



Evaluation of Forward and Reverse Mode Liquid Handling Techniques Using the ARTEL MVS[®]

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Abstract

This study illustrates the use of the Artel MVS[®] Multichannel Verification System to rapidly evaluate the accuracy and precision of dispensed volumes from the Caliper RapidPlate liquid handler. The volume transfer performance of the RapidPlate was directly compared for two commonly used liquid handling techniques: (1) direct dispense (forward mode) and (2) over-aspiration (reverse mode).

Introduction

Traditionally, *precision* of volume dispensing has received more focus than *accuracy*, making the latter overlooked. This study highlights the importance of understanding both accuracy (how close the transferred volume is to the target volume) and precision (how repeatable the volume transfer is) when evaluating the quality of instrument performance. Quantifying both parameters allows liquid handling techniques to be directly compared. Until now, however, a fast, easy way to measure both accuracy and precision of delivered volumes has been absent. The MVS streamlines the assessment of accuracy and precision into one measurement and facilitates liquid handler optimization through both manual and electronic method adjustments during assay development and on-the-fly assay validation.

Materials & Methods

The MVS simultaneously measures the accuracy and precision of volume dispense performance for target volumes between 10 nL and 200 μ L. A target volume of MVS Sample Solution is dispensed into a characterized microplate (Verification Plate) followed by the non-quantitative addition of Diluent.¹ After mixing, the absorbance values of the solutions in the plate are measured at two distinct wavelengths and used to determine the volume of sample dispensed by the liquid handler under test¹. This application note describes the use of the MVS to evaluate the following liquid handling methods:

Forward mode transfer method (Direct)²:

1. Aspirate a volume of air into the tip (pre-air gap)
2. Aspirate a desired volume of sample solution into the tips from a reservoir on the deck of the liquid handler under test
3. Dispense the entire contents of the tip (sample and air) into the microplate

Reverse mode transfer method (Over-asp)³:

1. Aspirate a pre-air gap
2. Aspirate the desired volume of sample *plus* an additional aliquot of sample into the tips from a reservoir on the deck of the liquid handler under test

3. Dispense a small portion of the sample back into the reservoir
4. Dispense the desired volume of sample into the microplate
5. Discard the remainder of the tip contents to waste

The MVS Sample Solutions and Diluent were transferred to 96-well Artel Verification Plates using the 96-tip Caliper RapidPlate (Caliper Life Sciences, Hopkinton, MA) at a variety of volumes spanning the specified performance range of the liquid handler. Tested volume transfers also included some that were lower and therefore outside of the specified volume range of the instrument. Each target volume was run in triplicate. For all replicates and all tips, the overall accuracy and precision values are reported herein.

The following liquid handler parameters were used for operation of the RapidPlate for both methods:

- 5 μ L air gaps
- Sample:
 - Aspirate height = 0.7 cm
 - Aspirate speed = 7 μ L/sec
 - Dispense height = 0.7 cm
 - Dispense speed = 8 μ L/sec
- Diluent:
 - Aspirate height = 0.7 cm
 - Aspirate speed = 10 μ L/sec
 - Dispense height = 0.7 cm
 - Dispense speed = 8 μ L/sec

Target (μ L)	Direct Actual (μ L)	Over-asp Actual (μ L)	Direct %CV	Over-asp %CV	Direct %Inacc	Over-asp %Inacc	Spec
200	191.84		0.44%		-4.08%		10%
100	96.18		0.47%		-3.82%		10%
50	48.22		0.64%		-3.57%		10%
40	38.22		0.37%		-4.46%		10%
25	24		0.49%		-4.01%		10%
10	9.47	9.8	0.69%	1.01%	-5.34%	-1.95%	10%
8	7.22	8.13	0.61%	0.92%	-9.79%	1.59%	10%
5	4.46	5.23	0.60%	1.06%	-10.74%	4.64%	10%
3	2.55	3.13	1.61%	1.65%	-14.89%	4.45%	10%
2	1.5	2.15	2.39%	1.97%	-25.17%	7.43%	10%
1	0.33	0.99	43.72%	7.68%	-66.70%	-1.49%	10%
0.5		0.51		24.83%		2.22%	10%

Table 1. Accuracy and precision of Direct and Over-aspiration methods measured by the MVS

For the reverse mode method, volumes as large as 20 μL were aspirated, 5 μL was returned to the reservoir followed by a dispense of the desired target volume into the microplate and discarding the remainder of the solution to waste. Once the solutions were dispensed to the plates, the accuracy and precision of the liquid handler were assessed using the MVS.

Results

The accuracy and precision of the RapidPlate, as measured by MVS at multiple target volumes using the two different dispense methods, are shown in Table 1 and represented in Figures 1 and 2.

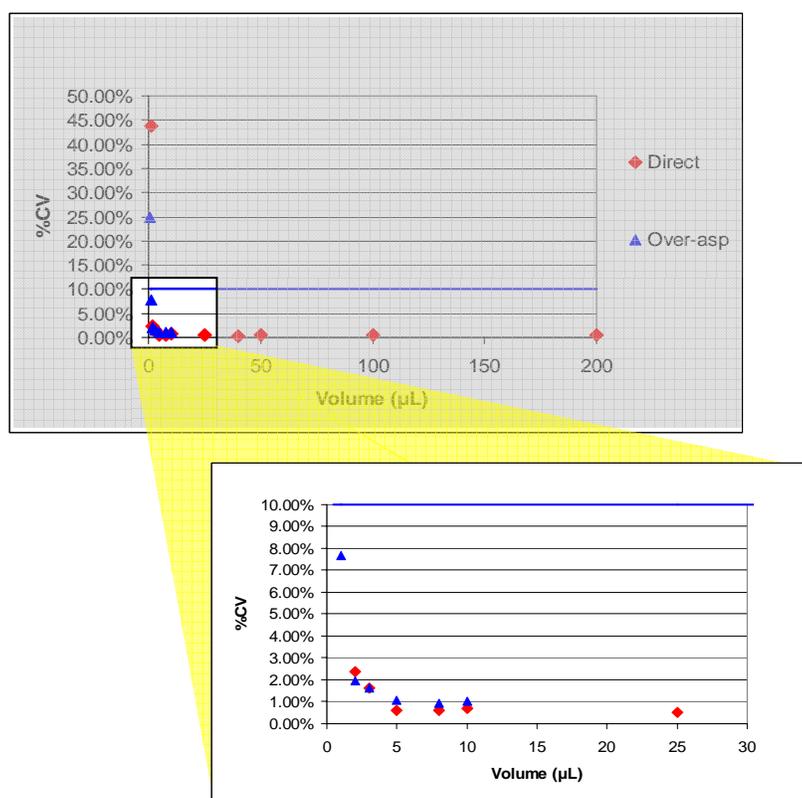


Figure 1. Comparison of precision (%CV) of Direct and Over-aspiration methods using the MVS. Volumes included in the graph are actual volumes delivered as opposed to target volume requested by the RapidPlate.

When comparing the precision data in Figure 1, it is notable that the performance at the nominal volume of 1 μL is significantly different between the two methods (Direct CV is 43.72% and Over-asp CV is 7.68%). Using the corresponding accuracy information provided by the MVS (Figure 2), it is clear that the actual volumes delivered are also significantly different between the two methods (Direct – 0.33 μL ; Over-asp – 0.99 μL). Because the actual volumes dispensed using the two methods are not equal, a direct comparison of the precision at that nominal volume is not appropriate. By using only precision information, it would be unclear that the performance of the liquid handler is being

compared for two different volumes.

Without knowing the accuracy of the dispensed volumes, incorrect assumptions about the equality of the performance of the two methods could be made.

As shown in Figure 2, there is a clear difference in accuracy of the transfer performance between the two liquid handling methods, which is not apparent when measuring only precision (as shown in Figure 1). For instance, between 2 and 10 μL , the precision values for both pipetting methods are nearly equal (Table 1, Figure 1), but in the same volume range, the Over-aspirate method is much more accurate at all target volumes (Table 1, Figure 2).

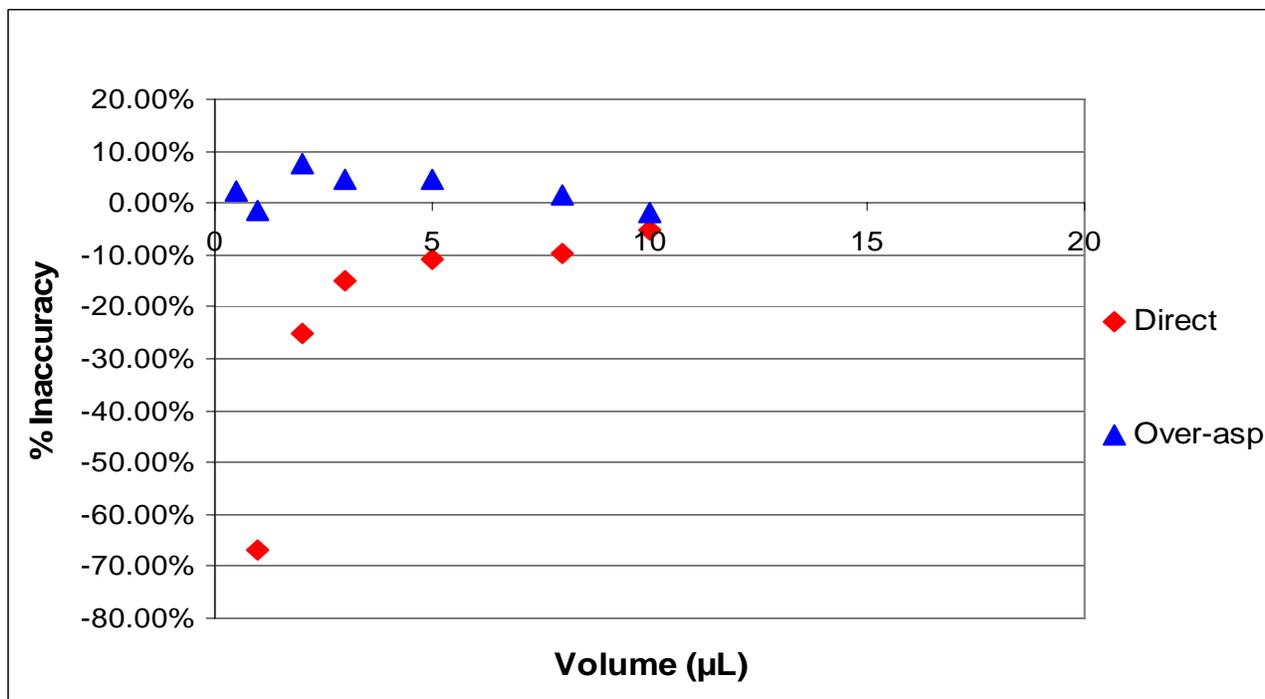


Figure 2. Comparison of the accuracy of dispensed volumes for Direct and Over-aspiration methods measured by the MVS (%CV in **Figure 1** was measured simultaneously)

Most software packages controlling liquid handler operation, including the software used to operate the RapidPlate, include adjustment features to allow the user to improve the volume transfer accuracy of the liquid dispense. These functions rely on the user to provide correct *actual* volumes in order to make the adjustments, which is information that MVS automatically reports. Absent an easy way to measure the accuracy of the volume transfer, correctly adjusting the software would be nearly impossible. The information the MVS provides allows the user to easily adjust the liquid handler to optimize the transfer performance. The performance information shown herein was all collected by the liquid handler in an “as found” state, meaning that the software was not adjusted to optimize the accuracy of the volume transfer.

Conclusion

The data contained herein illustrate the performance differences between two commonly used liquid handling methods as measured by the MVS. By comparing the precision and accuracy information directly, it can be concluded that liquid transfer accuracy is highly method dependent, especially at low volumes. In the case presented here, the reverse mode method was more accurate at the low volumes as compared to the direct mode method. The instrument’s volume transfer precision, however, is much less dependent on method. It is only possible to make the comparison of the two methods when the accuracy and precision parameters are simultaneously measured as they are with the MVS.

If, in the development and optimization of assays, a liquid handler’s pipetting technique and method are not assessed for volume transfer performance,

the assay integrity could be unknowingly flawed. Because concentrations of components in assays are volume-dependent, inaccurate volume delivery will lead to unknown sample concentrations, which could potentially lead to assay results that are misinterpreted. 

References

1. Bradshaw, J.T.; Knaide, T.; Rogers, A.; Curtis, R.H. Multichannel Verification System (MVS): A Dual-Dye Ratiometric Photometry System for Performance Verification of Multichannel Liquid Delivery Devices. *J. Assoc. Lab. Autom.*, **2005**, *10*, 35-42.
2. Direct mode pipetting technique is also known as forward-mode pipetting.
3. Over-aspiration pipetting technique is also known as reverse mode pipetting.

