

Five good reasons

# The argument for pipetting technique training

By A. Bjoern Carle, PhD

Liquid handling quality assurance is a critical component of an effective laboratory quality program. While quality assurance efforts in liquid handling typically place much emphasis on pipette calibration, repair, and maintenance, ensuring pipette operator competence is a crucial, albeit often neglected, area. Yet, improper pipetting technique can undermine the quality of a laboratory's liquid handling processes and potentially compromise the integrity of results as much as malfunctioning pipettes.

For a number of reasons, it is vital for laboratory managers to consistently and continually monitor pipette operator competence and provide scientifically based training.

### Reason one

**Common pipetting technique errors lead to volume discrepancies.** The amount of liquid dispensed by pipettes and other liquid handling instruments is influenced by a number of factors. Some of these are easy to control; others—including the properties of the liquids being handled and environmental conditions such as temperature and humidity<sup>1,2</sup>—are difficult or impossible to control. The pipetting skill of an operator, however, is one factor affecting the accuracy and precision of liquid delivery volumes that can be controlled.<sup>3</sup> Some operators are not aware that even minor alterations in pipetting technique can result in significant volume discrepancies. Even highly experienced laboratory technologists may never have received formal pipetting training and may be prone to routine errors. Some of the most commonly encountered pipetting technique errors that can cause volume transfer discrepancies include the following:

*Failure to pre-wet the pipette tip.* Aspirating and fully expelling liquid at least three times before aspirating for delivery increases the humidity within the pipette tip and reduces sample loss due to evaporation. Failure to pre-wet pipette tips prior to sample delivery may increase evaporation of sample into the tip air space, which will likely result in lower delivery volumes.

*Improper tip immersion depth.* Immersing the tip too deeply into the sample might force additional sample into the tip, leading to over-aspiration and hence over-delivery. It may also increase the risk of sample carry-over by droplets clinging to the outside of the tip. Touching the container

bottom with the pipette tip may restrict aspiration and reduce sample volume. Alternately, not immersing the tip deeply enough, particularly with larger pipettes, can lead to accidental aspiration of air and result in reduced volume delivery.

For accurate volume delivery, pipette tips should be immersed adequately below the meniscus before aspirating. Large volume pipettes (1-5 mL nominal volume) should be immersed 4 to 6 mm, while small volume pipettes should be immersed 2 to 3 mm below the meniscus.<sup>4,5</sup>

*Choosing the wrong pipetting mode for a sample type.* The choice of using reverse or forward mode during pipetting is often based on operator preference. When using forward mode (also referred to as standard mode) pipetting, the operator depresses the plunger to the first stop, immerses the tip in the liquid, and aspirates it by releasing the plunger. To dispense the entire volume, the operator must depress the plunger beyond the first stop, usually to a second stop, which is often referred to as blow-out mode. Using this mode yields better accuracy and precision except when handling viscous or volatile liquids.

In reverse mode pipetting, the operator depresses the plunger completely (past the first stop) to aspirate the sample and then depresses it only to the first stop to deliver the sample, leaving a small sample aliquot in the tip. If reverse mode pipetting is used with common aqueous solutions, the pipette tends to deliver more than the desired volume. On the other hand, using standard mode pipetting with viscous samples may result in under-delivery, as part of the aspirated sample may remain in the tip.

*Errors while aspirating.* A number of errors are common during aspiration of sample solution. First, failure to pause with the pipette tip in the liquid after sample aspiration may lead to under- or over-delivery. This variability arises from the fluctuation of the liquid level in the tip immediately following the aspiration of sample. For accurate dispenses, operators should allow the liquid level to equilibrate for approximately one second before removing the tip from the sample vessel.

Second, aspirating sample and removing the pipette at an angle from the sample container may cause volume variation due to surface tension effects. This will be particularly pronounced when pipetting small volumes. Touching the pipette tip to the container sides during aspiration may

also cause under-aspiration of sample due to flow restrictions and surface tension effects.

### Reason two

**Volume errors can lead to false assay results.** Volume delivery errors caused by common pipetting technique mistakes can erode data quality, especially when pipetting small volumes. As laboratories increasingly handle smaller sample volumes, which are often subjected to complex multipart tests, improper pipetting technique might have significant negative impacts on the final results.

Concentrations of assay components are directly correlated to the relative volumes of each reagent within the mixture. As assay reactions are concentration-dependent, the delivery of accurate volumes of all reactants is essential for achieving correct and reliable results.

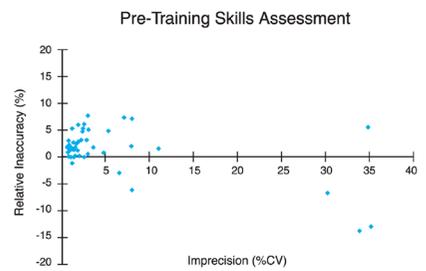
If assay interpretations are based on poor data, false conclusions may be drawn, which might have significant consequences for laboratories. In the clinical context, an incorrect test result may lead to a misdiagnosis, wrong treatment, or the unnecessary prescription of medications, for example.

### Reason three

**Technique training can improve volume accuracy and precision.** Comparing data on accuracy and precision of pipetted volumes by trained versus untrained pipette users strongly supports the case for pipetting technique training. Proper training of pipette operators yields significant improvements in volume delivery, accuracy, and precision, thereby enhancing laboratory data integrity.

The improvements in accuracy and precision resulting from technique training are evident when comparing **Figures 1 and 2** (p. 60), which show the pipetting results of 53 quality control laboratory technicians at four major biopharmaceutical laboratories before and after pipetting technique training.

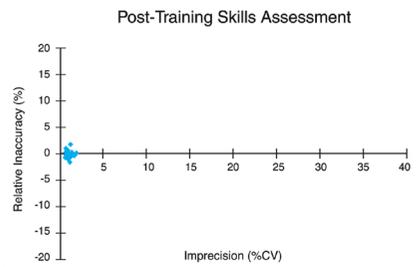
**Figure 1** shows pre-training data. Each technician's pipetting skill was evaluated by using a properly functioning and calibrated pipette to deliver five replicates of 10  $\mu$ L aliquots of sample. The relative inaccuracy and imprecision (coefficient of variation or %CV) were calculated ( $n = 5$ ) for each operator and are plotted as one data point in **Figures 1 and 2**. These data show that, without proper instruction, both



**Figure 1. Pipetting skills assessment of 53 QC technicians prior to receiving pipetting technique training, dispensing 10  $\mu$ L aliquots of sample (n = 5).**

imprecision and inaccuracy can be significant (up to 35% CV and 15% inaccuracy), even among professional technicians with many years of experience.

Quite a different situation is presented in **Figure 2**, which shows the volumes dispensed by the same technicians after having received training in pipetting technique, but otherwise following the same protocol as described above and using the same pipette. An improvement in accuracy and precision is evident in this post-training performance assessment, leaving inaccuracy and imprecision values below 2.5%.



**Figure 2. Post-training data for the same technicians, performing the same skills assessment protocol, with the same pipette, as described for Figure 1.**

## Reason four

**Regulatory bodies are focusing increasingly on personnel skills.** Regulatory bodies are including language in their standards and guidelines about requiring continuous assessment and documentation of personnel skills to ensure quality outcomes, reproducible results, and a high level of confidence in the performed work. Several standards published by the International Organization for Standardization (ISO) include references to operator competence, its assessment, and documentation.<sup>6-7</sup>

ISO 17025 (*General Requirements for the Competence of Testing and Calibration Laboratories*) stipulates that laboratories must prove their ability to generate technically valid results and demonstrate competent performance of their equipment, procedures, and personnel. This standard explicitly states<sup>6</sup> that “Personnel performing specific tasks shall be qualified on the basis of appropriate education, training, experience, and/or demonstrated skills, as required.” With this strong language, ISO 17025 goes beyond other published guidelines. For example, the Food and Drug Administration’s Good Manufacturing Practices (GMP) regulations require “education, training and experience,” but not demonstrated skills. “Demonstrated skills” means documented evaluation of personnel competency at important tasks, such as pipetting.

ISO 15189 (*Medical Laboratories - Particular Requirements for Quality and Competence*)<sup>7</sup> also calls for medical laboratory management to maintain records of personnel competency, including “demonstrated skills.” This standard is a guide for medical laboratories developing quality management systems to assess their own competence, and for accreditation bodies assessing and confirming their competence.

For clinical reference laboratories, ISO 15195 (*Laboratory Medicine - Requirements for Reference Measurement Laboratories*) is the relevant standard. It also requires the documentation of personnel qualifications and training.<sup>8</sup> This standard forms the basis for accreditation of a reference measurement laboratory that applies for official recognition of the performance of a given procedure.

Regardless of the specific standard, competency assessment programs should be designed to identify, monitor, and document the experience, qualifications, and abilities of personnel that allow them to perform an operation reliably and ensure the quality of a final product. It is useful to complement such competency assessment programs with regular training programs to refresh pertinent skills and prevent poor techniques from becoming established.

For regulated laboratories where liquid handling is a core function, pipetting competence assessment and training may be necessary for maintaining compliance. As an example, when the Food and Drug

Protection Division Laboratory at the North Carolina Department of Agriculture sought ISO 17025 accreditation, it implemented pipetting technique training and certification for its employees to demonstrate competence. After undergoing a full day of workshops and hands-on training, all employees passed written and practical exams and received objective documentation of their results.

## Reason five

**Operator competence makes good business sense.** Competent pipette users allow laboratories to minimize the need for costly and time-consuming assay repeats and eliminate reagent waste, an especially important benefit given the frequent use of scarce samples and expensive reagents. Improving operator competency optimizes staff assets and enhances efficiency and productivity for the long term.

Pipetting competence monitoring and training are essential components for ensuring overall laboratory quality. Just as pipettes and other instrumentation must be routinely calibrated and maintained, operator competence must be frequently assessed and the required operator skills fine-tuned with an effective pipetting technique training program. □



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## References

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