Low Volume Liquid Measurement Testing with Beckman Coulter's Biomek i-Series Automated Workstation using the Artel Multichannel Verification System (MVS®)

Dana Campbell
Artel

ABSTRACT

This application note provides performance testing results obtained at the low volume range capability of Beckman Coulter's Biomek i-Series Automated Workstation. The Artel MVS was used to measure the delivered volumes and to facilitate the optimization of pipetting techniques through Biomek Software. The results helped demonstrate a process for achieving optimized low volume pipetting performance with the Biomek i-Series.

INTRODUCTION

Automated liquid handling technology continues to evolve while increasing the productivity of laboratories that employ such dedicated systems. Beckman Coulter's newest liquid handling workstation was introduced during the 2017 SLAS conference held in Washington, D.C. and offers a variety of enhancements to both hardware and software when compared to its predecessors, Biomek NX^{P} and Biomek FX^{P}. A careful look at the publicly available pipetting performance specifications show both accuracy and precision claims at volume ranges applicable to the pipetting device and choice of tip type.[1] This information provides a conservative indication of pipetting performance expectations. However, it is quite common for a user to challenge the capability of performance at target volumes either at, or below, specified low volume claims. Therefore, the focus of this study was to use the MVS to measure very low volume aqueous transfers delivered from the Biomek i-Series. Various combinations of Biomek i-Series pipetting devices and disposable tip types were tested. The final reported data and the processes which were used to optimize the performance are provided.

The MVS is an easy-to-use, third party volume measurement system that enables rapid quantification of delivered liquid volume. The analysis performed is based upon Artel's dual-dye ratiometric photometry approach and provides measurements traceable to International System of Units (SI) through reference standards developed and maintained by the National Institute of Standards and Technology (NIST).[2] By applying the MVS as a verification tool, pipetting performance becomes clear and understood. Such valuable feedback can be applied to reduce troubleshooting efforts, resource requirements, and economic burden. It also ensures that all pipetting devices being monitored are verified under a standardized method of measurement helping ensure the quality needs of the lab environment are confidently controlled and documented.

Optimizing pipetting performance is a critical part of developing an automated liquid handling process. There are many factors to consider when choosing how to perform a pipetting operation.[3] Once the operation is optimized, the performance can be measured to see if a target volume is within an acceptable tolerance for both accuracy and precision. The Biomek pipetting technique applied to the aspirate and dispense operations contain the calibration parameters which can be adjusted, if necessary, to further fine tune accuracy.[4] Because only one target volume was evaluated per test, only the offset parameter within the calibration section of the Biomek technique was adjusted. When an accurate single target volume within an assay is desired, the offset parameter can simply be adjusted for that critical volume. If a single technique should be used for a range of volumes, then both slope and offset parameters are usually adjusted to account for the calibration curve.[5]

MATERIALS

- Artel MVS with version 3.2 Advanced software, MVS Sample Solutions, and Verification Plates
- Biomek i-Series Automated Workstation with Biomek Software version 5.0.97
- The following pipetting devices were tested:
  - Span-8 with 1 mL syringes
  - MC96 – 300 µL head
  - MC96 – 1200 µL head
  - MC384 – 60 µL head
- Biomek i-Series associated tips and reservoirs

PROCEDURE & RESULTS

The Span-8, with associated 1 mL syringes, allows a distinctive and versatile pipetting capability. Each independent channel can transfer a large volume range and access a variety of labware such as tubes and deep well plates. The lowest specified target volume with the 1 mL syringe is 10 µL using the T230 tips, which is only 1% of the nominal syringe capacity. During this testing, however, the Span-8 was evaluated at 1 µL and 5 µL, which is 0.1% and 0.5%, respectively, of the nominal syringe capacity.

A user often has a choice as to which tip type to use for a given pipette transfer operation. For the Span-8 testing, two Beckman Coulter tip types were evaluated at both 1 µL and 5 µL target volumes. A noteworthy enhancement to Biomek Software is having the capability to calibrate each of the 8 syringes directly within the hardware setup as shown in **Figure 1**. This independent control allows for improving the precision and accuracy across the eight Span-8 channels. Furthermore, each syringe can have up to 3 distinct calibration sets. This option helps to further improve the pipetting performance according to specified volume ranges. Changing the offset of a single Span-8 channel affects the accuracy. The approach used in this testing was to first adjust each Span-8 channel offset to bring it closer to a specific target volume. This helped increase the precision across all eight Span-8 channels. Next, the offset of the Biomek technique was adjusted to improve the overall accuracy and a final test of six transfers, or six data points per Span-8 channel, was collected. **Table 1** shows this final data and demonstrates the capability of the Span-8 to dependably pipette volumes less than 10 µL with the 1 mL syringes.

The MC96 – 300 µL head, MC96 – 1200 µL head, and the MC384 – 60 µL head were each tested at various volumes and tip types. The newer Biomek i-Series multichannel heads now have the capability to directly load and utilize various tip load configurations such as a single tip, a row or column of tips, and even partial or multiple rows or columns. This feature can simplify the initial testing process by using less tips and saving time and resources. For example, a single column of tips was frequently used as an initial test. Once final changes were made to the offset to improve accuracy, the full capacity of each head was evaluated with three full 96-w plates at each volume. **Table 2** shows this final data and demonstrates the capability of the heads to dependably pipette low volumes.

For many of the very small volume transfers with the multichannel heads, rather than only aspirating the requested target volume, a conditioning volume was applied within the technique. The conditioning volume instructs the robot to aspirate an overage, i.e., more than the target volume, which is then dispensed directly back into the source without the tips leaving the source liquid, so that the remaining target volume remains within the tip. This can be seen in the example Pipetting Template shown in **Figure 2**. Unfortunately, the highlighted text fields (**Figure 2, Label ① and ②**) are cut off in the Pipetting Template editor, but it can be copied and pasted into a text editor for ease of reading and editing. The full text for the original Aspirate volume is:

```
=C__Volume + (C__ConditioningSectionCount * C__ConditioningSectionVolume) + C__
ConditioningExcess
```

It was observed that when the low-volume pipetting technique and template were used, corrections for the calibration slope and offset needed to be applied to have the desired anticipated effect. Since the default volume calculation did not correct for calibration, the calibration values themselves needed to be adjusted to compensate. However, two simple changes were made to correct for the calibration so that slope and offset values were used without complication. The calibration correcting Aspirate volume formula (**Figure 3, Label ①**) is:

```
=C__Volume + ((C__ConditioningSectionCount * C__ConditioningSectionVolume) + C__
ConditioningExcess) / C__CalibrationSlope
```

The Dispense volume was also corrected. The calibration correcting Dispense volume formula (**Figure 4, Label ②**) is:

```
=(C__ConditioningSectionVolume - C__CalibrationOffset) / C__CalibrationSlope
```

These two corrections were applied for the testing reported in this paper.

It was also observed that better performance at very low volumes with the MC384 – 60 µL head was achieved with significantly slower aspiration speeds compared with the MC96 heads (1/20th speed). This makes sense considering the much smaller bore diameters; when considered as a percentage of the smaller channel volume, the speeds are similar.

CONCLUSIONS & CONSIDERATIONS

Many variables can affect pipetting performance, such as environmental factors, physical properties of liquids, tips, and labware. Biomek Software has many built-in parameters that a user can edit. This flexibility helps tailor a pipetting step to the circumstance so that optimal performance can be achieved, as long as the user has a method to measure the transferred volumes. However, care should be taken when attempting to transfer volumes lower than those outlined in a specification claim. Pipetting performance for specific test volumes and tip types was optimized relatively quickly and with minimal test runs by changing only the offset.

All tests were wet-well transfers, meaning that the low target volumes were dispensed into wells that had liquid. This wet-transfer approach that can also be combined with a mix step after dispensing to help ensure the entire content of the tip is expelled into each well. Also, new tips were used for every transfer and the results reported herein do not reflect tips being used more than once.

This study demonstrates a set of data achieved with a Biomek i-Series Automated Workstation using minimal calibration adjustments. It should also be noted that only one pipetting device and corresponding robot were tested, so that a general sense of performance at low volumes in a realistic lab environment could be evaluated.

Table 1. Span-8 Low Volume Data

Span-8 Syringe Volume	Tip Type	Transfer Volume (µL)	Relative Inaccuracy ± %	Imprecision CV (%)
1 mL	T80	1	3.93	3.81
1 mL	T80	5	0.02	1.53
1 mL	T90	1	2.98	4.74
1 mL	T90	5	0.9	1.54

Table 2. Multichannel Heads Low Volume Data

MC Head Type	Tip Type	Transfer Volume (µL)	Relative Inaccuracy ± %	Imprecision CV (%)
MC96-300 µL	T80	0.5	1.46	5.52
MC96-300 µL	T90	0.5	3.83	8.19
MC96-1200 µL	T80	1	0.19	5.03
MC96-1200 µL	T80	2	3.7	2.82
MC96-1200 µL	T90	1	0.21	6.25
MC96-1200 µL	T90	2	2.16	1.85
MC96-1200 µL	T1070	10	1.71	3.96
MC384-60 µL	T30_384	0.25	1.12	8.38
MC384-60 µL	T50_384	0.25	7.46	4.79

Figure 1. Span-8 Per Probe Volume Calibration > Hardware Setup Setting

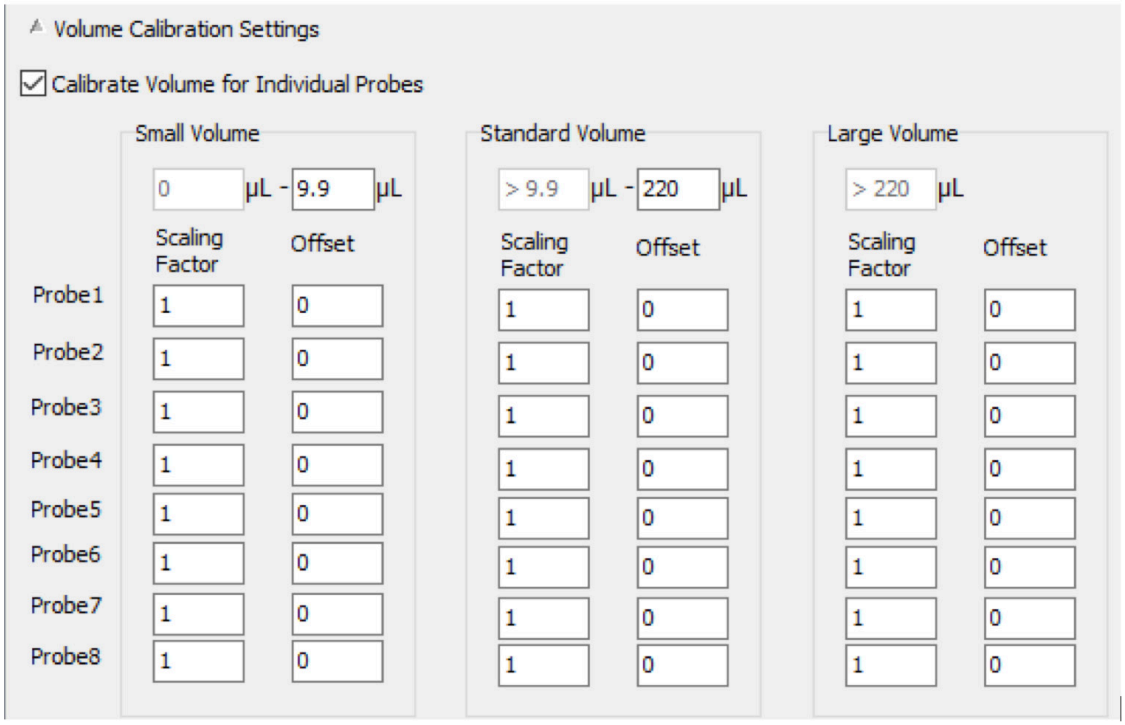


Figure 2. Pipetting Template with Conditioning (Original)

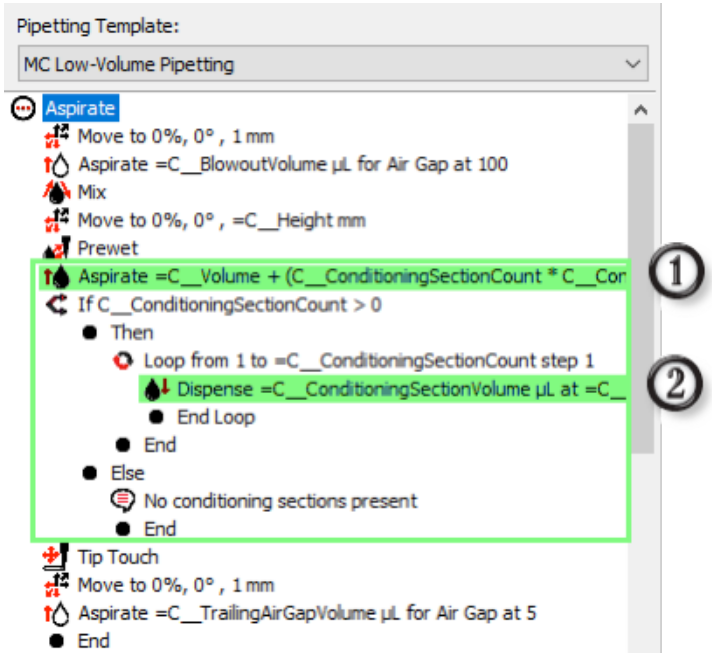


Figure 3. Pipetting Template with Calibration-Corrected Conditioning Volumes (Aspirate)

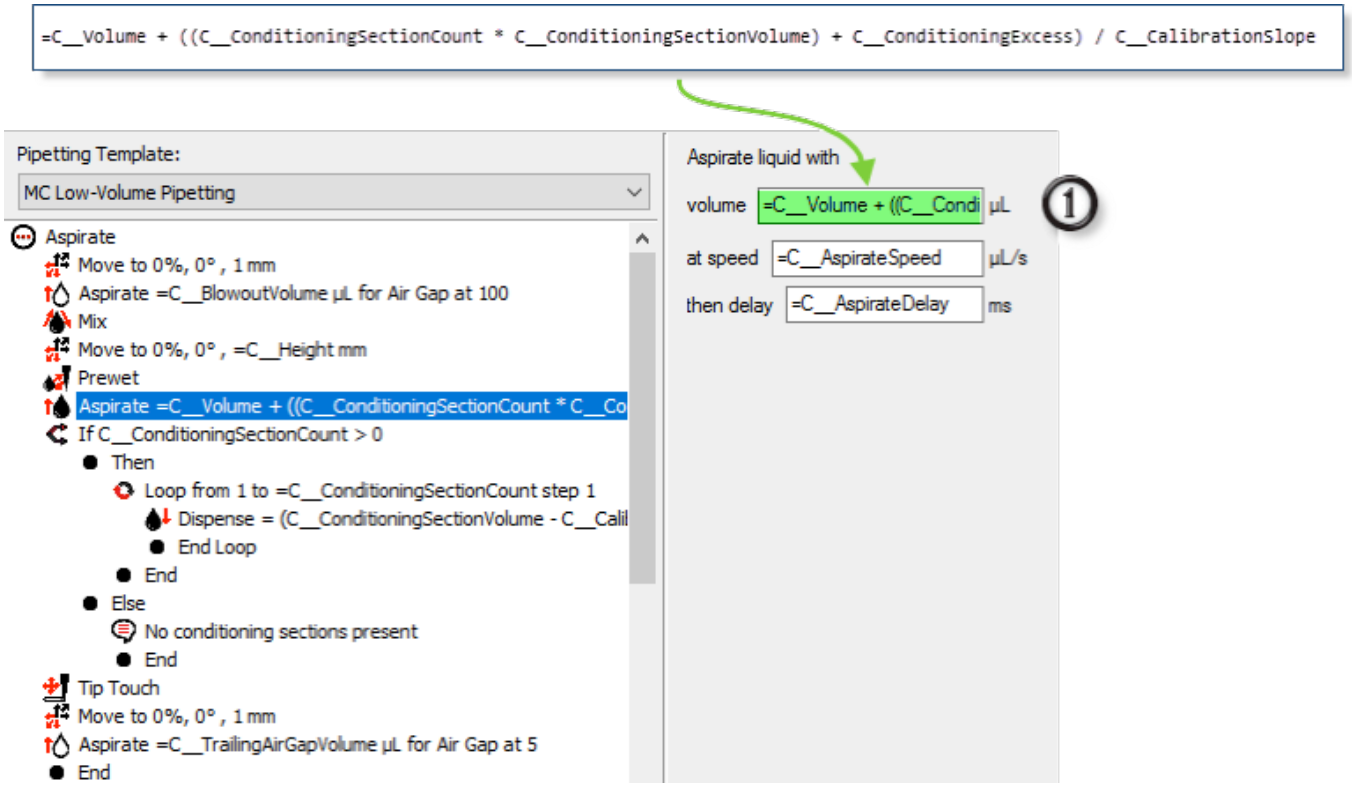
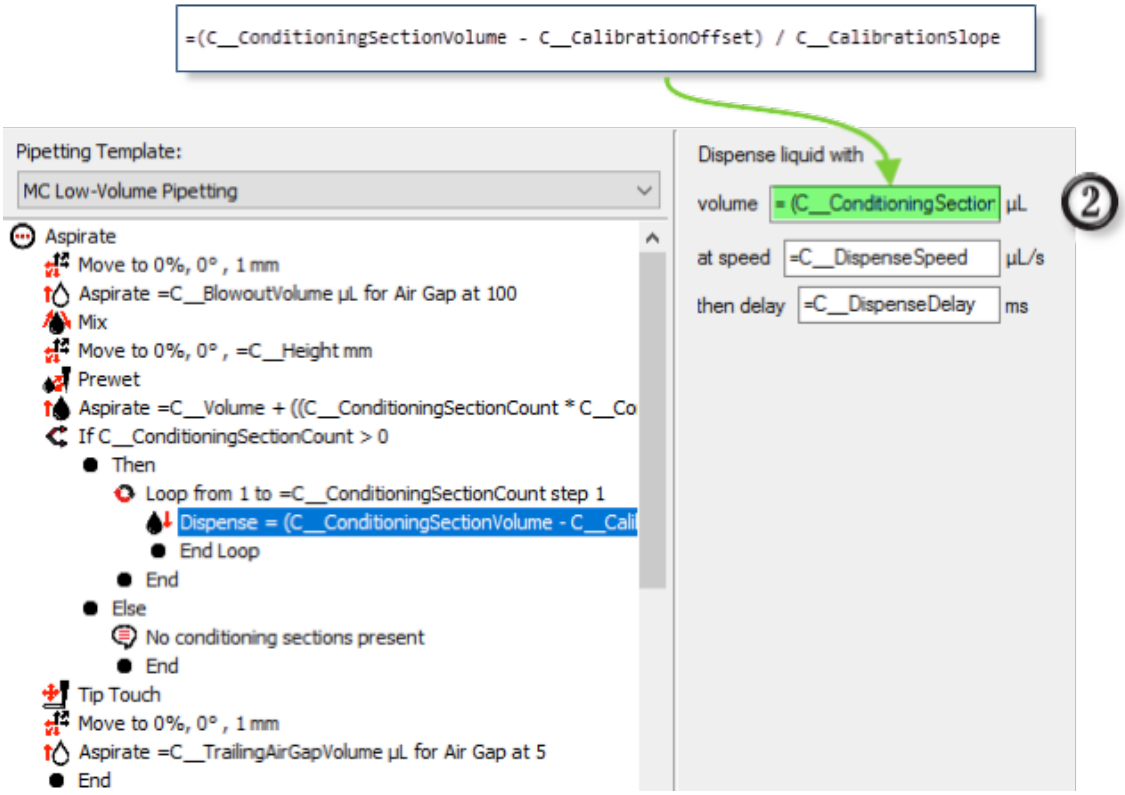


Figure 4. Pipetting Template with Calibration-Corrected Conditioning Volumes (Dispense)



REFERENCES

1. Beckman Coulter Life Sciences flyer entitled: “Biomek i-Series Pipetting Performance Capabilities.” Document # AAG-2254FLY01.17, 2017.
2. 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.
3. Artel application note entitled, “Optimizing Accuracy Performance on a Beckman Coulter Biomek Using the Artel MVS” Doc #12A6478, 2013.
4. http://www.artel-usa.com/resource-library/minimizing-liquid-delivery-risk-automated-liquid-handlers-as-sources-of-error/
5. http://www.artel-usa.com/resource-library/optimizing-accuracy-performance-on-a-beckman-coulter-biomek/

Thank you to both John Snider and Tina Kaiser from Beckman Coulter for contributing their time and technical review of this paper.