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Application Note

Optimizing Accuracy Performance on an Agilent Bravo Platform Using the Artel MVS®

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Abstract

This application note discusses one approach for optimizing pipetting accuracy on an Agilent Bravo liquid handler using MVS® Multichannel Verification System measurement information. Although the MVS system supports verifying both tip-by-tip precision and accuracy values for nearly all liquid handlers[1,2], the focus of this paper is accuracy measurement and optimization. This application note does not speak to the quality of volume transfer performance you should expect from any Bravo; instead it focuses on the optimization process only.

Pipetting accuracy can be improved in any number of ways, such as by adjusting individual pipetting variables (aspirate rates, dispense rates, air gaps, mix steps, etc.) and measuring transferred volume resulting after each successive adjustment. In the approach discussed here, the individual variables within a method are relatively unimportant to the optimization process. This application note is intended to show that volume transfer accuracy can be improved by simply adjusting the calibration factors within the method's liquid class, which is a process that also works for other liquid handler types.3,4 In the examples discussed, an Agilent 384ST Disposable Tip Pipette Head employing arbitrary liquid classes was used to transfer Aqueous QualAssure solution (3 – 9 µL.) and DMSO QualAssure solution (100 – 300 nL). In both the Aqueous and DMSO methods, the calibration factors of 1 and 0 (slope and offset volume, respectively) were employed. The MVS "as found" (pre-optimized) volume values were used to determine new calibration factors and, within minutes, the volume transfer accuracy was dramatically improved for both the aqueous and low-volume DMSO solutions.

Introduction

This application note is based on the same process as described in Reference 4. It should be known that the Bravo equation editor can incorporate multinomial equations, however, the process discussed here works when the highest order of polynomial within the liquid class equation is "1", so that the relationship is linear. Therefore, a Bravo liquid class using linear calibration will follow Equation 1 and can be further defined by Equation 23.

$$y = mx + b \qquad \textbf{eq 1}$$

$$\text{Displaced Volume} = (\text{Desired Volume}) * (\text{Scaling Factor}) + (\text{Offset Volume}) \qquad \textbf{eq 2}$$

In Equation 2, the slope and offset coefficients are shown as m and b, respectively, in Equation 1. By plotting theoretical-displaced volumes (y) vs. MVS measured volumes (x), a linear regression allows a new slope and offset to be determined. A new slope and offset volume are then entered for retesting with the goal of improving accuracy. Improving pipetting accuracy via calibration factor adjustment is a simple and effective process: (1) transfer target volumes and measure performance with the MVS (three or more target volumes is recommended); (2) plot theoretical-displaced volume vs. MVS volume; (3) determine the new slope and offset volume; (4) enter those new values into the equation tab within the liquid class; and (5) re-test.

Materials

- Artel MVS, QualAssure solutions, Diluent and Verification Plates
- Excel spreadsheet and/or other calculation template for determining scaling and offset values
- Agilent Bravo Platform
- Associated tips and reservoirs
- WellMate dispenser

Procedure & Results

A WellMate dispenser (Thermo Scientific, Hudson, NH) was employed to pre-load each 384-well Verification Plate with 50 µL. Diluent before positioning them on the Bravo deck. The deck was also loaded with new tip boxes and a Thermo low volume reservoir containing MVS QualAssure solution (Figure 1). For the aqueous testing, 30-µL tips (Agilent PN 11484-202) were employed and the reservoir contained Aqueous QualAssure B. For the DMSO testing, 10-µL tips (Agilent PN 10734-202) were employed and the reservoir contained DMSO QualAssure E. Two custom liquid classes were used to wet-dispense Aqueous QualAssure solution (3, 6 and 9 µL) and DMSO (100, 200, and 300 nL) using a Bravo 384ST Disposable Head. Portions of the aspirate and dispense graphical user interfaces are shown in Figures 2 and 3. New tips and plates were employed for both the pre- and post-optimization testing. For the purposes of this example and in an effort to save tips and plates, only one replicate was transferred at each volume.

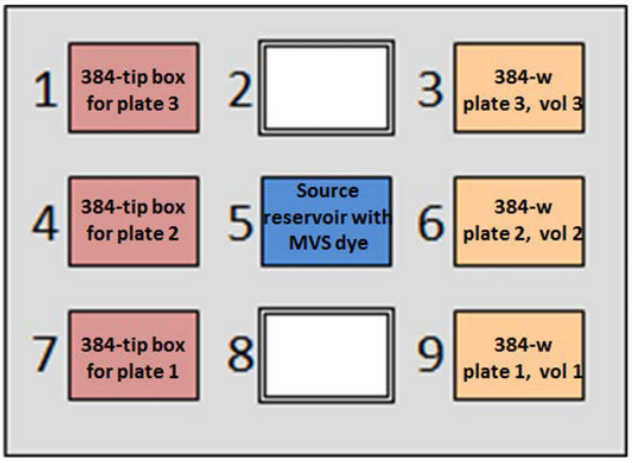


Figure 1. *Deck layout employed for both the pre-and post-optimization volume transfers.*

Volume	
Volume (0 - 72 µL):	9
Pre-aspirate volume (0 - 72 µL):	1
Post-aspirate volume (0 - 72 µL):	0
Properties	
Liquid class:	p30 384 disposable 2-10 uL water
Distance from well bottom (0 - 100 mm):	1
Dynamic tip extension (0 - 20 mm/µL):	0
Well selection:	1 selection: entire plate
Pipette technique:	
Tip Touch	
Perform tip touch:	☐

Figure 2. *The Aspirate Properties of the simple method employed for the three aqueous target volume transfers. For the DMSO testing, only the liquid class was different.*

Volume	
Empty tips:	☑
Volume (0 - 72 µL):	10
Blowout volume (0 - 72 µL):	0
Properties	
Liquid class:	p30 384 disposable 2-10 uL water
Distance from well bottom (0 - 100 mm):	3
Dynamic tip retraction (0 - 20 mm/µL):	.05
Well selection:	1 selection: entire plate
Pipette technique:	
Tip Touch	
Perform tip touch:	☐

Figure 3. *The Dispense Properties of the simple method employed for the three aqueous target volume transfers. For the DMSO testing, only the liquid class was different.*

In the Bravo software, the slope and offset coefficients can be determined by opening the Liquid Library Editor from the Tools menu, opening the specific liquid class (Figure 4) and then switching to the equation tab (Figure 5).

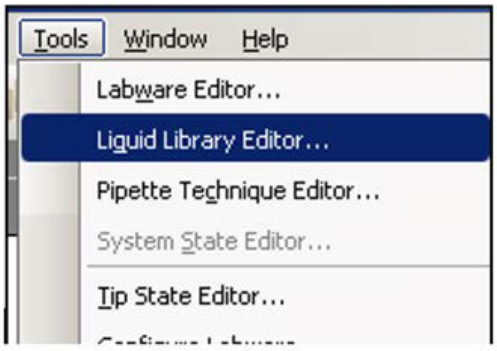


Figure 4. *top) Open the Liquid Library Editor to view the list of available liquid classes. (bottom) Images for the two liquid class IDs employed for this work.*

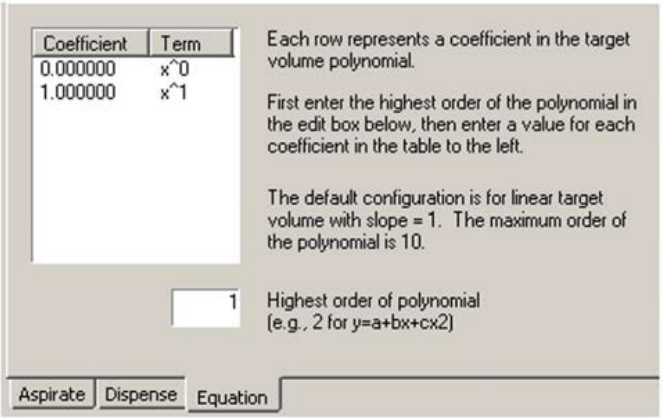


Figure 5. *The slope and offset coefficients were set to 1 and 0, respectively, for the pre-optimization testing for both aqueous and DMSO testing events.*

Once each of the target volumes was transferred and subsequently measured, the MVS test values as well as the slope and offset values were entered into an Excel workbook for further analysis. The target volumes, MVS measured mean volumes, theoretical-displaced volume (µL) [= (target volume µL) * (slope) + offset volume µL], and relative inaccuracy values were tabulated (Figure 6, top). Note that the theoretical-displaced volume is identical to the target volume when the scaling and offset are 1 and 0, respectively.

MVS Aqeuous Results: Pre-optimized

Target Volume (uL)	MVS Mean Volume for all Channels (uL)	Theoretical - Displaced Volume (uL; using Target, slope and offset)	Relative Inaccuracy %
3.00	2.87	3.00	-4.24%
6.00	5.86	6.00	-2.32%
9.00	8.92	9.00	-0.93%

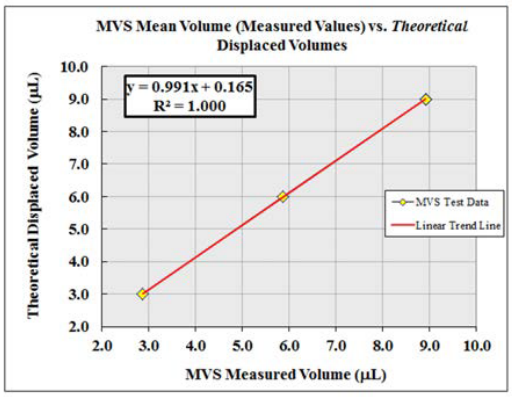


Figure 6. *(top) The aqueous pre-optimized mean volumes and inaccuracies were tabulated. (bottom) The middle two columns in the data table were plotted to determine the new slope and offset as 0.991 and 0.165, respectively.*

By plotting the MVS mean volume (x-axis) vs. theoretical-displaced volume (y-axis), a linear regression trendline can be used to show the slope and offset values of 0.991 and 0.165, respectively (Figure 6, bottom). These new factors were plugged into the equation tab in the employed aqueous liquid class prior to re-testing the same target volumes (Figure 7). Aside from the calibration factors, no other variables were adjusted before the second (final) round of testing was performed.

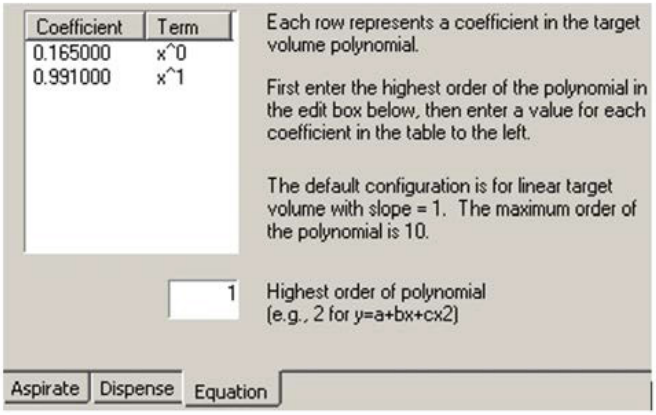


Figure 7. *The new slope and offset coefficients (0.991 and 0.165, respectively) were entered into the equation tab before completing the second (final) round of testing.*

Immediately upon retesting with the new calibration factors, it was obvious that the measurement results improved. The MVS overall relative inaccuracy and mean volume values for both the pre- and post-optimization testing are shown in Figure 8 and all aqueous testing statistics are shown in Table 1. This same optimization process was also performed with the 100% QualAssure DMSO E solution and all associated testing statistics for DMSO are shown in Table 2.

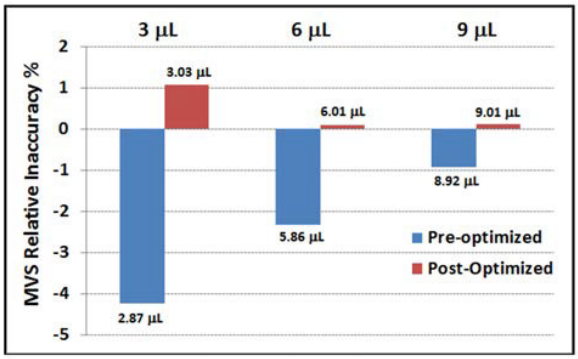


Figure 8. *Graphical representation of the mean and relative inaccuracy values for the pre- and post-optimization aqueous testing.*

Table 1. *AQUEOUS: MVS Results for the Pre- and Post- Optimization Testing*

	Pre-Optimization			Post-Optimization		
Target Volume (µL)	3	6	9	3	6	9
Slope and Offset Values (respectively)	1 and 0	1 and 0	1 and 0	0.991 and 0.165	0.991 and 0.165	0.991 and 0.165
Mean volume (µL)	2.873	5.861	8.917	3.032	6.006	9.011
Relative Inaccuracy %	***-4.24***	***-2.32***	***-0.93***	***1.07***	***0.10***	***0.12***
Standard Deviation (µL)	0.043	0.077	0.105	0.043	0.064	0.082
Coefficient of Variation %	***1.49***	***1.32***	***1.18***	***1.42***	***1.06***	***0.91***

Table 2. *DMSO: MVS Results for the Pre- and Post- Optimization Testing**

	Pre-Optimization *			Post-Optimization *		
Target Volume (µL)	0.1	0.2	0.3	0.1	0.2	0.3
Slope and Offset Values (respectively)	1 and 0	1 and 0	1 and 0	0.964 and -0.099	0.964 and -0.099	0.964 and -0.099
Mean volume (µL)	0.209	0.307	0.415	0.124	0.199	0.292
Relative Inaccuracy %	***109.48***	***53.26***	***38.84***	***24.08***	***-0.46***	***-2.22***
Standard Deviation (µL)	0.006	0.007	0.013	0.006	0.006	0.007
Coefficient of Variation %	***2.79***	***2.14***	***3.13***	***5.18***	***2.94***	***2.51***

* The test volumes employed here are outside of the specified volume range of an Agilent Bravo 384ST Disposable Tip Head, which is designed to operate in the range of 0.300 to 70 µL. Agilent also offers fixed-tip pipette heads and pin tools, which can transfer smaller volumes.

Conclusions & Considerations:

In a few quick tests, the methods employed on an Agilent Bravo Automated Liquid Handling Platform can be quickly improved by calibration adjustment using information garnered from the MVS test results.

There are a few things to consider before, and during, this type of optimization process:

• An iterative testing approach might be required to further improve accuracy.

• As noted above, this application note does not speak to the quality of volume transfer performance you should expect from any Bravo. This application note focuses on the optimization process only.

• The calibration factors are very likely to be labware dependent. For instance, changing disposable tips to a different type or a different vendor after an optimization is performed, may not yield the same volume transfer results.

• One should not adjust or optimize for accuracy without first knowing and ensuring the volume transfer precision is good/repeatable.

• This optimization process likely works on other liquid handling robots that employ calibration values based on the equation: $y = mx + b$ [3,4]

• Multiple liquid classes for different volume ranges may be required to reach accuracy goals. For instance, a volume range of 3 – 9 µL may have different calibration factors compared to other volume ranges, such as 10 – 50 µL or 10 – 100 µL.

• It is recommended, when possible and/or practical, to separate liquid classes for different critical volume transfers so that one optimization process does not affect another use/application (if multiple applications use the same liquid class).

• If a liquid class is only used to check one target volume, only adjust the offset volume by adding/subtracting the difference of actual vs. desired volume (µL) and re-test. Do not change the slope coefficient.

• Equation 2 is based on information from Reference 3:

• Displaced volume = volume displaced by the plunger inside the mandrel.

• Displaced volume ≠ desired volume when the scaling factor and offset are values other than 1 and 0, respectively.

• Displaced volume must be calculated with the new calibration numbers.

Acknowledgements

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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. https://journals.sagepub.com/doi/pdf/10.1016/j.jala.2004.08.012

(2) Albert, K. J. and Bradshaw, J. T. Importance of Integrating a Volume Verification Method for Liquid Handlers: Applications in Learning Performance Behavior, J. Assoc. Lab. Autom., 2007, 12, 172-180. https://journals.sagepub.com/doi/pdf/10.1016/j.jala.2006.10.005

(3) Beckman Coulter application note entitled "Improving Accuracy By Use of Technique Calibration" (T-1915A) (assessed in June 2013).

(4) Artel application note entitled "Optimizing Accuracy Performance on a Beckman Coulter Biomek Liquid Handler using the Artel MVS".

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