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.

This presentation offers specific information for optimizing the pipetting accuracy for the Beckman Coulter Biomek series (FX, NX, and 3000), 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 with the MVS® Multichannel Verification System[1]. Additionally, only 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. A 1-tip Qiagen QIAgility liquid handler was optimized to wet-dispense 2 µL into a 384-well plate by adjusting the p-value. *Experiment 2*. An 8-channel Thermo WellMate dispenser was optimized to dispense 25 µL into a 96-well plate by adjusting the cartridge assembly's set screws. *Experiment 3.* A 96-tip Beckman Coulter Biomek NXp was optimized for wet-dispensing three target volumes in a 96-well plate (2, 5 and 8 µL) by adjusting the scaling and offset factors as described in Beckman application note[2]. *Experiment 4*. A 96-tip PerkinElmer (Caliper) Sciclone was optimized to dispense 10 µL into a 96-well plate by sequentially adjusting pipetting variables[3].

Experiment 1 – Optimizing a Qiagen QIAgility

One can optimize volume transfer of a 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) re-test 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.

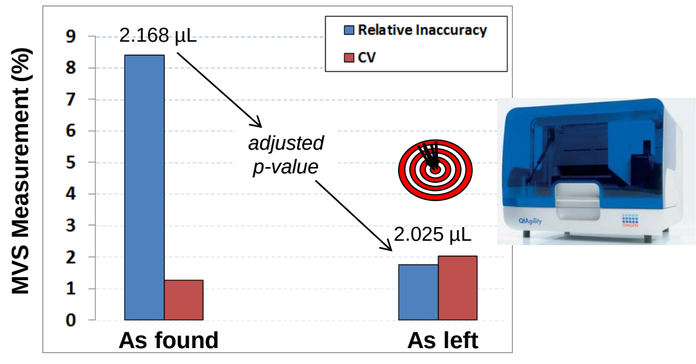

Figure 1. (left) Tweaking the p-value once for the 2-µL dispense improved the relative inaccuracy from 8.4% to 1.25%. (right) Image of Qiagen QIAgility from www.qiagen.com.

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.

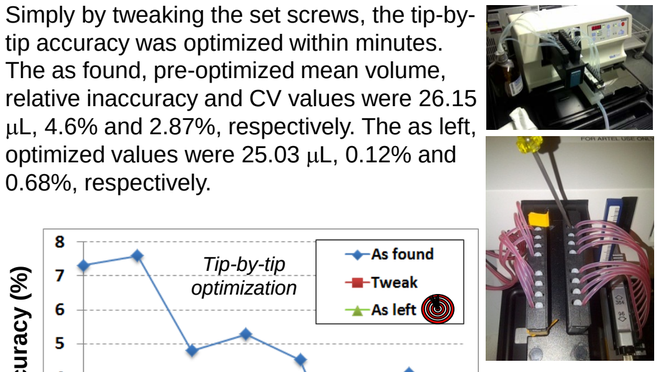

Figure 2. The WellMate can be optimized tip-by-tip by adjusting the set-screws within the cartridge assembly.

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 (y-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 displaced volume[2]; (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.

Figure 3. Image of Biomek NX from www.beckmancoulter.com.

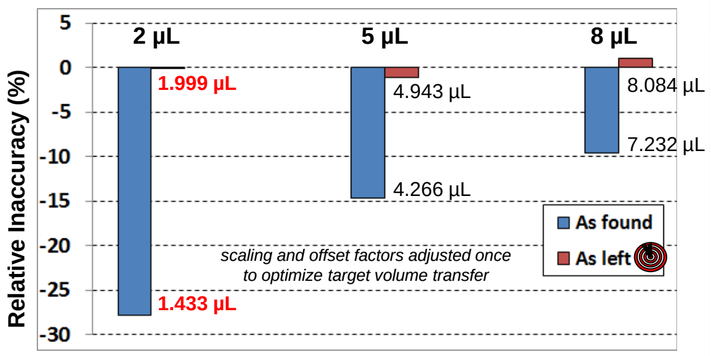

Figure 4. The measured volumes for the Biomek NX as tested before, and after, optimization. The scaling and offset values were changed from 1 and 0 to 1.032 and 0.548, respectively, for the as found and as left data sets.

Experiment 4 – Optimizing via Parameter Adjustment

The volume transfer performance of a 96-tip Sciclone was employed to show that sequentially tweaking volume transfer parameters, on nearly any type of air-displacement liquid handler with any tip configuration, can have a direct impact on the amount of liquid transferred[3]. **Table 1** shows the parameters and values used for each 10-µL target volume dispense, as well as the resulting volume measurement statistics for each trial (A-D). Of most important note, the original settings employed showed an inaccuracy of -32% (note that all CVs are within 4%). By introducing a 5-µL leading air gap, the accuracy immediately improved to -8.2%. Though this process for optimizing the automated task could have been performed with alternative approaches (different parameters, different order, defining liquid classes, etc.), it highlights the importance of performing a cause-and-effect volume verification method during method optimization. The results show that after three sequential parameter adjustments, the optimized data reflect mean volume, relative inaccuracy and CV values of 9.97 µL, -0.30% and 0.80%, respectively.

Table 1. Sequentially Adjusting Liquid Handler Parameters has a Direct Impact on the Transferred Target Volume *

Experimental Reference ID	A	B	C	D
Modified Parameters				
Aspirate Rate (µL/s)	5	5	5	5
Dispense Rate (µL/s)	5	5	5	5
Leading Air Gap (µL)	0	5	5	5
Trailing Air Gap (µL)	0	0	5	5
Requested Volume (µL)	10	10	10	10.88
MVS Measurement Results				
Mean volume (µL)	6.80	9.18	9.12	9.97
Relative Inaccuracy (%)	-32.01	-8.20	-8.83	-0.30
Standard Deviation (µL)	0.267	0.25	0.134	0.08
Coefficient of Variation (%)	3.93	2.72	1.47	0.80

* 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 running the final (optimized) transfer.

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

Experiment ID	Liquid Hanlder ID and Plate Type *	Target Volume of 10 mM Critical Compound (µL)	Theoretical Volume of Buffer Required to Reach Accurate Total Working Volume (µL)	Pre-Optimized Target as Measured (µL)	Post-Optimized Target as Measured (µL)	Model 1: Compound Concentration Error: inaccurate reagent and accurate buffer dispenses** (%)	Model 2: Compound Concentration Error: inaccurate reagent and inaccurate buffer dispenses *** (%)
1	1-tip QIAgility in 384-w	2	48	2.168	2.017	7.2	27.1
2	8-tip WellMate in 96-w	25	175	26.15	25.03	4.4	24.3
3	96-tip NX in 96-w	2	198	1.443	1.999	32.3	51.5
3	96-tip NX in 96-w	5	195	4.266	4.943	14.7	34.4
3	96-tip NX in 96-w	8	192	7.232	8.084	11.1	31.0
4	96-tip Sciclone in 96-w	10	190	6.8	9.97	37.8	56.7

* Total target working volume in 384-well and 96-well plates: 50 and 200 µL, respectively. Starting compound concentration is 10 mM. Percent Difference = | Conc1 - Conc2 | / [Conc1 + Conc2] /2 . ** [Pre-Optimized target in accurate buffer volume] minus [Optimized target in accurate buffer volume]. *** [Pre-Optimized target when buffer volume is off by 10%] minus [Optimized target when buffer volume is off by 10%]. *** The lower target is paired with the higher buffer volume, and vice versa.

Trust Your Results

References

(1) Bradshaw, J. T. et al. *J. Assoc. Lab. Autom.*, **2005**, *10*, 35-42.
(2) Beckman Coulter application note entitled "Improving Accuracy By Use of Technique Calibration" (T-1915A) https://www.beckmancoulter.com/wsrportal/bibliography?docname=T-1915A.pdf (assessed in December 2012).
(3) Albert, K. J. and Bradshaw, J. T. *J. Assoc. Lab. Autom.*, **2007**, *12*, 172-180

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