Painless Nanoliter-Transfer Accuracy Inspection for Busy Screeners

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Abstract

Acoustic droplet ejection first provided low-nanoliter transfers for high-throughput screening applications in 2004. At that time, there were no methods that could quickly and accurately determine the accuracy and precision of the transfer volume. As a result, existing quality control processes were modified by many screening facilities for these determinations. These modified processes have become time consuming and troublesome tasks. The ARTEL Multichannel Verification System (MVS®), which measures transfer volumes in microplates using the ratio of measured absorbance values between two dyes[1], is a complete system that enables aqueous-based and DMSO-based[2] volume verification on a rapid, standardized platform.

In this study, the MVS was used to measure transfer volumes of DMSO-based sample solutions down to 100 nL in a standard 384-well assay plate. In addition, an experimental DMSO-based sample solution with a higher concentration of dye was employed to show accurate and precise volume measurements at volumes as low as 5 nL. Here we compare accuracy and precision data for multiple Labcyte Echo® liquid handlers collected using the MVS system. Additionally, we compare the data collected using the MVS versus a fluorescein-based volume verification method[3].

Introduction

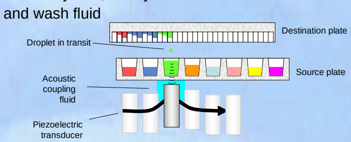
Artel MVS System Description

The Artel MVS contains the following components:

- Sample solutions, diluent, and baseline solutions containing known concentrations of dyes with distinct absorbance maxima, traceable to national standards.
- A specialized calibration plate consisting of sealed precision quartz cuvettes provides system traceability. Neutral density glass within each calibration plate provides an internal control to ensure proper performance.
- A plate shaker provides optimal mixing of solutions and diluent in the wells.
- A customized plate reader delivers reliable absorbance measurements at 520 nm and 730 nm.
- A notebook computer with MVS Data Manager software and a barcode reader.

Labcyte Echo Liquid Handler System Description "Move Liquids with Sound"™

- Use acoustic energy to transfer fluids with high precision and accuracy
- Transfer 2.5-1000 nL volumes
- Use true non-contact, direct plate-to-plate transfer
- Transfers any well to any well, at any volume
- Eliminates tips and wash fluid



Method and Materials

Artel MVS Method

1. Scan the MVS calibrator plate
2. Take a baseline measurement
3. Echo transfer 100 – 200 nL of Solution D (dual-dye in 75% DMSO), or transfer 5 – 100 nL of Solution E (dual-dye in 95% DMSO) into test plates
4. Fill test plates with diluent; shake plates for one minute
5. Begin photometric measurement
6. Generate report

Labcyte Fluorescein Measurement Method

1. Make stock solution (0.15 mM Fluorescein in DMSO)
2. Prepare two standard curve plates; one for 0 – 150 nM fluorescein in 10 mM NaOH, the other for 75 – 600 nM fluorescein in 10 mM NaOH
3. Read the two standard curve plates on PerkinElmer EnVision™ plate reader; graph fluorescent count versus concentration and record down the curve intercept and the slope
4. Read the Matech fluorescein reference plate (Matech cat# BR-517) before and after the standard curve plates and average the two readings
5. Use the Echo liquid handler to transfer 5 – 200 nL of 0.15 mM stock solution into 384-well test plates
6. Add 50 µL of 10 mM NaOH to test plates
7. Incubate test plates for 30 minutes; keep plates in dark
8. Read the test plates using the same reader protocol as step 3; read the Matech fluorescein reference plate before and after the test plates and average the two readings
9. Calculate Echo transfer precision (%CV)

   %CV = (STDEVP of one plate) / (Average of one plate) * 100%
10. Calculate reader PMT correction factor

    PMT Correction Factor = (Ave. Matech $_{curve}$) / (Ave. Matech $_{test}$)
11. Calculate Echo transfer volume

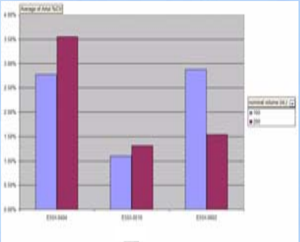
    Transferred Vol. = (PMT Correction Factor) * [(Fluorescent Count – Standard Curve Intercept) / (Standard Curve Slope)]
12. Calculate Echo transfer accuracy

    % Inaccuracy = (Expected Vol. – Transferred Vol.) / (Expected Vol.) * 100%

MVS Measurements

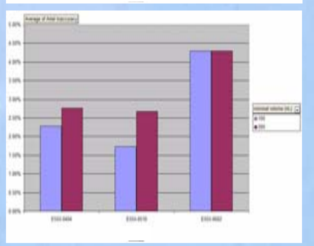
Transfer Precision

We used MVS Solution D to compare the transfer performance of three Echo liquid handlers. Transfer precision at 100, and 200 nL were monitored. Measured coefficient variations (CVs) were under 4% in all cases. Notice that the transfer precision measured for all three instruments were within 3% variation.



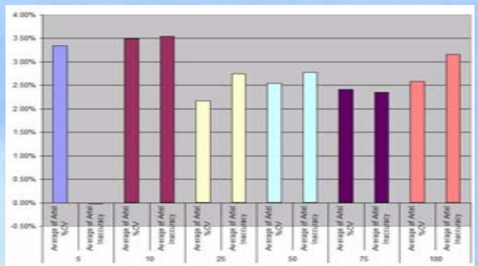
Transfer Accuracy

Using MVS Solution D, we compared the transfer accuracy of three Echo liquid handlers at 100, and 200 nL. Transfer inaccuracy was under 4.5% in all cases. The differences in accuracy for all three instruments were within 3%.



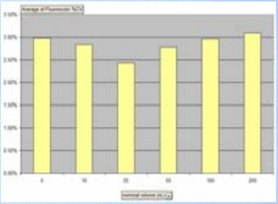
New MVS Solution for Low Nanoliter Measurements

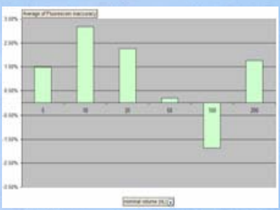
Solution E (in 95% DMSO) was developed to measure transfer performance at 5-100 nL range. Transfers made with an Echo liquid handler showed <4% CV and <4% inaccuracy at all volumes.



Fluorescein Measurements

Following the Labcyte fluorescein method described earlier, we determined the transfer performance from 5 to 200 nL for two Echo units. The average precision and accuracy were <3.5% CV and <3.5% inaccuracy.





Timing Comparison

We compared the time to complete transfer accuracy analysis for one test plate (one transfer volume) using the MVS method and the fluorescein method:

MVS	
Step	Time
Calibrate Plate Reader	1 minute
Fill, Shake & Read "Baseline" Plate	6 minutes
Transfer Dyes to 1 Test Plate	1.5 minutes
Fill, Shake & Read Test Plate	6 minutes
Software Output Final Results	Immediately
Total Time	**14.5 minutes**

Fluorescein Method	
Step	Time
Make Stock Solution	10 minutes
Prepare Standard Curve Plates	40 minutes
Transfer Dyes to 1 Test Plate	1.5 minutes
Fill, Incubate & Read Test Plate	33 minutes
Calculate Data	10 minutes
Total Time	**94.5 minutes**

The Artel MVS system measured the precision and accuracy of transfers performed with an Echo liquid handler in less than 15 minutes. The fluorescein method took over 90 minutes to complete. The MVS method is simple to follow and saves time.

Conclusion

- **The precision of low nanoliter transfers performed with an Echo liquid handler were measured to be under 4% using either the MVS or the fluorescein method**
- **The accuracy of low nanoliter transfers performed with an Echo liquid handler were measured at greater than 96% by both the MVS and the fluorescein methods**
- **The MVS system is easy and ready to use and requires no additional preparation**
- **The MVS system provides the same results as the fluorescein measurement in much less time**

Discussion

When measuring nanoliter-range transfer performance, the researcher must consider the linearity of the standard curves, the stability of the dye solutions, the inherent signal drift of the plate reader, and the dispensing accuracy of the bulk filler used to add diluent. The fluorescein method developed by Labcyte has taken all of these factors into consideration but still requires a skilled technician. The standard MVS method, however, reports similar test results similar to the fluorescein method, is more convenient and does not require a skilled technician.

References

1. Bradshaw *et al.* *JALA*, 2005, 10, 35-42
2. Albert *et al.* *JALA*, 2006, 11, 172-180
3. Harris and Mutz, *JALA*, 2006, 11(4), 233-239

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