Best Practices for Assessing Nanoliter Transfer Performance for Labcyte Echo Liquid Handlers Using the Artel MVS

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Abstract

Acoustic drop ejection (ADE) technology enables low-nanoliter transfers for high-throughput screening applications. Verifying the absolute transferred volume and the well-to-well reproducibility of the ADE liquid handler is important for quality control, but there are few methods available that can quickly and accurately interrogate dispensed volumes in this range. When the volume verification method used to assess transfer performance is not analytically implemented and properly executed, or when it is rushed through due to time constraints, unseen errors may occur in the results which may introduce a false sense of transfer performance.

This presentation discusses the best practices for assessing liquid transfer accuracy and precision for the Echo® 555 liquid handler (Labcyte Inc.) with the Artel Multichannel Verification System (MVS®), a standardized volume verification platform using a dual-dye absorbance-based method. Some of the best practice topics discussed herein include test solution preparation, source plate preparation and assay plate reagent mixing. By following these recommended practices, optimal conditions for measuring the Echo performance can be achieved. Using the best practices, the measured accuracy and precision of the Echo were below 5% inaccuracy and 5% CV for transfer volumes of 50 to 300 nL of DMSO solutions.

Introduction

A volume verification method, which can be used to quantify the amount of transferred volume from a liquid handler, is an essential component that enables proper interpretation of experimental results (reference 1). If the volume verification procedures are scientifically-based and the methods are properly executed, then the verification method can be used to increase confidence in assay integrity and liquid handler performance. A very important facet of understanding liquid handler performance is by properly executing *both* the liquid handler task *and* the volume verification method. 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 Echo ADE performance, the sample solution must be bubble free, must be in intimate contact with the bottom of the wells in the source plate, and must be near thermal equilibrium with its surroundings. For photometric verification methods, such as with MVS (reference 2), best performance also requires bubble free, properly mixed solutions which are in thermal equilibrium with the lab environment. For optimal MVS performance, proper sample mixing is important and the key to accurate dispensing performance confirmation for a liquid handler (reference 3). This presentation demonstrates Echo volume transfer performance with the MVS when both methods are properly implemented and executed.

Experimental Section

A dye-based DMSO test solution is prepared and used to fill an Echo source plate. Per instructions through ARTEL's *Alternative Solution Helper* software, DMSO test solutions can be prepared by gravimetrically combining concentrated MVS dye stock solution with DMSO (reference 4; **Figure 1**).

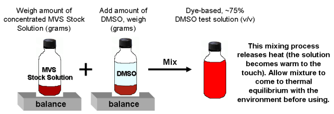

Figure 1. Schematic highlighting the simple process of preparing a dye-based DMSO test solution for MVS volume checks, as described in greater detail in reference 4.

Using the weight of the MVS stock solution, the weight of the added DMSO, and the density of DMSO (1.1 g/mL), MVS system software calculates the volume range for the test solution, which is based on dye concentration. Current measurable volume ranges for DMSO test solutions are approximately 0.4 – 50 µL in a 96-well plate or 0.04 – 10 µL in the various 384-well plate formats. The detailed process is discussed in reference 4. After allowing the DMSO test solution to come to thermal equilibrium, a 384-well polypropylene source plate (Labcyte P-05525) is prepared for the Echo by dispensing 30 µL/well to the plate with a hand-held pipette. The source plate is then centrifuged using a Beckman Coulter Allegra 25R plate centrifuge to remove any bubbles and any air gap between the solution and the plate bottom, thereby ensuring intimate contact between the solution and the well bottom (**Figure 2**). This intimate contact allows for proper acoustic energy transfer into the test solution, which in turn provides for accurate ADE dispensing.

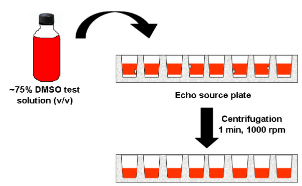

Figure 2. The equilibrated DMSO test solution is added to each well of the Echo source plate with another liquid handler or by hand pipetting. The source plate is centrifuged to remove any bubbles and to ensure intimate contact between the dye solution and the well bottom.

The source plate is inserted into the Echo 555 liquid handler for target volume transfer via ADE into a 384-well destination plate (**Figure 3**).

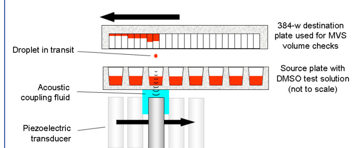

Figure 3. Echo ADE volume transfer into the optically-clear, flat-bottom 384-well destination plate for MVS measurement.

Once the transfer is completed, the destination plate is removed from the Echo. A 55-µL aliquot of diluent is manually added to each well of the destination plate, and the plate is centrifuged and mixed on the MVS plate shaker before volume measurements commence (**Figure 4**). Sufficient mixing of solutions is a key performance parameter that must occur.

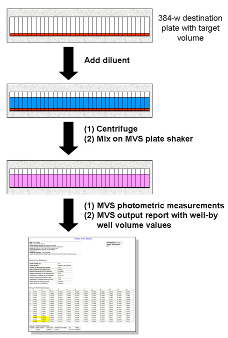

Figure 4. After the target volumes are transferred, 55 µL diluent is added to each well of the destination plate before it is centrifuged (1 min, 1000 rpm) and mixed on the MVS plate shaker (110 s, 2750 rpm). The destination plate is measured on the MVS to determine well-by-well volumes and an output report is generated for immediate review.

Results and Summary

MVS was used to rapidly assess Echo performance for various target volumes (50 nL, 100 nL, 200 nL, 300 nL) with a 75% DMSO test solution (v/v). For the target volumes transferred, the performance of the Echo was tested for linearity over the entire volume range of interest (50 nL – 300 nL). **Figure 5** demonstrates a significantly linear volume delivery, as measured by the MVS for each of the 96 replicate wells, with a R^2 value of 0.99995. Note that the individual data points presented in **Figure 5** also contain error bars representative of the standard deviation of the 96 measurements made at each test volume, which are too small to see.

Additional analysis shows that both the relative inaccuracy and CV for each of the four target volumes are all below 3% (**Figures 6-7**). A summary of all data for the Echo performance is presented in **Table 1**. As mentioned, the test solutions were allowed to come to thermal equilibrium before they were dispensed into the Echo source plate, and the source plate was centrifuged before placing into the Echo. To reach an MVS working volume in each of the 384 wells, a 55-µL addition of diluent was conducted using a handheld multichannel pipette before MVS measurement. Because the MVS process is based upon dual-dye ratiometric photometry, the accuracy of the diluent addition is not important, so long as the total working volume is within ~± 20% of the recommended 55 µL total working volume. Further details of MVS performance can be found in reference 2. After the addition of diluent, the destination plate was centrifuged and then mixed on the MVS plate shaker before using the MVS for volume measurements.

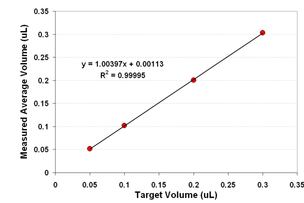

Figure 5. MVS measured volumes vs. theoretical target volumes delivered by the Echo show a linear relationship over the tested volume range for the 75% DMSO test solution.

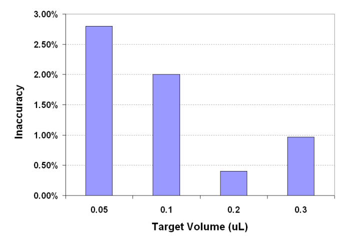

Figure 6. The relative inaccuracies for the four target volumes transferred by the Echo, as measured by the MVS.

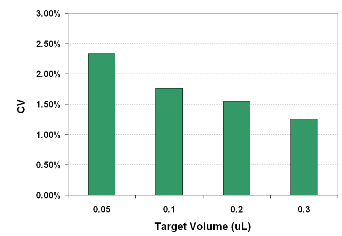

Figure 7. The %CV (coefficient of variation) for the four target volumes transferred by the Echo, as measured by the MVS.

Table 1. Echo transfer performance summary, as measured by the MVS, when the experimental methods for both are properly executed

Target Volume (µL)	*n* *	Average Volume (µL)	Relative Inaccuracy %	Standard Deviation (µL)	CV%
0.05	96	0.0514	2.80%	0.0012	2.33%
0.1	96	0.102	2.00%	0.0018	1.76%
0.2	96	0.2008	0.40%	0.0031	1.54%
0.3	96	0.3029	0.97%	0.0038	1.25%

* n = number of replicate measurements

Discussion

If automated liquid handlers are not dispensing the exact, or desired, volume for critical reagents, then it is likely that unseen error can increasingly propagate as a process continues. Without knowing the exact volume transferred at each step from a liquid handler, for instance, true concentrations of species in solution may be unknown and assay results could be falsely interpreted. As process control within a laboratory continues to be emphasized, a volume verification method should be properly implemented so that liquid handler behavior is known and optimized to deliver the desired target volumes throughout all levels of assay development.

In addition to the need for a robust and reliable volume verification method, implementing proper protocol is also key in achieving optimal performance from any liquid handler, or volume measurement approach. Users should follow "best recommended practices" when conducting any type of testing with scientific equipment. If said practices are ignored, suboptimal performance can result, and may not be immediately recognized.

Conclusions

The data presented herein were collected following "best recommended practices" for both the Labcyte Echo 555 and the Artel MVS. As pertaining to these experiments, some of the key points are:

- The test solution was allowed to cool and come to thermal equilibrium with the lab environment prior to dispensing with the Echo.
- The source plate was centrifuged before dispensing from the Echo to remove any bubbles in the sample solution and to ensure intimate contact between the test solution and the well bottom.
- The sample solution and diluent were fully mixed in the test plate prior to MVS measurements.

When these practices are followed, the Echo 555 is capable of achieving the performance demonstrated, and the MVS measurements of the delivered volumes can be trusted to the highest degree. If these practices are not followed, non-ideal performance of the Echo and/or the MVS may be unknowingly observed in the final results.

References

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