Measuring the performance of microplate dispensers as part of a method scale-up process

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Abstract

Liquid handling is often an overlooked and underappreciated factor in the success of assay transfer from the bench to automation. Often as part of the scale-up process, intermediate automation is implemented to aid in the transfer process where systems such as BioTek's MultiFlo™ Microplate Dispenser are implemented. However, because of the viscous or sticky properties of the liquids commonly transferred by these types of instruments, it is imperative to have the capability of monitoring performance to identify channel build-up and clogs, or even to understand discrepancies in the volume dispensing performance on a channel-by-channel basis.

This presentation discusses how the measurement technology from Artel can be used to help laboratories specifically identify and troubleshoot mis-performing channels in microplate dispensers allowing users to save time and avoid costly repetitive testing. As is with any type of liquid delivery device, proper care and maintenance are often required to promote long-term, error-free volume dispensing. The Artel method of measurement goes a step further to generate more inclusive and informative performance measurement answers in a channel-by-channel manner. The use of gold standard measurement technology allows for easy visualization of performance information and allows for optimization, where applicable, of liquid handling instrumentation when assays are transferred to automated platforms.

Materials & Methods

During this study, the accuracy and precision of dispensed volumes from the MultiFlo were compared using three different methodologies:

(1) the Artel MVS®;
(2) gravimetry; and
(3) the BioTek recommended volume measurement procedure at volumes for which the manufacturer specifies performance.

From BioTek Instruments:

MultiFlo Dispenser
Dual Syringe Module - Autoclavable
8-channel manifold
16-channel manifold
BioTek Liquid Test Solutions

From Artel:

Artel MVS
96-Well Verification Plates
Sample Solutions and Diluent
MVS Calibrator Plate

Procedure

The MultiFlo was configured for syringe pump dispensing, and the initial performance at each specified volume was assessed with the three measurement methods mentioned previously. Two different syringe pumps and two manifolds were tested, i.e., the MultiFlo has two distinct syringe-pump dispensers, each with an 8-, 16- or 32-tube manifold (only the 8- and 16-channel manifold were tested).

It should be noted that the initial evaluation did not include any method optimization. Initially, each microplate was pre-filled with the Artel Diluent blue dye and placed directly onto a Sartorius R160D balance and tared. Each plate was then placed onto the deck of the MultiFlo where the specific target volume of Artel Sample Solution was dispensed. Once the target volume was dispensed, each filled plate was placed back on the balance where the overall weight of the target transfer was recorded. After recording the weight, each plate's channel-by-channel dispensed volume of sample solution was measured in every well with the MVS. Dispensing parameters and instrument components were subsequently adjusted based on the volume measurement results obtained.

The performance of the MultiFlo was also measured using BioTek's recommended Syringe Dispense Precision and Accuracy Test, as described in the instrument User Manual, and as performed by a qualified field technician. In the 96-w plate, 40 and 160 µL were measured and in the 384-w plate, 20 µL was measured. The MultiFlo Dispenser was equipped with either the 8- or 16-channel manifold. In the testing described below, both manifolds were employed to test the same target volumes using both syringe pumps in distinct testing events. In all cases, the instrument was employed to dispense the same specific target volume to each well of the microplate.

The following parameters were tested:

- 8-channel manifold (repeated using Syringe A *and* Syringe B pumps at each volume):
  - 96-well plates
  - 12 reps per channel
  - 20, 40, 80, and 160 µL
- 16-channel manifold (repeated using Syringe A *and* Syringe B pumps at each volume):
  - 96-well plates
  - 2-channels per well; 12 full reps across the plate
  - 20, 40, 80, and 160 µL
- 16-channel manifold (repeated using Syringe A *and* Syringe B pumps):
  - 384-well plates
  - 24 reps per channel
  - 20 µL

BioTek MultiFlo Instrument Performance Specifications	
	Dispense Precision
8-Tube:	< 2% CV when dispensing 100 µL/well
	< 5% CV precision at 20 µL/well
	< 5% CV precision at 40 µL/well **
	** Tested in-house to < 1.6% CV
16-Tube:	< 2% CV when dispensing 100 µL/well
	< 2.5% CV precision at 80 µL/well***
	< 5% CV precision at 20 µL/well **
	< 10% CV precision at 5 µL/well*
	*unspecified for non-autoclaveable syringe pumps
	Dispense Accuracy
8-Tube:	For all volumes 2 µL or 1%, whichever is greater, at flow rate 2
16-Tube:	For all volumes 2 µL or 1%, whichever is greater, at flow rate 2

Table 1. Manufacturer's Performance Specifications

Results

In all cases, the dispensed volumes as measured with three different methodologies were within the manufacturer's specifications for precision and accuracy, as referenced in **Table 1**. Channel dispensing patterns from one specific MVS pre-optimized test run were used to identify clogs or under-performing channels, as observed for channel 5 (row E, **Figure 1**). The overall averaged performance measurements for each test case are shown in **Figures 2 – 4**, where there is very good agreement between all measurement methodologies.

	1	2	3	4	5	6	7	8	9	10	11	12
A	19.88	19.98	19.94	19.94	19.95	19.83	19.89	19.93	19.90	19.96	19.88	20.01
B	20.61	20.65	20.64	20.61	20.66	20.63	20.65	20.65	20.67	20.62	20.65	20.61
C	20.79	20.87	20.82	20.82	20.81	20.84	20.80	20.84	20.84	20.87	20.80	20.93
D	20.60	20.60	20.63	20.54	20.64	20.64	20.64	20.56	20.60	20.59	20.58	20.54
E	21.02	21.07	21.03	21.03	21.07	21.08	21.07	21.03	21.06	21.07	21.04	21.10
F	20.63	20.66	20.69	20.68	20.66	20.71	20.72	20.65	20.67	20.66	20.63	20.59
G	21.79	20.64	20.65	20.60	20.63	20.62	20.62	20.65	20.65	20.66	20.62	20.61
H	20.65	20.68	20.70	20.64	20.68	20.66	20.67	20.66	20.71	20.66	20.69	20.68

Figure 1. MVS results illustrating one channel which required method adjustments. Orange coloring indicates performance outside of tolerance.

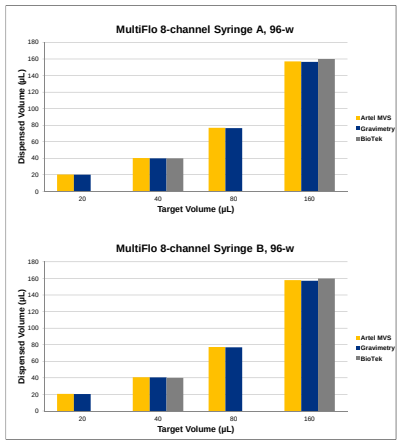

Figure 2. Overall averaged measured volume for: (top) 8-channel manifold with Syringe A into 96-w; (bottom) 8-channel manifold with Syringe B into 96-w.

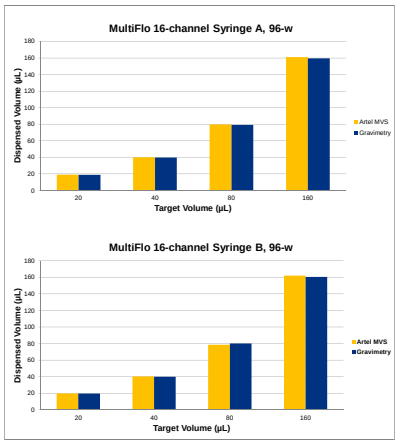

Figure 3. Overall averaged measured volume for: (top) 16-channel manifold with Syringe A into 96-w; (bottom) 16-channel manifold with Syringe B into 96-w.

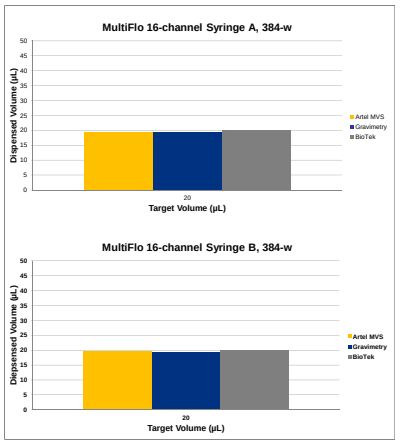

Figure 4. Overall averaged measured volume for: (top) 16-channel manifold with Syringe A into 384-w; (bottom) 16-channel manifold with Syringe B into 384-w.

Conclusion

This study assessed the performance of one BioTek MultiFlo Microplate Dispenser, which met manufacturer's specifications for precision and accuracy, after liquid handling method optimization steps were performed. Three methods for evaluating performance were tested side-by-side showing very comparable results.

Using a tool, such as the MVS, for channel-by-channel performance assessment, allows for easy identification of issues and specific problems. Identifying mis-performing channels in microplate dispensers improves assay consistency and avoids costly retesting due to liquid handler error.

When evaluating which measurement methodology would be a better fit, the user would additionally want to evaluate:

- the amount of information gained by the test (i.e., well-by-well and channel-by-channel accuracy information can be garnered via MVS but not via gravimetry);
- skill/time required to complete the measurements;
- the traceability or standardization of results.

Evaluating and optimizing the precision and accuracy performance of these often overlooked instruments is critical to effectively reducing the overall variability of assay results. The channel-by-channel information, as well as the overall dispensing performance metrics at assay-specific volumes, allows a dispenser's behavior to be understood, which is critical during method transfer and scale up. Additionally, knowing dispenser performance will allow the laboratory to make well-informed decisions on use, application and *potential risk* of implementing the dispensers in certain work-flow situations.

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