on pipette calibration, repair, and maintenance, and rarely include protocols on verifying operator technique. Yet, just as using malfunctioning pipettes and automated liquid handlers can result in inaccurate delivery volumes and laboratory results, so too can poor pipetting skills.

Laboratory technicians must be trained in proper pipetting skills, and techniques must be standardized within and across laboratories to minimize error and facilitate data comparability. Otherwise, data integrity may be at risk.

This article discusses common pipetting- technique errors, their effects on pipetting accuracy and precision, and how effective training programs can minimize the risk of error.

How operator technique contributes to error

Laboratorians pipette every day, yet many have never received formal training in the process. Like most repetitive, common tasks, pipetting is often taken for granted. It's important to understand the steps that laboratorians can take to improve pipetting skills. It's also helpful to evaluate common pipetting errors that can contribute to volume variation, all of which can be avoided with proper training.

Common pipetting errors include:

• **Failure to pre-wet the pipette tip**. If pipette operators don't pre-wet the pipette tip prior to initial

It's easy to overlook laboratory environmental conditions and the effects that they can exert on the quantitative dispensing of liquids with hand-held pipettes.

To explore the effect of environmental conditions on pipetted volumes, ARTEL launched the Extreme Pipetting Expedition. During this multiphase, year-long scientific study, ARTEL visited locations with extreme ranges of commonly encountered laboratory conditions. Pipette performance was measured at each location using the ARTEL Pipette Calibration System to identify the resulting volume variability.

Mission No. 1: Mount Washington

To kick off the expedition, ARTEL trekked to the Mount Washington Weather Observatory, situated at an altitude of 1,917 meters (6,288 feet). The barometric pressure at this altitude was measured at 805 millibar, well below the sea-level average of 1,013 millibars.

Data collected by ARTEL show that at this altitude, air-displacement pipettes (the type most commonly used) underdelivered by 1 to 10 percent, depending on the pipette's make, model, and target volume. This volume variation is explained by the lower density of air at higher altitudes. To transfer liquid volumes, today's laboratories largely rely on pipettes and liquid handlers that function by air-displacement mechanisms. If air is less dense, less liquid is aspirated into the pipette tip and subsequently dispensed, resulting in underdelivery and possible test failure.

Laboratories can compensate for repeatable volume variation caused by barometric pressure by adjusting the internal mechanism of the pipette or by adjusting the delivery setting.

Mission No. 2: Yellowstone

Yellowstone National Park was chosen as the next site for the Extreme Pipetting Expedition because it is emblematic of thermal variation and disequilibrium. The studies at Yellowstone provided clear evidence that pipettes show a bias in volume delivery when dispensing fluids at temperatures different than the pipette itself, specifically underdelivery of warm liquids and overdelivery of cold liquids. The error was especially significant when handling small liquid quantities and, as expected, error was present but lessened when working with larger liquid volumes.

Pipette calibration regulations stipulate stringent control of temperature during calibration (20°C ± 0.5°C), and that all materials, including pipettes, tips, and liquids, be thermally equilibrated prior to calibration. Such thorough thermal equilibration is difficult to achieve in a typical laboratory setting. Many common assay protocols require the dispensing of reagents that are not in the specified temperature equilibrium. Common examples are tissue culture applications, which employ reagents at 37°C, assays with nucleic acid-based reagents at 4°C or lower, and polymerase chain reaction samples transferred at 60° C or higher.

Since the magnitude of error caused by thermal variation is dependent on a number of protocol-specific details, such as pipette set volume, tip size, and temperature of the sample, a one-size-fits-all correction factor does not currently exist. ARTEL will continue to study thermal disequilibrium to help laboratories develop strategies to minimize this source of error.

delivery, sample volume can be lost due to evaporation within the tip. Aspirating and expelling sample liquid at least three times before delivery can lessen this risk. Pre-wetting is especially important when handling volatile solutions such as organic solvents.

• **Disregarding temperature**. Sample volume can be altered if the pipette and the liquid being dispensed aren't temperature-equilibrated. This is because air-displacement pipettes are affected by air pressure, relative humidity, and the liquid's vapor pressure, all of which are temperature-dependent. Human body heat can also be transferred from the user to the pipette if it's handled for too long, which also causes volume variation. The problem of hand warming can be mitigated by relaxing the grip and holding the pipette loosely while pausing between repeated dispenses. Similarly, the pipette should be returned to its stand, rather than held in the hand, during longer breaks in pipetting.

• **Tip wiping**. Unnecessary tip wiping can lead to sample loss, especially if the technician is wiping with absorbent materials, which can remove sample from the pipette tip. To avoid sample loss, minimize tip wiping and use great care when wiping. Liquids that cling to the outside of a pipette tip can often be removed without any problem if the absorbent material used to wipe the tip is kept clear of the pipette tip orifice. When wiping the pipette, use the cloth's edge to dab the droplet.

Mission No. 3: Death Valley

For Mission No. 3, ARTEL went to Death Valley National Park to test the effect of dry and hot environmental conditions on pipetted volumes. Dry heat was chosen as a scientific focus because many laboratory technicians encounter dry conditions due to the type of analytical instruments and other laboratory equipment (such as ovens, incubators, and freezers), and the lack of humidifiers in their work environments.

At Death Valley, ARTEL found that pipettes underdeliver by up to 35 percent in dry and hot environments. While volume-delivery errors were partially reduced by pre-wetting pipette tips, underdelivery still persisted, and the pipettes were found to operate outside of manufacturers' specifications in most instances.

As found in previous missions, errors were greater when pipettes were set to their minimum volumes than when they were set to their maximum volumes. When working with larger liquid volumes, errors were still induced but were of a smaller magnitude.

Liquid-handling error in dry heat is largely due to evaporation. The evaporation of a miniscule amount of liquid inside the pipette tip has a large effect on pipetted volumes, especially when target volumes are in the microliter range. When one microliter of liquid evaporates, it converts into more than 1,000 microliters of gas, expanding by a factor of 1,250 to 1,450, depending on temperature. This expansion prevents the pipette from aspirating the desired target volume.

Based on the data found in Death Valley, pre-wetting pipette tips at least five times is highly recommended to reduce the error when pipetting in dry and hot environments.

• **Choosing the wrong pipetting mode**. The choice of reverse or forward mode during pipetting is often arbitrary or based on operator preference. However, reverse mode should be used only for samples that leave a film inside the tip. During reverse mode, the plunger is depressed completely (i.e., past the first stop) to aspirate the sample and then depressed only to the first stop to deliver it. Therefore, using reverse mode with aqueous solutions leads to overdelivery; using forward mode with viscous solutions results in underdelivery.

Forward-mode pipetting is the preferred mode that nearly all manufacturers specify for use. Reverse-mode pipetting can introduce as much as a 5-percent error when compared to normal-mode pipetting using the same conditions. For liquids that stick to plastic tips and leave a noticeable film, or for organic solvents with high vapor that present a dripping problem, reverse mode is recommended.

• **Working too quickly**. After aspirating, failing to pause with the pipette tip in the liquid can lead to underdelivery. This is because the liquid isn't still at first insertion and requires about one second to settle. It's recommended that the user pause for one to two seconds after aspiration before removing the tip from the liquid being aspirated to allow the solution in the tip to come to a complete standstill. This pause also ensures that the operator isn't prematurely withdrawing the pipette tip from the solution, which can cause underdelivery of or disruption within the sample.

• **Pipetting at an angle**. Touching the pipette tip to the container sides during aspiration results in loss of sample. In addition, removing the pipette at an angle can cause volume variation due to surface tension effects, especially when pipetting small volumes. Pulling the pipette straight out of the container minimizes error.

• **Using the wrong pipette tips**. Choosing an incorrect tip for a given type of pipette can lead to an inadequate seal between the pipette and tip, causing leakage and sample loss.

Pipette tips come in many shapes and sizes. When trying to obtain the most accurate and precise results possible with a given pipette, the quality of the tip fit is what matters most. Tips made by the pipette manufacturer often yield the best fit because they're specially made to provide a tight seal between the tip and the pipette shaft.

Tips purchased from third-party vendors may be desirable due to their lower price, but the difference in material and seal quality may cause inaccuracies in pipetting results.

Why training is important

The need to include pipetting training in laboratory quality assurance programs is growing due to several trends in today's life sciences industry. First, laboratories are working with smaller liquid volumes and more complex multipart tests, including serial dilution protocols and polymerase chain reaction (PCR) assays, where inaccuracies of one microliter can be detrimental. Because microliter quantities are more sensitive to volume variation, operator variability can significantly alter research results.

Another trend is a shortage of trained laboratory professionals, and a corresponding increased focus by regulatory bodies on ensuring operator competency. International standards such as ISO/IEC 17025--"General requirements for the competence of testing and calibration laboratories," and ISO 15189--"Medical laboratories--Particular requirements for quality and competence," place a strong and appropriate emphasis on assessment of operator competency.

Minimizing risk through training

By providing technicians with proper training from expert professionals in liquid handling, laboratories can minimize the risk of volume variability caused by operators. However, to reduce the risk of laboratory error, a comprehensive and standardized method of training is essential.

The first step in improving operator technique is educating users about the mechanical function of modern pipettes, which work through air displacement, and the variables that can affect accuracy and precision. This portion of the training should also include an overview of the various types and brands of pipettes and when it's best to use each, as well as how to select the proper tips. It's also important to review existing regulations and quality standards and to illustrate how technique can alter laboratory data.

After providing a foundation of knowledge about pipettes, demonstrating proper technique and hands-on coaching should follow. This stage of the training is most effective when coupled with measurement technologies that can provide immediate feedback, so operators can instantly verify how various physical actions alter final volume and change their technique accordingly.

As with any class or seminar, measuring learning and proficiency is essential. With pipetting technique-training programs, pre- and post-training skills assessment is necessary to gauge improvement in pipetting accuracy and precision.

Standardized measurement tools that document the effectiveness of a training program, along with standardized training, can be used to facilitate certification. This can provide laboratory managers with objective evidence and documentation to support the pipetting competency and consistency of their technicians and will prove useful as regulations continue to advance.

It's not enough to train just the pipette operators. Training is important for laboratorians at all levels, whether they're on-the-bench scientists and technicians or laboratory managers. Process-control-focused training can also be beneficial for quality and laboratory managers and supervisors. This level of training can include information about pipette repair and maintenance, detailed information about relevant regulations and quality standards, and calibration technologies. Best practices for development and implementation of liquid-handling quality assurance programs must also be addressed.

Finally, pipetting-technique training shouldn't be a one-time event. Because operators contribute to laboratory error through inconsistent pipetting technique, instrumentation isn't the only thing that needs frequent calibration or checks. Annual or semiannual training is optimal to ensure that operators pipette consistently.

About the author

Wendy Vaccaro is technical services manager at ARTEL. She's responsible for all after-sales support as well as developing and delivering training programs and services that educate customers on the proper usage of volume-measurement products, pipetting-technique certification seminars, and other services to ensure quality and promote best practices in liquid handling. Visit online at www.artel-usa.com.

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