Optimize Volume Transfer Methods to Avoid Reagent Concentration Errors of > 50%

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Abstract

Laboratories utilizing liquid handling devices appreciate the efficiency benefits that these important instruments provide. Fast and repeatable volume transfers, however, do not always contribute to accurate assay results. Assays are dependent on reagent concentrations and reagent concentrations are volume-dependent. If a liquid handler is inaccurate in the volumes it dispenses, reagent concentrations can be very different in the reaction vessel when comparing theoretical and experimental concentration values. Undoubtedly, most liquid handlers can be highly precise, but some can also be very inaccurate when default methods/settings are employed. When errors are discovered a game of finger-pointing often occurs – is it the reagent kit? The robot? The tips? The method? The user?

This presentation highlights: (a) the importance in checking volume transfer accuracy for all pipettors associated with a specific assay using a standardized metric; (b) how reagent concentrations are critically affected by as much as 50% when as few as two successive volume transfer steps are inaccurate; and (c) how with simple tools/knowledge, liquid handlers and their associated methods can be quickly optimized to deliver accurate volumes to ensure proper reagent concentrations in a reaction vessel.

The presentation offers specific information for optimizing the pipetting accuracy for the Beckman Coulter Biomek series (FX, NX, and x3000), the Qiagen QIAgility, PerkinElmer Sciclone and a Thermo WellMate bulk dispenser. It is hoped the information provided will help users, programmers and overseers of automated liquid handlers to gain control of liquid handling QA & QC processes to help eliminate liquid handlers as sources of error.

Materials & Methods

All sample solutions were aqueous and all target volumes were measured using the MVS® Multichannel Verification System. Additional MVS Verification Plates were employed. The MVS was employed to show simple and quick optimization processes for four different liquid handlers. The details pertinent to each optimization process are included, whereas some of the experimental information (tip type, environmental conditions, etc.) are not discussed herein.

Experiment 1 – Optimizing a Qiagen QIAgility

One can optimize volume transfer of a Qiagen QIAgility by adjusting the p-value, which changes the amount of liquid pipetted by approximately 0.04 µL/step. Without a “measurement stick” for determining volume transferred to a vessel, however, one cannot make p-value adjustments to improve accuracy. The process for optimizing accuracy is quite simple: (1) perform “as found” testing; (2) determine the volume difference for measured vs. desired; (3) convert the volume difference to p-value steps; (4) add/subtract steps to/from current p-value; (5) repeat with new p-value. In the data presented here, the pre-optimized mean volume was 0.168 µL higher than the desired target of 2 µL (Figure 1). The p-value was decreased by 4.2 steps to 71.16 ([0.168 µL] / [0.04 µL/step] = 4.2) and the 2-µL target was retested and optimized in one simple experiment.

Experiment 2 – Optimizing a Bulk Dispenser

The volume transfer performance of an 8-tip Thermo WellMate, fitted with a standard bore cartridge, was monitored at 25 µL. Interestingly, though the target volume is relatively high, the performance was pretty good for this common bulk dispenser. Initial testing showed an overall accuracy to be within 5%. Two additional tests were conducted after the set screws were either tightened (to decrease volume transfer) or loosened (to increase volume transfer). Two of the tips (6 and 8, Figure 2) were initially adjusted in the wrong direction, which was immediately obvious in this cause-and-effect testing process. Simply by tweaking the set screws, the tip-by-tip accuracy was optimized within minutes. The as found, pre-optimized mean volume, relative inaccuracy and CV values were 26.15 µL, 4.6% and 2.87%, respectively. The as left, optimized values were 25.03 µL, 0.12% and 0.68%, respectively.

Experiment 3 – Optimizing a Beckman Coulter Biomek

A Biomek liquid handler (Figure 3) can be optimized for pipetting accuracy by adjusting the scaling factor (slope, m) and the offset (p-intercept, b) in the Calibration tab within the Technique Editor. The calibration is based on y = mx + b. However, the scaling factor and offset values are meaningless without a way to measure volume transfer accuracy. Adjusting pipetting accuracy is a simple and effective process: (1) measure volume transfer performance (three or more target volumes is recommended); (2) plot measured vs. theoretically dispensed volume; (3) determine the new slope and offset values; and (4) enter values and re-test. In the example shown, a default non-optimized universal technique was employed to dispense 2, 5 and 8 µL. The as found performance was (linearly) inaccurate: -27.85%, -14.68% and -9.6% (respectively for the three volumes; Figure 4). New scaling and offset factors were calculated and employed, and in one test, the relative inaccuracies improved to -0.05%, -1.14% and 1.05%, respectively.

Accuracy Matters in Liquid Handling – Resulting Reagent Concentrations are Very Different When as Few as One or Two Transfers are Inaccurate

Compound concentration values can vary by > 30% if one transfer is inaccurate and by > 50% if two successive transfer steps are inaccurate (Table 2). The mean pre- and post-optimization volumes from Experiments 1 to 4 were employed in two simple calculation models that assess the effect of the dispensing errors described would have on the final concentration of a compound after just two liquid delivery steps. Model 1 was designed to assess the effect that an inaccurate dispense of reagent into an accurate amount of buffer would have on reagent concentration. Model 2 assesses the maximum effect that inaccurate dispensing of both reagent and buffer would have on reagent concentration.

Liquid handlers can be inaccurate, but with tools and know-how, simple adjustments can ensure volume transfer accuracy, and confidence in assay results.

Table 2. Theoretical Percent Differences in Compound Concentrations: Pre- and Post-Optimized Target Volumes With Both Accurate and Inaccurate Buffer Additions

<table>
<thead>
<tr>
<th>Experiment ID</th>
<th>Liquid Handler Type</th>
<th>Target Volume (µL)</th>
<th>Theoretical Percent Difference in Concentration</th>
<th>Pre-Optimized Target as Measured (µL)</th>
<th>Post-Optimized Target as Measured (µL)</th>
<th>Model 1: Compound Concentration Error: Inaccurate Reagent and Accurate Buffer Additions (%)</th>
<th>Model 2: Compound Concentration Error: Inaccurate Reagent and Inaccurate Buffer Additions (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-tip QIAgility in 96-w</td>
<td>150</td>
<td>0.00%</td>
<td>150.0</td>
<td>150.0</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>2</td>
<td>8-tip Thermo WellMate in 96-w</td>
<td>150</td>
<td>0.00%</td>
<td>150.0</td>
<td>150.0</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>3</td>
<td>1-tip QIAgility in 96-w</td>
<td>150</td>
<td>±0.05%</td>
<td>149.5</td>
<td>150.5</td>
<td>±0.05%</td>
<td>±0.05%</td>
</tr>
<tr>
<td>4</td>
<td>8-tip Thermo WellMate in 96-w</td>
<td>150</td>
<td>±0.05%</td>
<td>149.5</td>
<td>150.5</td>
<td>±0.05%</td>
<td>±0.05%</td>
</tr>
</tbody>
</table>

* Subset of data from reference 3. The mean volume in trial C was 9.12 µL, so the requested volume in the software for trial D was increased by 0.88 µL before the final (optimized) transfer.

References

(3) Albert, K. J. and Bradshaw, J. T. J. Assoc. Lab. Autom., 2007, 12, 172-180