



Techniques on the RapidPlate

Comparison of Liquid Handling Techniques on the Caliper RapidPlate using the Artel Multichannel Verification System (MVS®)

Abstract

Different liquid transfer techniques can be used on the Caliper Life Sciences' RapidPlate Liquid Handler. This study used the Artel Multichannel Verification System (MVS) to rapidly evaluate and compare the dispensing accuracy and precision for two different liquid transfer techniques. The current study shows that technique has a significant impact on accuracy, but that precision is relatively independent.

I. Introduction

Just as with hand-held pipettors, automated liquid handlers also require attention to technique. In this study, we compare two transfer techniques using the RapidPlate liquid handler. In automated liquid-handling systems, precision currently receives more attention than accuracy. However, since precision performance is related to the volume transferred, we were interested in evaluating both simultaneously. We therefore used the MVS to rapidly establish NIST traceable accuracy data concurrent with the collection of precision data. We found that the two liquid transfer techniques employed had a significant effect on the accuracy. However the precision was almost unaffected.

II. Methods and Materials

MVS dyes were transferred from an open reservoir to a MVS 96-well "characterized" microtiter plate¹ using the RapidPlate.



Figure 1: Caliper RapidPlate Liquid Handler.

The two liquid transfer techniques used were run at a variety of volumes, including volumes below the 5 μL manufacturing specification limit. The methods consisted of the following steps:

Method "A" (one-to-one transfer)

- 1) Aspirate a pre-air gap.
- 2) Aspirate the desired amount of dye from the dye reservoir.
- 3) Dispense dye into MVS characterized microtiter plate containing MVS Diluent solution.

Volumes were run in triplicate (three plates per volume). Tips were new for the first dispense and reused for each subsequent dispense. For the 200 μL dispense, 200 μL tips were used. All other sample dispenses were completed using 100 μL tips. All dispenses were completed by aspirating the desired volume and dispensing the contents of the tips with a 5 μL air gap. Operation parameters included: Diluent aspirate height = 0.7 cm, aspirate speed = 10 $\mu\text{L}/\text{sec}$, air gap = 5 μL , dispense height = 0.7 cm, dispense speed = 8 $\mu\text{L}/\text{sec}$. Sample aspirate height = 0.7 cm, aspirate speed = 7 $\mu\text{L}/\text{sec}$, air gap = 5 μL , dispense height = 0.7 cm and dispense speed = 8 $\mu\text{L}/\text{sec}$.

Method "B" (over-aspiration transfer)

- 1) Aspirate a pre-air gap.
- 2) Aspirate more than the desired amount of dye from the dye reservoir.
- 3) Dispense a portion of dye back to the dye reservoir.
- 4) Dispense dye into MVS characterized microtiter plate containing MVS Diluent solution.
- 5) Dispense any remaining dye to waste.

The pipetting speed, pre-air gap, and height settings were the same as for Method "A", however, an over aspiration protocol was employed during which as much as 20 μL was aspirated, 5 μL was then returned to the source, then the desired volume was transferred to the MVS plate. Any dye remaining in the tip was discarded.

After the methods were run, plates were analyzed using the MVS.

Results

In Table 1 we present results from both Techniques.

Target (µL)	Method A Actual (µL)	Method B Actual (µL)	Method A % CV	Method B % CV	Method A % Inacc	Method B % 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	24.00		0.49%		-4.01%		10%
10	9.47	9.80	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	1.50	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. Comparison of Method A and B, Accuracy and Precision.

This data is visualized in the following graphs. In Figure 2 the flat blue line is the manufacturer’s specification.

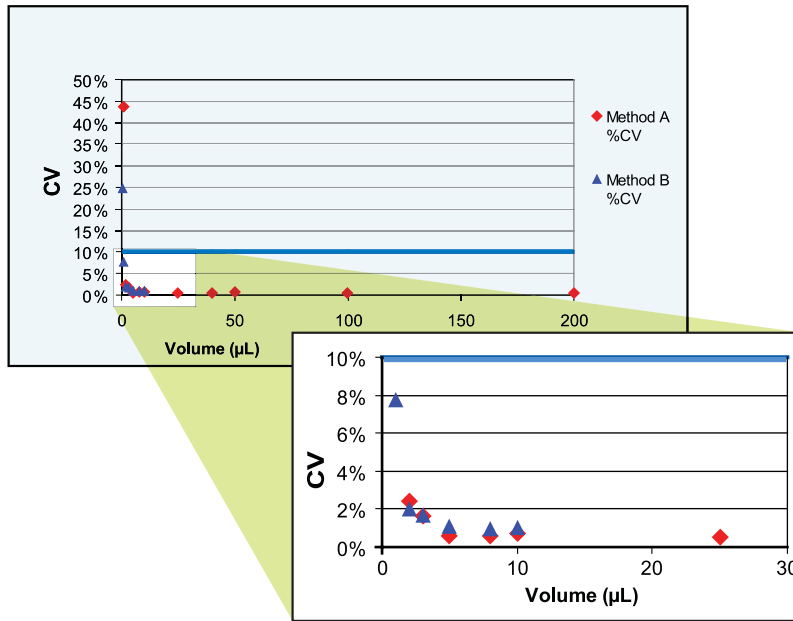


Figure 2. Precision Comparison for Method A vs. B

The precision performance is under 10% for some volumes below the RapidPlate’s specified minimum operating volume (5 µL). We also see that below 2 µL, the RapidPlate begins to exceed the manufacturer’s precision specification regardless of pipetting technique.

To compare precision performance between the two methods, we considered the actual volume delivered rather than the requested volume, and compared their corresponding precision. Although there is a significant difference in the precision results for Method A versus Method B at the nominal volume of 1 μL , this is not a proper comparison; since the actual volume transferred in Method A was only one third of the volume transferred in Method B. Therefore, a direct comparison of precision requires using actual rather than nominal volume data.

This brings up the important point that, when evaluating various liquid transfer techniques, accuracy as well as precision should be simultaneously tracked in order to make a fair comparison.

In Figure 3 we see that there is a significant difference in accuracy depending on the pipetting technique. After choosing a liquid transfer technique, a liquid class calibration of the instrument can be used to improve accuracy. The results in Figure 3 are uncorrected "out-of-the-box" accuracy results.

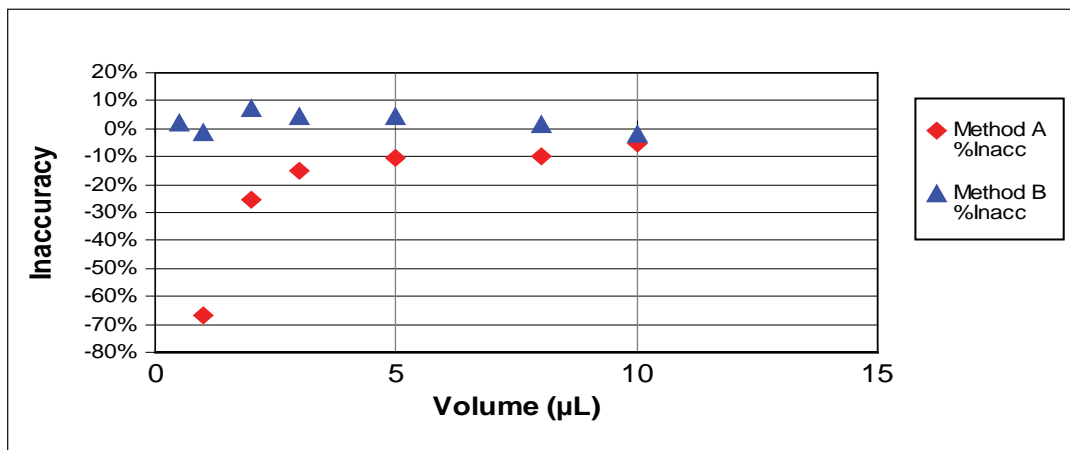


Figure 3. Inaccuracy Comparison for Method A vs. B

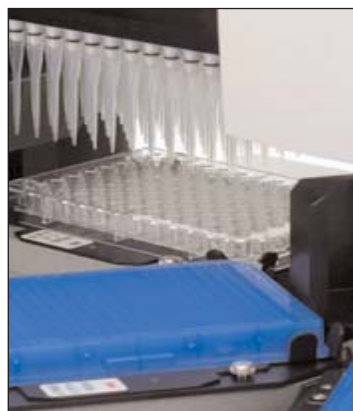
Conclusion

As expected, pipetting techniques can have a significant impact on accuracy. The data shows that although accuracy is technique dependent, precision is relatively technique independent. It is only possible to make this comparison when both accuracy and precision are being simultaneously determined.

We found the use of the MVS facilitated rapid, simultaneous generation of precision and accuracy data. We believe this quick evaluation (<1 day) is sufficient for many applications. Additional calibration of liquid classes could further improve the accuracy.

The implication of our findings suggest that for validating liquid transfer methods, a consideration of pipetting technique, when coupled with liquid class calibration, will yield the best liquid handling results.

We also found that the performance of the RapidPlate used exceeded the manufacturing specification. Although this was a single unit, it may be inferred from the data that the manufacturing specifications could be safely re-evaluated and lowered to include both a wider range (2-200 μL rather than 5-200 μL) and a tighter precision specification (5% rather than 10%).



¹Bradshaw, J.T.; Knaide, T.; Rogers, A. and Curtis, R. *Multichannel Verification System (MVS): A Dual-Dye Ratiometric Photometry System for Performance Verification of Multichannel Liquid Delivery Devices*, JALA 2005; 10(1): 35-42.



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