ADVANCED INSTRUMENTS

Application Note

Optimizing Accuracy Performance on a Beckman Coulter Biomek Using the Artel MVS®

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Abstract

This application note discusses one approach for optimizing pipetting accuracy on a Beckman Coulter Biomek liquid handler using the MVS Multichannel Verification System. Pipetting accuracy can be improved in any number of ways, such as by adjusting individual liquid class variables (aspirate rates, dispense rates, air gaps, mix steps, etc.) and measuring transferred volume resulting after each successive adjustment. In the optimization approach discussed here, however, the individual and unique liquid class variables within a technique are relatively unimportant to the optimization process. The focus of this application note is to show that pipetting accuracy can be improved by simply adjusting the linearly-based calibration factors within the technique1 by first knowing: (1) the scaling factor and offset volume employed within the technique for the volume transfer task and (2) the amount of volume transferred to the test vessel as measured with the MVS. In the example discussed, a pre-optimized arbitrary "universal" pipetting technique was employed to dispense 2, 5 and 8 µL (scaling and offset were 1 and 0, respectively). The "as found" performance was (linearly) inaccurate: -27.85%, -14.68% and -9.6% respectively for the three volumes. New scaling and offset values were calculated and the instrument was retested with the new values to show an improved relative inaccuracy at each volume: -0.05%, -1.14% and 1.05%, respectively.

Introduction

This application note is based on the same process as discussed in Reference 1, but instead uses MVS test results rather than a QC process requiring dye preparation, handheld pipetting of standard curves, comparing absorbance values, etc.1 The technique calibration of a Biomek is based on Equation 1 and in Beckman software terminology, this linear relationship is further defined as Equation 2.

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

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

The scaling factor and offset volume are the slope (m) and y-intercept (b) values, respectively, from 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 scaling factor and offset volume are calculated and employed for retesting with the goal of improving accuracy. Optimizing pipetting accuracy via calibration factor(s), 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 scaling factor and offset volume; (4) enter those new values into the technique's calibration tab; and (5) re-test.

Materials

- Artel MVS, QualAssure solutions and Verification Plates
- Excel spreadsheet and/or other calculation template for determining scaling and offset values
- Biomek NXp liquid handler and Biomek Software v3.3.14 (BiomekNX-MC)
- Associated tips and reservoirs

Procedure & Results

A Thermo Wellmate dispenser was employed to pre-load each of the three 96-well Verification Plates with 195 µL Diluent before positioning them on the Biomek deck. The deck was also loaded with 3 x p50 new tip boxes and a Thermo low volume reservoir containing Aqueous QualAssure C **(Figure 1)**. The 96-tip Biomek was employed to wet-dispense three target volumes (2, 5 and 8 µL) using a Default (Pipetting) Template within a "Universal Technique" **(Figure 2).** 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.

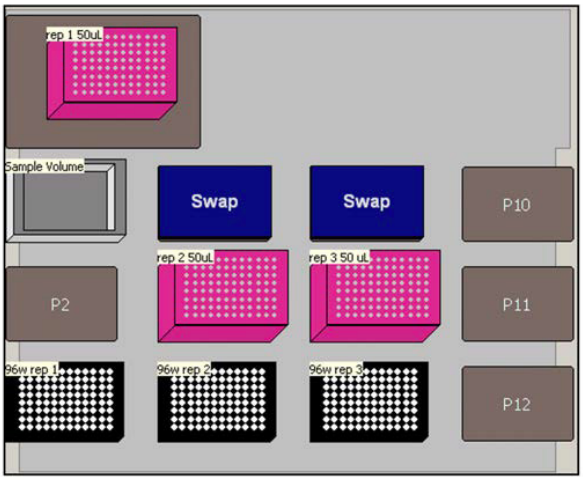


Figure 1. *Deck layout employed for both the pre-and post-optimization volume transfers.*

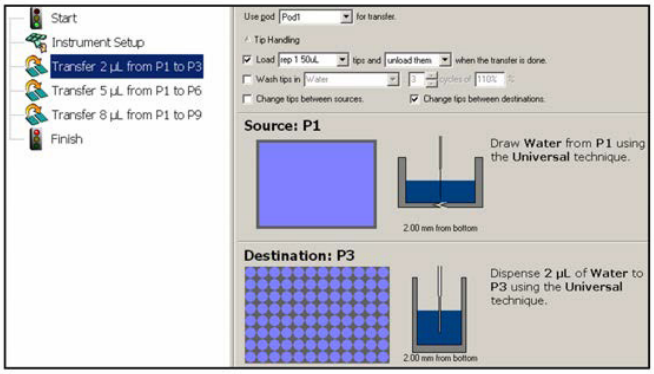


Figure 2. *The simple method employed for the three target volume transfers showing use of the Universal technique (this technique was used for all three transfers).*

Settings for scaling factor and offset volume employed by the technique can be determined by opening the Technique Browser from the Project menu, opening the specific technique **(Figure 3)** and then switching to the calibration tab **(Figure 4)**.

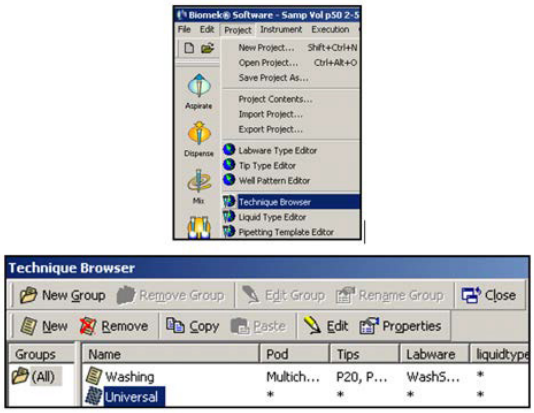


Figure 3. *Open the Technique Browser to view the list of available techniques (bottom image).*

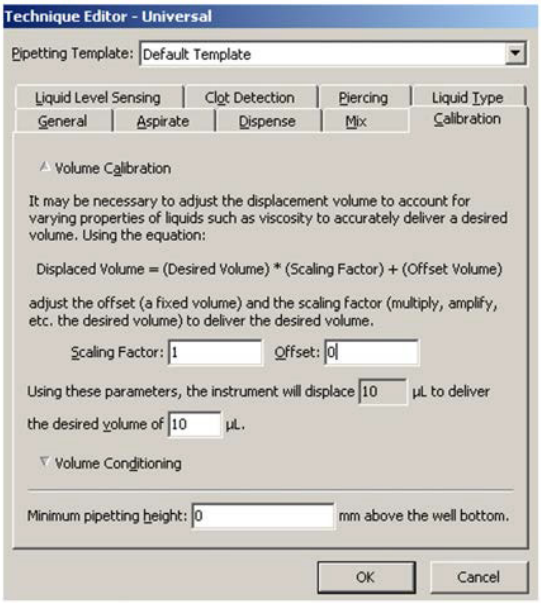


Figure 4. *Open the Technique Browser to view the list of available techniques (bottom image).*

Once each of the target volumes was transferred and subsequently measured, the MVS test values, scaling factor and offset volume were entered into an Excel workbook for further analysis. The target volumes, MVS measured mean volumes, theoretical-displaced volume [= (target) * (scaling factor) + offset], and relative inaccuracy values were tabulated (Figure 5, top).

Note that the theoretical-displaced volume is identical to the target volume when the scaling and offset are 1 and 0, 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 (scaling factor) and y-intercept (offset volume) of 1.032 and 0.548, respectively **(Figure 5, bottom).**

MVS Results: Pre-optimized			
Target Volume (uL)	**MVS Mean Volume for all Channels (uL)**	**Theoretical - Displaced Volume (uL; using Target, slope and offset)**	**Relative Inaccuracy %**
2.00	1.433	2.00	-28.35%
5.00	4.266	5.00	-14.68%
8.00	7.232	8.00	-9.60%

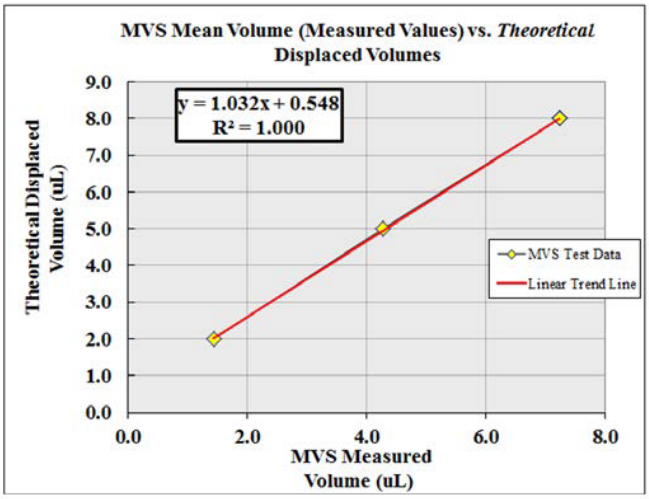


Figure 5. *(top) The pre-optimized mean volumes and inaccuracies were tabulated (bottom). The middle two columns in the data table were plotted to determine the new scaling factor and offset volume as 1.032 and 0.548, respectively. Following data collection, it was determined that there was data entry error for the target[2].*

These new factors were plugged into the technique editor's calibration tab prior to retesting the same target volumes, with the same deck layout and the same Universal technique **(Figure 6).** Aside from the calibration factors, no other variables were manipulated before the second (final) round of testing was performed.

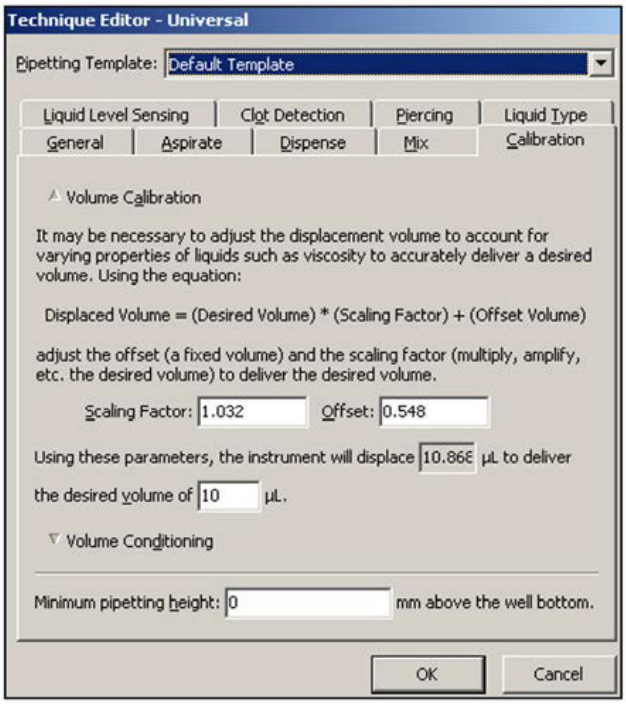


Figure 6. *The new scaling factor and offset volume (1.032 and 0.548, respectively) were entered into the calibration tab before completing the second (final) round of testing.*

Immediately upon retesting with the new factors, it was obvious that the measurement results dramatically improved. The overall relative inaccuracy values measured by MVS for both the pre- and post-optimization testing are shown in Figure 7 and all testing statistics are shown in **Table 1.**

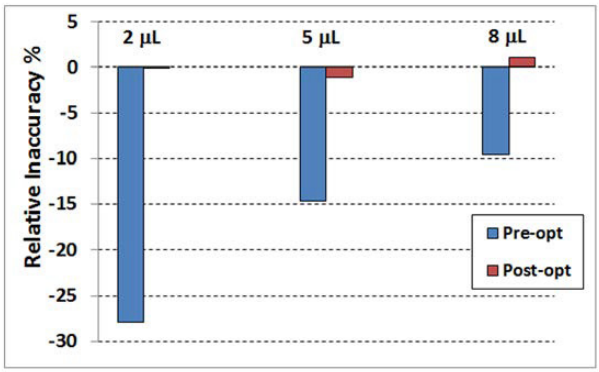


Figure 7. *Graphical representation of the relative inaccuracy values for the pre- and post-optimization tests.*

Table 1. *All MVS Measurement Results for the Pre and Post-Optimization Testing*

	Pre-Optimization			Post-Optimization		
Target Volume (µL)	2	5	8	2	5	8
Scaling and Offset Values (respectively)	1 and 0	1 and 0	1 and 0	1.032 and 0.548	1.032 and 0.548	1.032 and 0.548
Mean volume (µL)	1.443	4.266	7.232	1.999	4.943	8.084
Relative Inaccuracy %	-27.85%	-14.68%	-9.60%	-0.05%	-1.14%	1.05%
Standard Deviation (µL)	0.125	0.053	0.042	0.104	0.066	0.037
Coefficient of Variation %	8.66%	1.24%	0.58%	5.20%	1.34%	0.46%

For all three target volumes, the relative inaccuracy data are now all within 1.14%, which is a vast improvement from the pre-optimized results *(note the data entry error for the pre-optimized 2 µL testing in Figure 5, top vs. Figure 7).*

Conclusions & Considerations:

In a few quick tests, the techniques employed on a Biomek series liquid handler can be quickly optimized via technique calibration 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.

• This application note does not speak to the quality of volume transfer performance you should expect from any Biomek. This application note focuses on process f optimization 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 was 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: $y = mx + b$.

• Multiple volume ranges may be required to reach volume accuracy goals, especially if there is a large range of target volumes and the performance is non-linear over the range. For instance, a volume range of 2 – 8 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 techniques for different critical volume transfers so that one optimization process does not affect another use/application (if multiple applications use the same technique).

• If a technique is only used to check one target volume, only adjust the offset volume by adding/subtracting the difference of actual vs. desired and re-test as employed in the example in Reference 3. Do not change the scaling factor.

• Equation 2 above is found in the Technique Browser under the Calibration Tab and further information from Reference 1 is as follows:

• 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.

References

(1) Beckman Coulter application note entitled “Improving Accuracy By Use of Technique Calibration” (T-1915A).

(2) It should be noted that the 2-µL measured volume in Figure 5 should have been 1.443 µL (and not 1.433 µL). Additionally, the relative inaccuracy for the measured volume of 1.443 µL should be -27.85%, as shown correctly in Table 1. This data entry error went unnoticed but was employed to determine the scaling factor and offset volume with no detriment to the actual optimization process. Using the correct value of 1.443 µL in the Excel analysis (plot) would have changed the slope and offset to 1.033 and 0.538, respectively. The author felt that the procedure describing the optimization process is educationally more important to the theme of this application note as compared to the very small differences between calibration values determined using the wrong target volume at 2 µL.

(3) https://www.artel.co/learning_center/using-the-artel-pcs-to-calibrate-the-beckman-coulter-biomek-3000/

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