Artel Lab Report 7

This publication provides technical information regarding the use, application, and metrology related to liquid handling instrumentation.

"The difference between failure and success is doing a thing nearly right and doing it exactly right."

—Edward Simmons

Facilitating **Assay Transfer** by Controlling Liquid Handling Variables

Assay transfer is one of the most complex activities in laboratories today.

Successful assay transfer ensures that new results are comparable to historical data. Whether it involves scaling an assay up to a higher throughput platform or transferring an assay from development to a QC environment, assay transfer is challenging because of the large number of variables involved. Differences in equipment, reagents, technique and interpretation of methodology are typically the focus of preparation before assay transfer. Less obvious variables, such as those that involve liquid handling, are often the cause of assay transfer failures. These frequently ignored variables are difficult to identify and, therefore, to troubleshoot.

Liquid handling instruments, such as pipettes and automated liquid handlers, are routinely used to perform assays in laboratories. Their accuracy is often taken for granted and, therefore, not given proper attention during assay transfer work. Understanding the importance that liquid handling plays in assay transfer work and taking measures to control liquid handling variables will ultimately facilitate assay transfer.

For example, errors in liquid delivery can often be unnoticed, undocumented, or misunderstood. These errors may come from one or more sources, such as pipettes, liquid handlers, operators, environmental conditions, and labware.[1-4] Liquid handling errors propagate and can significantly impact the assay. These errors result in the time-consuming challenges of diagnosing and troubleshooting the problem, hence jeopardizing the process before the assay is successfully transferred.

Successful assay transfers are a product of careful planning and advance preparation. Preventing liquid handling problems during execution involves pre-transfer work, such as understanding the assay, focusing on training and calibration, and generating effective documentation.

Table 1: Parameters that affect a working assay

Assay Parameters
Plate or tube format (96, 384, etc.)
Selected analyte concentration
Number and type of dilution steps
Number of liquid transfers
Number of incubation or centrifugation steps
Number of wash steps
Dispense order
Liquid properties (viscosity, temperature, etc.)
Instrument Parameters
Target or off-set volume
Single vs. multi-sequential dispenses
Reagent mixing protocol
Aspirate/dispense rate
Aspirate/dispense height
Pertinent liquid class settings
Accuracy of volume transfer
Overall speed between transfers (time delays)
Precision of volume transfer
Wet vs. dry dispense
Type of liquid handler/pipettor:
• Number of tips
• Air displacement, system fluid, acoustic ejection
Types of Tips:
• Max/min volume capacity
• Fixed vs. disposable
• Dry tip vs. wet tip
• New tip vs. used tip
• Carryover
• Tip touches
• Sterilized vs. unsterilized disposable tips
• Use of filter or specialty tips
• Tip quality
Laboratory Parameters
Operator skill and technique standardization
Environment, temperature, and humidity

Table 2. Common methods used to evaluate volumetric performance

Method	Description
Gravimetry	The liquid is dispensed into a container and the container is weighed on an analytical balance; the liquid density value is used to calculate volume transferred by the pipette.
Absorbance	A dye-based solution is dispensed with the liquid handler and, depending on the extent of the methodology, the absorbance data are correlated to compute volume.
Fluorescence	A fluorescent dye-based solution is dispensed with the liquid handler and, depending on the extent of the methodology, the fluorescence data are correlated to the volumetric precision.
Dual-dye Photometry	Dual-dye, dual-wavelength ratiometric photometry is used to assess accuracy and precision of volume transfers in one measurement.
Combination of Methods	Combining some of the above methodologies helps achieve precision and accuracy information. For instance, an absorbance method to determine precision can be combined with a gravimetric method to determine trueness.

Pre-Transfer Activity #1
Understand the Assay

Understanding the liquid handling steps in the assay is important because variable liquid delivery will alter analyte and critical reagent concentrations, which in turn affect the accuracy of the assay.

Begin by identifying the critical liquid handling steps in the assay. To accomplish this, list all the liquid handling steps performed and describe their execution in detail, drawing on the parameters compiled in Table 1.[4] Using this detailed list, evaluate the risk at each liquid handling step, and pinpoint the areas that could be problematic during transfer.

Next, evaluate the accuracy of the volume delivery that will occur at the critical steps between the assay transfer sites.[5] Table 2 lists the common methods for evaluating volumetric performance. Implementing a volume verification process ensures that the performance of liquid handling instrumentation is known, which allows laboratories to achieve the same relative analyte and reagent concentrations in the assay at both the transferring and receiving sites.

Pre-Transfer Activity #2
Implement Calibration and Training Programs

Pipettes and liquid handlers require calibration and preventive maintenance schedules that ensure the accuracy, precision and trueness of each volume dispensed under normal operating conditions. Properly maintained and calibrated volume delivery instrumentation at the transferring and receiving sites is an easy-to-control variable that minimizes assay transfer failures. Calibrating to a known standard available at each site will further reduce the potential for failures.

Further, technologists pipetting skills can be a relatively large unknown source of error[2] encountered during assay transfer. Operator error may go undetected, resulting in poor assay performance and failed assay transfers. Figures 1 and 2 illustrate the differences in pipetting skills in terms of precision (%CV) and trueness (%SE), before and after training for fifty-three QC technologists. A significant improvement in pipetting skill was observed post-training. Hence, a comprehensive training program is necessary to control the variable of technologist pipetting skills, and to standardize pipetting technique, prior to assay transfer.

Pre-Transfer Activity #3
Develop Effective Documentation

Generating effective documentation is another critical part of pre-transfer work. A detailed standard operating procedure (SOP) describing the assay, as it is executed in the transferring site, is key because it provides a standard for training and executing at the receiving site.

Robust and detailed SOPs created during or after assay development at the transferring site provide information that can further define assay steps to reduce problems at the receiving site.
For instance, detailing the liquid handling steps in Pre-Transfer Activity #1 will allow the receiving site to focus on details that may have otherwise been overlooked, and will ultimately lead to a successful transfer.

Another important documentation component is the transfer protocol.
A formal protocol defines the expectations for the transfer exercise, provides agreement between the sites on how the transfer will be executed, and defines the criteria for a successful assay transfer. A comparison between the results for identical samples at both sites is required, and an equal number of determinations for each sample at each site is advisable to simplify statistical analysis. Analytical precision studies including repeatability, intermediate precision and inter-laboratory reproducibility are recommended.[6] Poor precision in liquid handling can have a direct effect on repeatability, while poor liquid handling accuracy would contribute to poor reproducibility. Finally, analytical accuracy is evaluated by determination of the percent difference for results for identical samples at both sites using the results of the transferring site as the "true value."[6] So, it is important that liquid handling accuracy be consistent at both laboratories.

Figure 1: Pre-training skills assessment

Figure 1. Pipetting skills assessment of fifty-three QC technologists prior to receiving pipetting technique training, dispensing 10 µL aliquots of sample (n=5) using the same calibrated pipette.

Figure 2: Post-training skills assessment

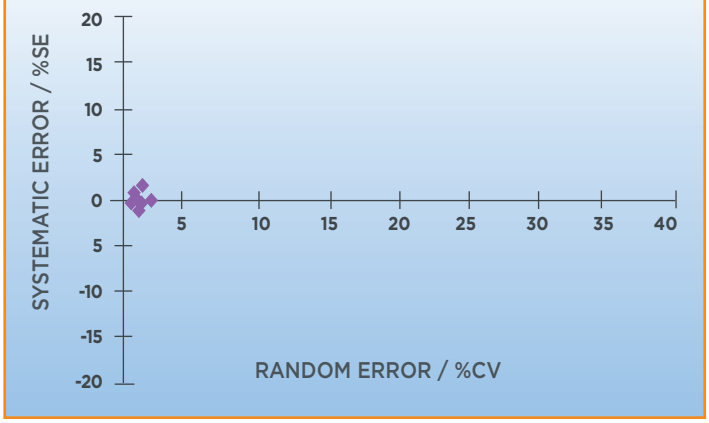

Figure 2. Post-training data for the same technologists, performing the same skills assessment protocol, with the same pipette, as described for Figure 1.

Post-Transfer Activities

Sustaining Knowledge Transfer

A complete, successful assay transfer is the foundation for ensuring that comparable, high quality results are generated at the receiving site. Sustaining the knowledge obtained during the execution of a method transfer in a new site involves robust SOPs, method maintenance and, as mentioned in Pre-Transfer Activity #2, strong training and calibration programs.

Proper and detailed documentation captures method nuances discovered during the transfer exercise. SOPs are typically refined after the assay is transferred to clarify and expand on the instructions provided in the original document. Furthermore, assay controls implemented as part of the transfer process must be added to the SOP to guarantee the assay is executed correctly.

Maintaining a transferred method can be accomplished in many ways, depending on the purpose of the assay. Comparability studies, trending assessments and regular revalidation schedules are typically put in place to ensure that the transferred assay continues to perform as expected.

Conclusion

Liquid handling steps are a controllable but often ignored variable in assay transfers. Understanding the impact of volume delivery on the assay, implementing appropriate pipette and liquid handler calibration and training programs, and developing effective documentation that addresses the details of liquid handling steps are pre-transfer preparation activities that are essential to the success of an assay transfer. They allow the transferring and receiving sites to approach the assay transfer with confidence by eliminating variables and, therefore, improving the chances for success. Once an assay is successfully transferred, these strategies are still applicable to sustaining the assay at the new site by, for example, keeping the equipment functioning properly and capturing method details in SOPs.

Preventing liquid handling problems during execution involves pre-transfer work, such as understanding the assay, focusing on training and calibration, and generating effective documentation.

Q:What are some common operator pipetting technique errors?
A: Several pipetting actions can contribute error to a laboratory process. Failing to pre-wet the pipette tip can lead to inaccurate liquid delivery by delivering lower volumes than expected. Excessive tip wiping can also cause sample absorption and under-delivery. Choosing the wrong pipetting mode is another common error. For instance, reverse mode pipetting can be more ideal for viscous samples, whereby the plunger is depressed past the first stop to aspirate the aliquot from the sample, and depressed only to the first stop to deliver the aliquot. Finally, variability in the temperature of the liquid significantly contributes to error in pipetting. For more information, see Reference 2.

Q: Will the disposable tips on automated liquid handlers contribute error during assay transfer?
A: When using disposable tips, the tip quality and characteristics are critical to the integrity of the volume transfer. Vendor-approved tips, as opposed to the less expensive 'bag of tips' option, should always be employed to minimize volume transfer error and optimize liquid delivery quality. Disposable tip performance has been found to be directly related to quality because tip material, shape, properties, fit and wet-ability are all important factors for repeatability. The cheaper, bulk tips may not be manufactured with the highest precision manufacturing and may have variable characteristics that affect delivery, such as differences in upper diameter, virgin plastic content and presence of plastic residue inside the tip, known as "flash." These tips also might not fit well on the liquid handler and they may have variable wetting/delivery properties. Without using approved tip types, accuracy may be at risk. See Reference 1 for more information.

Q: Can fixed tips on liquid handlers contribute error during assay transfer?
A: Fixed tips are usually stainless steel pipetting channels which are used repeatedly, often with wash steps between pipetting steps. These tip types may also present sources of variability in liquid delivery with automated liquid handlers. Continued use of fixed tips with caustic reagents may degrade the inside surface of the tips and create grooves or pockets in the tip material. Liquid may fill the formed cavities during volume transfers resulting in carry over, contamination or even over-delivery of solution to critical assays. Additionally, many liquid handlers that use fixed tips require system fluid to aspirate and dispense the sample volume (and the system fluid aids with the washing steps between pipetting events). Error can creep into a process when insufficient air gaps are employed between the system fluid and sample volume, which results in a diluted sample, i.e., less concentrated sample volume and cross-contaminated system fluid. For more information on identifying sample dilution from system fluid as well as circumventing such issues, see References 7 and 8.

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