Ensuring your Automated Liquid Handler Works for your Assays – Understanding Device Behavior, Optimizing Methods, and Uncovering Errors

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Abstract

The focus of this presentation is to highlight the need of ensuring quality in important assays performed with automated liquid handlers. Nearly all assays performed within a laboratory are volume-dependent. In turn, all concentrations of biological and chemical components in these assays, as well as the associated dilution protocols, are volume-dependent. Because analyte concentration is volume-dependent, an assay's results might be falsely interpreted if liquid handler variability and inaccuracies are unknown or if the system(s) go unchecked for a long period. Measuring and knowing the exact volumes transferred, for specific and/or routine methods, will inherently lead to confidence in the experiment, *i.e.*, the results can be trusted.

This presentation highlights the importance of monitoring, evaluating, and optimizing liquid handler performance so operators can get the most out of their liquid handlers and the associated assays. To understand and assess liquid handler performance in each case demonstrated herein, a standardized volume verification method was employed (MVS® Multichannel Verification System, Artel, Westbrook, ME). For instance, it will be presented how the liquid handling steps were measured, diagnosed, understood, and/or optimized for: (1) on-board mixing efficiency; (2) finding a bad "tip-in-the-box"; (3) highlighting the differences between accuracy and precision; (4) comparing individual volume transfers over multi-sequential dispenses; (5) optimizing an automated method for a specific target volume; and (6) directly comparing performance between liquid handlers from multiple locations.

Is Your Liquid Handler Working Properly?

Assays are dependent on reagent concentration(s) and reagent concentrations are volume-dependent. Therefore, assay integrity can be dependent on volume delivery performance.

To understand liquid handler behavior, it is important to measure both the accuracy and precision in the volume transfer steps, on a tip-by-tip and/or well-by-well basis. The terms *accuracy* and *precision* often get incorrectly 'swapped' when discussing volume transfer performance and it is important to know the difference (see the schematic below). If reagent concentration in the assay is critical, then all of the liquid transfers must be accurate. If all reactions/wells need to have the "same" amount of reagent regardless of the difference from desired, or theoretical, concentration(s), then precision in the volume transfer(s) is critical.

accurate, imprecise — *5-µL target volume; 5 µL dispensed without repeatability*

precise and accurate — *5-µL target volume; 5 µL dispensed with repeatability*

precise, inaccurate — *5-µL target volume; 8 µL dispensed with repeatability*

imprecise, inaccurate — *5-µL target volume; 8 µL dispensed without repeatability*

Tweaking and measuring – optimizing methods to ensure improved accuracy and precision

As shown in Table 1, there are many different liquid handler parameters that affect the accuracy and precision of a volume transfer. In seven different experiments (A-G; Figure 1), the liquid handling parameters were arbitrarily, and sequentially, tweaked to monitor how the performance of a 96-tip Caliper Sciclone was affected when transferring a *presumed* 10-µL target volume into a 96-well, flat-bottom plate. This experiment demonstrates the importance of knowing both accuracy and precision. *In all seven experiments, the coefficient of variation (CV) values, which are used to monitor precision, are all < 5%. The measured accuracy values, however, vary by more than 30% for the same set of experiments* (see Figure 1, right). **Good precision does not indicate good accuracy.**

Table 1. Liquid Handler Parameters That Affect Performance

- Tips/cannulas
  - max/min volume capacity
  - fixed vs. disposable
  - tip-touches
  - dry tip vs. wet tip
  - carry-over
  - new tip vs. used tip
- pre- and post-air gaps
- target, or off-set, volume
- on-board mixing
- aspirate/dispense height
- aspirate/dispense rate
- wash steps
- dispense order
- wet vs. dry dispense
- overall speed

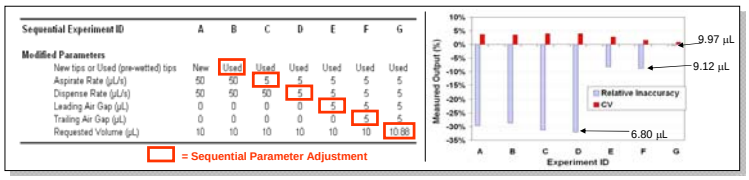
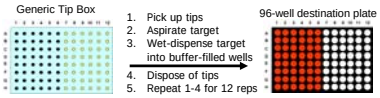


Figure 1. Performance optimization by sequential tweaking of liquid handling parameters of a Caliper Sciclone 96-tip liquid handler.

Diagnosing volume transfer errors due to a "bad tip-in-the-box"

Liquid handling errors can result from the type of materials, *i.e.*, labware, employed during an experiment. In a forensics institution's "AMP" laboratory, a Biomek 3000 equipped with an 8-tip (MP-20) head was used to wet-dispense either 1 µL or 15 µL across a flat-bottom, 96-well plate using disposable **generic** tips. New tips were used for each replicate as indicated in the schematic below.



Using heat maps (directly from the MVS output reports; as shown to the right), the performance data were analyzed immediately after all replicates had been completed. **Four different experiments were collectively used to show that a lot of generic, disposable tip racks had one tip (location 9D) that introduced errors in the volume transfer.** Refer to Figure 2 for test details.

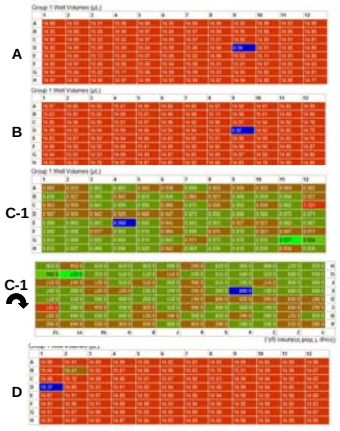


Figure 2. Heat maps. (A) a new tip rack was used for a 15-µL transfer; well 9D is low by 10 µL. (B) repeat run with a new tip rack; well 9D is low again. (C-1) A new tip rack was used for a 1-µL transfer; well 4E "low" by nearly 1 µL. (C-1) Same data as C-1, but rotated 180 degrees; upon inspection, it was discovered that the tip box was *mistakenly* rotated 180 degrees on the deck; well 4E = well 9D after a 180-degree rotation. (D) The tip in location 9D was swapped with the tip in location 1D before transferring 15 µL. Note – the inventory of this tip lot was depleted during testing so more comprehensive experiments could not be conducted.

Diagnosing errors associated with "on-board" mixing in microplate wells, *i.e.*, well contents are mixed using aspirate/dispense cycles on the deck of the liquid handler

There are many assays that require the liquid handler to perform "on-board" mixing where multiple aspirate/dispense cycles are used to create a homogenous solution within a microplate well. These methods are typically used for sequentially transferring aliquots of 'mixed' solution to different wells, such as when performing dilution protocols. In the situation discussed below, a forensics laboratory's aspirate/dispense on-board mixing method was compared to a known, efficient mixing method (see Figure 3). For each target volume, the CV improved after the plate was shaken with the MVS plate shaker, **indicating that the on-board mixing method was not efficient or optimized** (see Figure 4). Just as the MVS was used to measure mixing efficiency, it could also have been used to facilitate mixing optimization (not shown here).

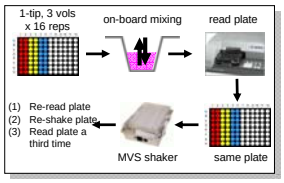


Figure 3. A Biomek 3000 with P20 head was used to transfer 16 reps of three different target volumes into a 96-well plate (wet-dispense). After each dispense, three "common" aspirate/dispense cycles were used to mix each well. When the 48 wells were completed, the plate was measured using the MVS. The same plate was then shaken using the MVS shaker before re-reading a second time. Finally, the plate was re-shaken on the MVS shaker and read in the plate reader for a third time.

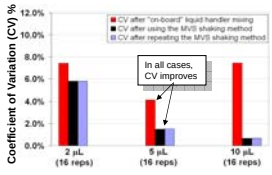


Figure 4. In all cases, the CV improves and stabilizes after mixing the plate with the MVS shaker – indicating that the original on-board mixing step was not optimized or efficient. Note – the CV improves noticeably more with higher target volumes due to diffusion time before reading the plate, *i.e.*, the 2 µL wells were transferred first, followed by 5 µL, and finally with 10 µL before the entire plate was read. Reagents aspirated from wells that underwent on-board mixing, therefore, would not have had their proper concentrations, and if these methods are not corrected, **it is possible that assay errors would propagate simply due to inefficient mixing**.

Diagnosing errors during volume transfer: aspirating a larger, initial volume followed by multi-sequential dispensing of smaller volumes

To save time compared to individual aspirate/dispense steps, liquid handlers are often programmed to perform sequential dispenses after an over-aspiration step. Without knowing the actual volume transferred for each replicate – or at least understanding the dispense pattern of the method/script for the liquid handler method under test, assay integrity could be questioned. For instance, one should not assume that the robotic liquid handler will always dispense the same volume during sequential dispenses (see Figures 5 and 6).

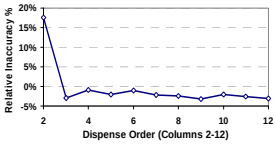


Figure 5. The first dispense for this fixed-tip Tecan liquid handler was ~20% high, with more uniform performance for subsequent deliveries. This protocol to sequentially dispense 10 µL was improved by dispensing the first target volume into a waste reservoir before proceeding with the remaining samples.

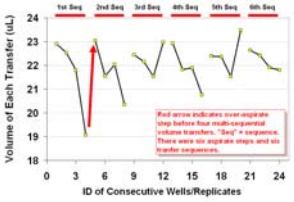
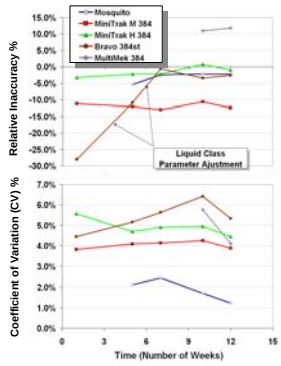


Figure 6. In a forensics laboratory, a Biomek 3000 with a P200L head was used to aspirate 92 µL and then dispense 23 µL four times in a row before repeating the sequential dispense cycle, *i.e.*, before aspirating another 92 µL. In all, there were six aspirate steps and six sequential (Seq) volume transfers. In all six sequences, the amount of volume transferred for each replicate is different and though some of the dispense patterns for the six sequences are similar to each other, the volume transfer performance on a whole was not reproducible. This dispensing method was not optimized with further testing.

Monitoring multiple liquid handlers in one laboratory for dispensing the same target volume over a 3-month period

One pharma R&D laboratory is trying to get the most out of their liquid handlers – by comparing and understanding volume transfer behavior for the same task: dry-dispensing 0.5 µL into 384-well, flat-bottom plates (see Figure 7). This laboratory is monitoring, evaluating, and optimizing their liquid handling scripts, as well as testing older robots to bring them back on-line, to get the best performance from the equipment that they own.

Figure 7. In this R&D lab, many different liquid handlers are used to dry-dispense 0.5 µL DMSO reagents. The data shown here are the *overall* relative inaccuracy (*top*) and CV values (*bottom*) for two replicate plates during each QC interval. The frequent QC testing (some of these liquid handlers were checked 5 times in 12 weeks), emphasizes less time between failure(s). Because the performance data can show trends, operators can react by using the liquid handlers that best suit their needs or they can choose to tweak methods used by *their* favorite liquid handler(s). Previously, this laboratory did not have a standard procedure to assess and compare liquid handlers. Now this lab is proactively monitoring performance instead of reacting to *unknown* variability that can creep into their process.



Comparing identical liquid handlers running the same methods at three different locations

By using the NIST-traceable measurement results from the MVS, three different forensics laboratories were able to directly compare the volume transfer performance of identical liquid handlers running the same methods. See Table 2 below for a partial summary of the results. The methods were written at one site and then rolled out to the other sites for implementation. **Even though the methods, labware, and equipment are the same, the volume transfer performance varies from site to site**, *i.e.*, compare the P200L results at 10 µL or the P20 results at 2 µL or the MP20 results at 2 µL – as highlighted in red in Table 2.

Table 2. Site-to-site comparisons

Biomek 3000	P200L			P20			MP20		
	Location 1	Location 2	Location 3	Location 1	Location 2	Location 3	Location 1	Location 2	Location 3
Target Volume (µL)	10	10	10	2	2	2	2	2	2
Number of data points per tip	16	16	16	16	16	16	5	10	10
Mean volume for all tips (µL)	10.04	8.16	9.28	2.18	1.90	1.77	2.03	2.12	1.54
Relative Inaccuracy for all tips	0.43%	-18.35%	-6.45%	7.90%	-4.23%	-11.55%	1.51%	6.15%	-22.80%
Standard Deviation for all tips (µL)	0.22	0.44	1.95	0.05	0.05	0.04	0.02	0.02	0.03
Coefficient of Variation for all tips	2.14%	5.38%	20.84%	2.15%	2.52%	2.46%	1.33%	1.51%	1.87%
Target Volume (µL)	30	30	30	5	5	5	10	10	10
Number of data points per tip	16	16	16	16	16	16	5	10	10
Mean volume for all tips (µL)	30.25	29.37	28.88	5.25	5.04	4.85	10.14	9.52	10.11
Relative Inaccuracy for all tips	0.82%	-2.10%	-4.41%	5.08%	0.88%	-2.92%	1.36%	-4.80%	1.10%
Standard Deviation for all tips (µL)	0.34	2.50	0.23	0.77	0.59	0.22	0.06	0.05	0.10
Coefficient of Variation for all tips	1.11%	8.53%	0.79%	14.72%	11.65%	6.67%	0.61%	0.53%	1.01%
Target Volume (µL)	60	60	60	10	10	10			
Number of data points per tip	16	16	16	16	16	16			
Mean volume for all tips (µL)	61.65	57.51	59.01	10.42	10.17	10.18			
Relative Inaccuracy for all tips	2.75%	-4.15%	-1.65%	4.22%	1.69%	1.83%			
Standard Deviation for all tips (µL)	1.71	3.24	1.20	0.22	0.36	0.58			
Coefficient of Variation for all tips	2.78%	5.63%	2.03%	2.15%	3.54%	5.71%			

Discussion

It is *very* important to get the best performance out of your liquid handlers. By understanding the *behavior* of your liquid handlers it is possible to monitor trending, evaluate accuracy and precision, diagnose errors, and optimize methods. The behavior of a liquid handler can be assessed using multiple methods, such as gravimetry or the MVS (as discussed for all portions of this poster). Regardless of the method employed, *knowing your liquid handler* will ultimately have an impact on: (1) experimental, assay and data integrity; (2) confidence in the assay results and process; (3) laboratory productivity and motivation; (4) creating and maintaining standardized protocols; (5) compliance with internal/external regulations; and (6) economics, where instrument downtime is minimized, lab-to-lab efforts are streamlined, downstream costs are reduced, and resources (time, labor) are minimized.

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