Simple and Fast Performance Verification for Ultra-Low-Volume Liquid Handlers: Nanoliter Aqueous Fluid Transfers Assessed with Dual-Dye Technology

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Abstract

Poor liquid handling performance can lead to poor assay results. When the volume verification methodology used to assess liquid handler performance is not properly executed, errors will enter into the process and the results could lead to incorrect conclusions regarding instrument performance. This presentation discusses proper techniques and best practices for assessing aqueous liquid transfer accuracy and precision for low-volume liquid handlers. These practices were established using both acoustic droplet ejection (Echo® 555 liquid handler, Labcyte Inc.) and a feedback-controlled, tip-based dispenser (Deerac™ GX reagent dispenser, Labcyte Inc.). Volumetric accuracy and precision were measured using a standardized volume verification platform based on a dual-dye absorbance technology (MVS®, Artel) and compared to fluorescence-based tests. For absorbance testing, manufactured aqueous-based dye solutions were employed for all target volumes and diluents. For fluorescence testing, sodium fluorescein (150 mM) in water was used for target volumes and sodium hydroxide (10 mM) was used as diluent. In both cases, the dye solution was transferred into a 384-well test plate with the Echo (30 – 200 nL) or the Deerac GX system (200 – 800 nL) followed by the addition of buffer. Using the MVS, the measured accuracy and precision for both the Echo and Deerac GX Series were below 5% inaccuracy and 5% CV for the volume transfers indicated. Some of the best practices developed and discussed herein include source plate preparation, assay plate preparation and assay plate reagent mixing. By following these recommended practices, optimal conditions for measuring liquid handler performance can be achieved.

Introduction

If a volume verification process is scientifically-based and the methods are properly executed, then the verification method can be used to increase confidence in liquid handler performance (reference 1). This presentation shows liquid handler performance for two different liquid handlers (Echo and Deerac GX) when two different volume verification methods are properly implemented and executed (MVS and a fluorescence method). For background information on the absorbance-based MVS system, see reference 2 and for the fluorescence method protocols, see reference 3. Additionally, using the Echo and MVS, purposely flawed methodologies were used to emphasize how the liquid handler's performance might be perceived, or interpreted, if the tasks are not properly executed. Understanding liquid handler performance comes when *both* the liquid handler task *and* the volume verification method are executed properly. If care is not taken when performing the liquid handler dispense protocol, or the volume verification method is not properly implemented, *true* liquid handler performance may not be measured.

For best practices and assessment of Echo system performance as determined by the MVS, see reference 4. Briefly, the sample solution and source plate must be bubble free and must be near thermal equilibrium with their surroundings. The source plates should therefore be centrifuged before use and for the 384-well destination (test) plates, the well contents should also be centrifuged before efficient mixing and subsequent readout. See reference 5 for methods to evaluate mixing efficiency.

Experimental

The MVS Sample Solution or the fluorescence test solution was transferred to a 384-well source plate (see **Figure 1**) with a hand-held electronic pipette before centrifugation in a Beckman Coulter Allegra 25R plate centrifuge. After the source plates were centrifuged, they were placed in, or on, the liquid handler for volume transfer performance measurement. In the case with MVS, after target was transferred from the source plate to the empty (dry) 384-well Corning 3711 destination (test) plate, 55 µL diluent was added, the plate was centrifuged and then shaken on the shaker before readout (**Figure 2**).

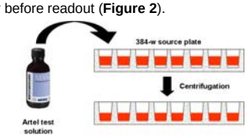

Figure 1. Schematic highlighting the simple process of preparing a 384-well source plate with Artel aqueous test solution. For Echo testing, ~ 10 µL of test solution per well was pipetted into a 384-well polypropylene source plate (Labcyte P-05525) before centrifuging for 3 min at 3000 rpm. For Deerac GX testing, 50 µL of test solution was pipetted per well into a 384-well Corning 3711 plate before centrifuging at 1000 rpm for 1 min.

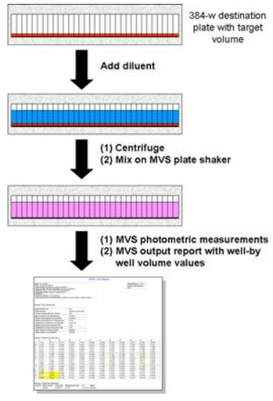

Figure 2. Testing the liquid handler with the MVS method. After the target volumes are dry-dispensed into the destination plate, 55 µL diluent is added to each well of the destination plate before it is centrifuged (1 min, 1000 rpm) and mixed on the MVS plate shaker (ramping with high and low cycles for 110 s at 2750 rpm). The destination plate is then measured on the MVS plate reader to determine well-by-well volumes and an output report is generated for immediate review.

Results

Properly Executing Liquid Handler Tasks and Volume Verification Methodology

MVS and the fluorescence volume verification methods were both used to assess Echo and Deerac GX performance for various nanoliter target volumes with aqueous sample fluids. All collected data are shown in **Tables 1 – 2** (Echo) and **Tables 3 – 4** (Deerac GX).

Table 1. Echo Performance via MVS

Plate ID (nL)	30	50	75	100	150	200	250
Average Calculated Volume (nL)	31.0	49.8	72	96.2	144.6	200.2	254.6
Standard Deviation (nL)	0.7	4.4	2.2	1.3	2.3	4.7	7.8
CV	**2.42%**	**2.61%**	**2.36%**	**2.39%**	**2.49%**	**2.35%**	**3.06%**
Relative Inaccuracy	**3.17%**	**-0.40%**	**-4.00%**	**-3.80%**	**-3.60%**	**0.10%**	**1.84%**
Min measured volume (nL)	28.8	46.5	67.4	89.7	133.6	185.6	237.9
Max measured volume (nL)	32.7	53.7	75.6	101.6	155.2	213.4	276.3
Total number of data points (*n*)	192	192	192	192	192	192	192

Table 2. Echo Performance via Fluorescence

Plate ID	2.5 nL	30 nL	50 nL	75 nL	100 nL	150 nL	200 nL
Average Calculated Volume (nL)	2.51	30.98	51.48	75.12	97.22	145.00	189.69
Standard Deviation (nL)	0.09	1.33	2.01	2.61	2.95	6.03	8.07
CV	**3.73%**	**4.29%**	**3.91%**	**3.48%**	**3.03%**	**4.16%**	**4.25%**
Relative Inaccuracy	**0.39%**	**3.26%**	**2.95%**	**0.16%**	**-2.78%**	**-3.34%**	**-5.16%**
Min measured volume (nL)	2.19	25.17	43.66	63.71	84.44	109.63	151.26
Max measured volume (nL)	3.03	36.08	55.89	80.70	107.64	156.07	210.79
Total number of data points (*n*)	384	384	384	384	384	384	384

Table 3. Deerac GX Performance via MVS

Plate ID (nL)	200 nL	400 nL	600 nL	800 nL	1000 nL
Mean volume for all Channels (nL)	205.2	399.4	606.5	825.9	1026.8
Standard Deviation for all Channels (nL)	9.1	14.2	18.6	23.7	33.7
Coefficient of Variation for all Channels	**4.43%**	**3.56%**	**3.07%**	**2.87%**	**3.28%**
Relative Inaccuracy for all Channels	**2.60%**	**-0.15%**	**1.08%**	**3.24%**	**2.68%**
Min measured volume (nL)	166.1	348.2	559.3	767.9	874.2
Max measured volume (nL)	231.8	433.5	665.2	910	1120.9
Total number of data points (*n*)	384	384	384	384	384
Number of data points *n per channel*	48	48	48	48	48

Table 4. Deerac GX Performance via Fluorescence

Plate ID	50 nL	100 nL	200 nL	400 nL	800 nL	1000 nL
Average Calculated Volume (nL)	48.38	98.46	209.56	410.93	816.89	966.36
Standard Deviation (nL)	4.37	7.20	9.00	11.12	20.35	35.15
CV	**9.03%**	**7.32%**	**4.29%**	**2.71%**	**2.49%**	**3.64%**
Relative Inaccuracy	**-3.24%**	**-1.54%**	**4.78%**	**2.73%**	**2.11%**	**3.36%**
Min measured volume (nL)	37.17	60.27	114.17	364.14	740.01	852.06
Max measured volume (nL)	69.19	141.93	246.83	442.12	869.74	1046.26
Total number of data points (*n*)	384	384	384	384	384	384

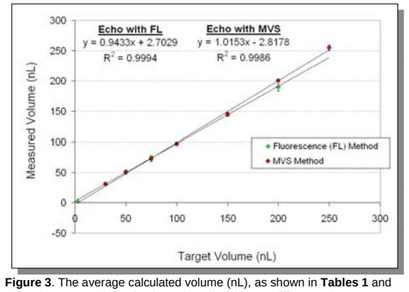

Figure 3. The average calculated volume (nL), as shown in **Tables 1** and **2**, vs. the target volume for the Echo using the two different volume verification methods. Error bars represent the standard deviation at each data point. (FL = fluorescence)

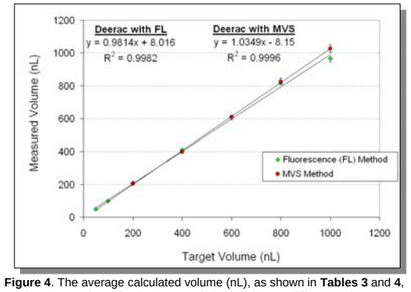

Figure 4. The average calculated volume (nL), as shown in **Tables 3** and **4**, vs. the target volume for the Deerac GX using the two different volume verification methods. Error bars represent the standard deviation at each data point. (FL = fluorescence)

The above data represent typical results when the liquid handling tasks and volume verification methodology are properly executed. Both the MVS and fluorescence methods are comparable for the two different liquid handlers with 100% aqueous sample fluids. The MVS method requires little skill and takes only minutes to execute per target volume. The fluorescence method takes longer and requires careful pipetting, however, it can be used to verify transfer volumes as low as 2.5 nL whereas the MVS methodology can only measure down to: 30 nL in a standard 384-w plate; 19 nL in a low-volume 384-well plate; or 10 nL in a 384-well low-profile plate.

As shown in **Figures 3** and **4** for the Echo and Deerac GX liquid handlers, respectively, volume delivery is linear over the volume range as measured with both methods (and R^2 values are ≥ 0.998). Note that the individual data points presented in **Figures 3** and **4** contain error bars representative of the standard deviation of the *n* measurements made at each test volume (in many cases, the error bars are too small to see).

The MVS and Echo were then used to demonstrate what happens to liquid handler CV performance when the tasks are not properly implemented and executed (**Table 5**).

Table 5. Assessing Echo Performance with MVS Methodology: INCORRECTLY (A – D) and CORRECTLY (E)

A. Source Plate NOT Centrifuged [a]

	n	CV	Notes	What to do next time
Source plate NOT centrifuged before tranferring test solution	384	131.61%	Souce plate had air pockets, which caused repeatability errors during transfer	Spin source plate before transfer, recommended 3000 rpm for 3 min

[a] After 50 nL target transfer, diluent added; destination plate centrifuged and shaken on MVS shaker before reading

B. Destination Plate Read WITHOUT Centrifugation or Efficient Mixing [b]

	n	CV	Notes	What to do next time
Destination plate not centrifuged or shaken	384	98.67%	Addition of Diluent to target does not efficiently mix contents; large CV resulted from dye pockets not being mixed	After diluent added, the plate should be centrifuged (approx 1000 rpm for 1 min), shaken on MVS shaker, then read
Same destination plate centrifuged and immediately read (not shaken)	384	11.40%	Addition of Diluent followed by spinning does not mix well contents in a 384-w std plate	same as above

[b] Source plate centrifuged; 50 nL target transferred to destination plate; 55 uL diluent added

C. Destination Plate Read WITHOUT Efficient Mixing - Trial 1 [c]

	n	CV	Notes	What to do next time
Destination plate immediately read (centrifugation, but no shaking)	384	14.34%	Centrifugation of destination plate but no shaking step; plate read before mixing by diffusion could occur	After diluent added, the plate should be centrifuged (approx 1000 rpm for 1 min), shaken on MVS shaker, then read
Same destination plate re-read 30 mins after initial read	384	4.43%	Diffusion after 30 mins improved response but still not efficiently mixed (note - reading moves plate for 4 mins, which facilitates mixing and this read was second pass through reader; this the plate 'moved' for 8 mins - so diffusion helped by plate reader	same as above
Same destination plate re-read 60 mins after initial read	384	3.51%	Diffusion is slow. After an additional 30 mins, the CV is improved but the wells are still not efficiently mixed (note - as above, this read was third pass through reader; therefore this plate 'moved' constantly for 12 mins - so diffusion helped by plate reader	same as above

[c] Source plate centrifuged; 50 nL target transferred to destination plate; diluent added; destination plate centrifuged

D. Destination Plate Read WITHOUT Efficient Mixing - Trial 2 [d]

	n	CV	Notes	What to do next time
Destination plate centrifuged and read 30 mins after initial dispense (no shaking)	384	8.12%	High CV indicated that well contents are not properly mixed after 30 mins of waiting before read	After diluent added, the plate should be centrifuged (approx 1000 rpm for 1 min), shaken on MVS shaker, then read
Same destination plate re-read 30 mins after initial read (60 mins from transfer)	384	4.21%	CV further improves after 60 mins of diffusion (note - the plate reader moves plate for 4 mins per read, so there is some added plate-motion mixing to the diffusion)	same as above
Same destination plate re-read 60 mins after initial read (90 mins from transfer)	384	3.36%	CV further improves after 90 mins of diffusion (see note above)	same as above

[d] Source plate centrifuged; 50 nL target transferred to destination plate; diluent added; destination plate centrifuged

E. Volume Verification Performed Correctly [e]

	n	CV	Notes	What to do next time
No mistake. Source plate centrifuged; Diluent added to destination plate, then centrifugation, mixing and reading (in that order)	192	2.61%	No mistake (repeated results from Table 1)	NA

[e] Source plate centrifuged; 50 nL target transferred to destination plate; diluent added; destination plate centrifuged

Discussion & Conclusion

Good lab work generates results that can be trusted.

- Both the Echo and Deerac GX liquid handlers perform well within their stated specifications for low-nanoliter aqueous target volumes when using best practices for checking liquid handler performance.
- When care is taken with liquid handler QC, the performance of the liquid handler, good or bad, can be accurately assessed.
- Both the MVS and manual fluorescence-based methods yielded comparable results.
- The fluorescence method takes much longer and requires pipetting skill, whereas the MVS method is simple and requires little skill.
- The fluorescence method can be used for volumes as low as 2.5 nL, whereas the MVS can be used for volumes as low as 30 nL in the same plate type (384-well standard plates).
- Using flawed methodologies for QC'ing a liquid handler, *i.e.*, to save time, will result in a false-sense of liquid handler performance, which may lead to assay results that are *unknowingly* misleading.

It should be noted that the same Deerac GX was assessed two months apart with the two different methodologies and the same Echo was assessed one month apart at two different locales (east coast with MVS, west coast with fluorescence). In the work presented in Table 5, we took advantage of the ease at which the MVS can be quickly used to assess liquid handler performance when methods are performed correctly and incorrectly. By systematically changing parameters and subsequently measuring the contents in the microtiter plate, we could observe how odd or inconsistent methodology changes ultimately affected the *perceived* liquid handler repeatability (as noted by the CVs in **Table 5**).

References

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(6) *Painless Nanoliter-Transfer Accuracy Inspection For Busy Screeners*, at http://www.artel-usa.com/resources/posters.aspx or www.labcyte.com

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