Accuracy Matters When Quantitative, Manually-pipetted Assays Graduate to Automation – A Story in Diagnosing and Troubleshooting

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Abstract

It is often the case that assays are initially performed on the benchtop using handheld pipettes before they graduate, or transfer, to an automated liquid handler. Automating a manual method may take time and patience, but automation will help lower costs, increase throughput, and potentially avoid errors associated with a manual method. During the transfer process, however, the manual assay should be directly compared to the automated assay for consistencies in pipetting performance. What if the volumes of reagents pipetted to and from the assays are only compared for precision and not accuracy? An undetected variability in accuracy will impact the integrity of the assay as the automation process continues. Even if the manual method and automated method both have highly precise liquid delivery, they might not be pipetting the same volume of reagent to the assay. Accuracy information is critical to determine the deviation of the dispensed volumes from the target volume when transferring or scaling-up an assay. Validating the liquid delivery steps for each assay using a robust measurement method should be implemented to uncover discrepancies in pipetting performance. This presentation discusses the importance of knowing both accuracy and precision information when a manual method is transferred to a Biomek® liquid handler. By using a standardized volume verification methodology, it was determined that the rate-limiting reagent was not being accurately pipetted between the manual and automated methods per the protocol, and the automation was not to blame.

Introduction

The need to ensure quality in a laboratory process has become increasingly important, especially as it relates to the accurate volume transfers within microplate-based assays (ref 1). Measuring and knowing the exact volume transferred will inherently lead to accurate and precise analysis of the experiment, *i.e.*, the results can be trusted. Many assays, such as the highlighted real time polymerase chain reaction (RT-PCR) assay, depend on **accurate** volume delivery with either a handheld pipette (benchtop assay) or a liquid handler (automated assay). Because the concentrations of components in assays are volume dependent, inaccurate volume transfers will directly impact assay results. In our story, the RT-PCR assay was being transferred from a manual pipetted method to an automated liquid handler (Biomek FX). The technician was observing *acceptable* results with the manual assay, but as the method graduated, or transferred, to the Biomek, the results could not be repeated and were deemed unacceptable, *i.e.*, the liquid handler was producing errant results for the same RT-PCR assay that worked with a handheld pipette. The liquid handler was blamed for performing poorly and a service call was initiated. A senior applications scientist was called-in to diagnose, troubleshoot and *correct* errors in the automated liquid handling method. The field service engineer employed the MVS® Multichannel Verification System (**Figure 1-left**), which is a standardized volume measurement platform, to quantify the volume transfer performance of the Biomek FX (**Figure 1-right**).

Introduction (cont'd)

Figure 1. The ARTEL MVS® Multichannel Verification System (left) was used to assess the volume transfer performance for the Beckman Coulter Biomek FX (right) for the RT-PCR assay.

Accuracy required for the RT-PCR assay

This RT-PCR assay was primer-limited, so the volume transfer of 15 µL master mix to the assay was a critical step and therefore needed to be accurate.

RT – PCR assay
- Assay Plate
  - *10 µL Sample* – *In Excess; not a critical volume transfer step*
  - *15 µL Master Mix* – ***CRITICAL STEP; accuracy required***
- Place in thermocycler for 1.5 hours
- Results obtained

The applications scientist used the MVS to characterize the Biomek's performance when transferring the 15-µL target volume. As discussed in reference 2 and shown in the schematic in **Figure 2**, the MVS dye-based sample solutions are dispensed into the microplate by the liquid handler, and the target volume is quantified well-by-well and tip-by-tip using dual-dye ratiometric photometry with a foundation based on the Beer-Lambert Law. Immediately after the absorption measurements are collected, the MVS is used to determine the volume dispensed in each well of a microplate (ref 2).

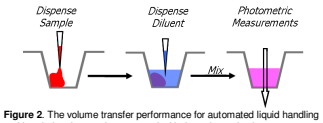

Figure 2. The volume transfer performance for automated liquid handling and hand pipetting can be assessed with the ratiometric photometry implemented by the MVS. A target volume of sample solution is dispensed into a microplate well before, or after, the non-quantitative addition of diluent (ref 2). The solutions are mixed and the absorbance values are used to determine the well-by-well volume values using a series of equations. The MVS is a standardized, universal platform for measuring both accuracy and precision of volume delivery in 96-well or 384-well plates for volumes between 10 nL – 200 µL. Such measurements allow for directly comparing pipetting equipment regardless of tip number, model, make, manufacturer or location.

"As Found" Performance Assessment

The Biomek's "as found" volume transfer performance was determined with the MVS for the 15-µL target volume of aqueous solution in a 96-well plate. The initial *Scaling Factor* and *Offset* for the Biomek were set at 1 and 0, respectively.

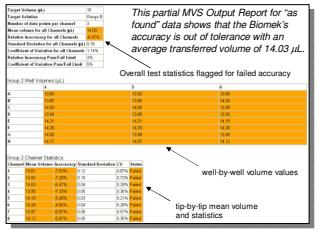

Figure 3. A partial MVS output report for the initial, "as found", performance assessment shows highlighted (flagged) tips and wells that perform outside of the assay specifications. Assay specifications for relative inaccuracy and CV were set at 3% and 5%, respectively. Though the CV for all tips was within specification (at 1.14%), the relative inaccuracy for all tips was failing at -6.47% with an average dispensed volume for the 8 tips and 3 reps of 14.03 µL.

Adjusting the Software for Accuracy

In the Biomek software, the *Scaling Factor* and *Offset* were adjusted to 1.045 and 0.354, respectively and the 15-µL target volume was re-tested to show that the instrument was dispensing within the assay's specifications (**Figure 4**).

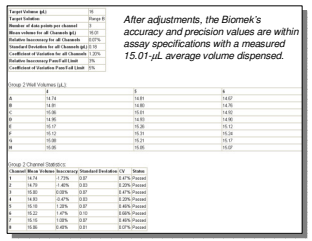

Figure 4. After making software adjustments to the *Scaling Factor* and *Offset* (see text), the Biomek met assay specifications for both precision and accuracy. The measured CV was 1.20% and the relative inaccuracy for all tips was 0.07% (with an average dispensed volume for the 8 tips and 3 reps of 15.01 µL).

End of story?

No. The results obtained for automated RT-PCR assay on the Biomek still did not match those obtained with manually pipetted method. What went wrong? The applications scientist decided to use MVS alternative test solution functionality (refs 3 and 4) to assess Biomek performance when transferring a 20% glycerol in water (v/v) test solution. The glycerol test solution is *more like* the master mix as compared to an aqueous test solution.

Testing with a 20% Glycerol Solution

Custom test liquids, such as 20% glycerol in water (v/v), can be prepared and used to assess a liquid handler's volume transfer performance for assay-specific reagents (see refs 3 and 4). Per user-friendly instructions, concentrated MVS dye stocks are gravimetrically added to the solvent-of-interest (**Figure 5**) to prepare a dye-based test solution that can be assessed for transfer statistics with the MVS.

Figure 5. Schematic highlighting the simple process of preparing a dye-based glycerol test solution for MVS volume checks, as described in greater detail in references 3 and 4.

Once the dye-based glycerol test solution was prepared, it was used to check the Biomek's transfer performance for 12 reps of 15 µL when using the same *Scaling Factor* and *Offset* (1.045 and 0.354, respectively). The accuracy was shown to be slightly out of tolerance for the 3% assay specification (**Figure 6**).

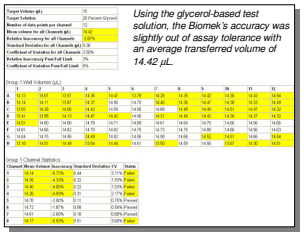

Figure 6. Using the glycerol-based test solution, the Biomek was shown to be out of the accuracy specifications with a measured relative inaccuracy of -3.87%.

After software adjustments to the *Scaling Factor* and *Offset* (1.052 and 0.135, respectively), the Biomek was determined to be performing within the assay specifications (**Figure 7**).

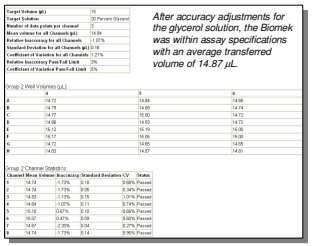

Figure 7. After adjusting the *Scaling Factor* and *Offset* for the glycerol test solution, the Biomek's overall accuracy and precision values were -1.07% and 1.21%, respectively (with an average volume of 14.87 µL).

Round 2 – End of Story?

No. Even after the Biomek was optimized to transfer the correct volume of the glycerol test solution, **the RT-PCR assay results still did not match those obtained with the manual method.**

Assessment of Hand Pipetting

The technician's pipetting proficiency was assessed with the MVS. Using a single channel pipette, the technician transferred 15 µL of the 20% glycerol solution into a 96-well plate (8 reps) and the volumes were measured (**Figure 8**).

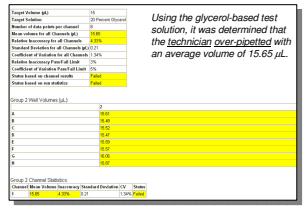

Figure 8. The MVS was used to determine that the technician was over-pipetting master mix, with an average volume dispensed of 15.65 µL (relative inaccuracy and CV were 4.33% and 1.34%, respectively).

Problem Solved!

The Biomek's software was subsequently modified to pipette 15.65 µL (to match the hand pipetting of the technician) and the **results of manual assay and modified automated assay were now the same**. The RT-PCR assay was successfully automated on the Biomek after using the MVS to diagnose and troubleshoot the method transfer.

Discussions and Conclusions

This poster shows the importance of volume measurement in the transfer of assays. Accuracy matters! Too often, however, the importance of accurate delivery is overlooked. The goal is to achieve liquid delivery quality assurance through accurate and precise volume transfer. Using a standardized volume measurement method or platform, such as the MVS, is an easy way to meet that goal (ref 1). When transferring a method or assay, *e.g.*, bench-to-automation, lab-to-lab, liquid handler-to-liquid handler, group-to-group, *etc.*, it is extremely important to standardize volume measurements between the two or more pipetting systems to ensure, and have confidence in, the assay results. In this case, inaccurate hand pipetting was the root cause of error. Once the error was determined and the Biomek was adjusted to match hand pipetting accuracy, the automated RT-PCR assay was successfully implemented.

References

(1) Albert and Bradshaw, *J. Assoc. Lab. Autom.*, **2007**, *12*, 172-180; **(2)** Bradshaw *et al. J. Assoc. Lab. Autom.*, **2005**, *10*, 35-42; **(3)** Albert *et al. J. Assoc. Lab. Autom.*, **2006**, *11*, 172-180; **(4)** ARTEL application note entitled "Glycerol Solutions for MVS® Volume Verifications" is available by contacting ARTEL at the information below or at http://www.artel-usa.com/.

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