FORMULATRIX®

Application Note

An Assessment of the Volumetric Performance of the MANTIS® Liquid Handler Using the Artel MVS® Multichannel Verification System

Introduction:

Since the inception of the variable volume pipette in the 1960s, researchers have been sacrificing the precision of their liquid transfers for ease of use, efficiency, and speed. However, a new trend towards decreasing reaction volumes in genomics laboratories has called into question the capabilities of various liquid handling solutions, especially since lower volumes tend to increase the impact of liquid handling imprecision on experimental results. With precision medicine and drug discovery relying so heavily on the findings published by translational genomics laboratories in academia and biotechnology, the reproducibility of data has never been so salient and highly scrutinized.

Today, researchers generally regard automated workstations as the most reproducible liquid handling solutions, as automated liquid handling (ALH) systems remove many of the obstacles associated with user-variability. Researchers assess the volumetric performance of these devices mainly by measuring the imprecision of liquid transfers, expressed quantitatively through coefficients of variation (CVs). ALH platforms often dispense extremely precisely, and published CVs for target liquid deliveries in the range of tens to hundreds of microliters (µL) tend to be less than 1%. However, for most ALH systems these specifications deteriorate as target volumes approach 1 µL. In fact, the most widely utilized liquid handling platforms publish specifications including CVs as high as 6% at 0.5 µL (Table 1).

Volume	Traditional Liquid Handler #1	Traditional Liquid Handler #2	ISO 8655 Maximum Permissible Random Error[1]
10 µL	1.0%	0.8%	1.5%
5 µL	1.5%	No Spec	2%
1 µL	4.0%	No Spec	5%
0.5 µL	6.0%	5.0%	No Spec
0.1 µL	No Spec	No Spec	No Spec

Table 1: Published CVs of two common ALH systems (10 µL disponsable tips) compared to ISO 8655 maximum permissible errors for manual pipettes.

FORMULATRIX® and Artel present this application note in order to showcase the precision of the MANTIS® Liquid Handler, a novel solution designed to supplement traditional liquid handling platforms. By implementing the MANTIS, researchers can increase experimental reproducibility in genomics workflows that involve dispensing small volumes of valuable reagents with a high degree of precision.

At the core of the MANTIS is a patented microfluidic valve cluster that measures and dispenses discrete volumes of liquid (Figure 1). Pressure and vacuum open and close each valve on the silicone valve cluster. The positive displacement chips have two micro diaphragms that can fill and dispense as rapidly as 10 times per second. The low volume (LV) chips contain 0.1 and 0.5 µL diaphragms, while the high volume (HV) chips contain 1 and 5 µL diaphragms.

The Continuous Flow (CF) chip utilizes a distinct valve based technology that allows for dispensing of volumes from 5 µL to 2000 µL in a continuous stream.

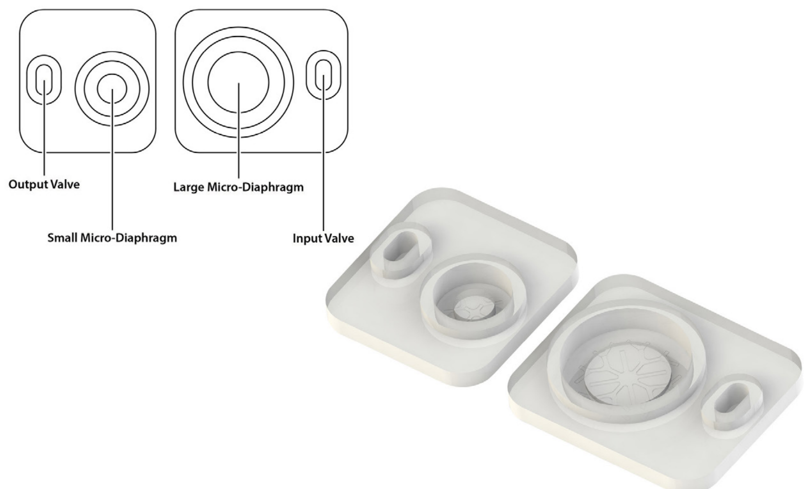

Figure 1: FORMULATRIX® patented microfluidic dis-pensing technology

Materials:

Artel ELx800 NB Plate Reader (Figure 2)

Artel MVS Calibrator Plate (Figure 2)

Artel Sample Solutions (Figure 2)

21x Artel 384-well Verification Plates

12x Artel 96-well Verification Plates

Figure 2: Artel Multichannel Verification System (MVS)

Q-Instruments Bioshake 3000 (Figure 2)

Grant-bio LMC-3000 Laboratory Centrifuge

HONEYWELL Xenon 1902 Barcode Reader (Figure 2)

FORMULATRIX MANTIS Liquid Handler (Figure 3)

Figure 3: FORMULATRIX® MANTIS® Liquid Handler

Methods:

In this experiment, the MANTIS Liquid Handler delivered target volumes of Artel Sample Solutions into each well of meticulously characterized Artel 96- or 384-well Verification Plates. Following centrifugation and mixing, the Artel MVS read the concentration of the sample solution and provided, through the graphical user interface of the Artel MVS Software, a CV value for each plate. One discrete target volume was selected for each 96- or 384-well microplate in order to provide a compelling set of liquid deliveries.

Eleven target volumes were chosen over the three microfluidic MANTIS chip types in order to provide a comprehensive review of the liquid handling imprecision associated with the MANTIS. In accordance with best practices, each run for each target volume was tested in triplicate to ensure statistical significance.

All volumetric tests performed with the HV and LV chip types utilized Artel 384-well Verification Plates. All tests perfomed with the CF chip type utilized Artel 96-well Verification Plates. Lower density plates were utilized for all CF testing to accommodate the 200 µL dispense volume and ensure consistency across all testing for this chip type.

Results:

Random error of each run was assessed by calculating the standard deviation of the series of 96- or 384- measured volumes under repeatability conditions, as shown in Formula (1).

$$S_{r(l,m)} = \sqrt{\frac{\sum_{n=1}^{N}(V_{(l,m,n)} - \overline{V}_{(l,m)})^2}{N-1}} \quad (1)$$

where

Sr(l,m) - is the random error of channel 'l' during run 'm'

V(l,m,n) - is a single measured volume

V(l,m) - is the average of all measured volumes from channel 'l' during run 'm'

N - is the number of replicate deliveries in the run

This standard deviation was used to calculate the coefficient of variation expressed as a percentage as shown in Formula (2).

$$C_{v(l,m)} = \frac{S_{r(l,m)}}{\overline{V}_{(l,m)}} \; x \; 100\% \quad (2)$$

where

Cv(l,m) - is the coefficient of variation of channel 'l' during run 'm'

CVs were averaged over three separate runs for each target volume using a root-mean-squares approach as shown in Formula (3) below. This formula was selected since the number of replicate deliveries was consistent across each run.

$$C_{v(l)} = \sqrt{\frac{\sum_{m=1}^{M} {C_{v(l,m)}}^2}{M}} \quad (3)$$

where

Cv(l) - is the coefficient of variation of channel 'l' combining data from multiple runs

M - is the number of runs included in the average[2]

Over the entire volume range, all CVs, and Root Mean Squared (RMS) of CVs, were less than or equal to 1.5% (Table 2, Figure 4).

LV Chip*	$CV_{(1,1)}$	$CV_{(1,2)}$	$CV_{(1,3)}$	$CV_{(1)}$
0.1 µL	1.3%	1.5%	1.4%	1.4%
0.5 µL	0.5%	0.5%	0.6%	0.5%
1 µL	0.4%	0.4%	0.4%	0.4%

HV Chip*	$CV_{(1,1)}$	$CV_{(1,2)}$	$CV_{(1,3)}$	$CV_{(1)}$
1 µL	0.8%	0.8%	0.8%	0.8%
5 µL	1.1%	1.1%	1.1%	1.1%
10 µL	0.5%	0.6%	0.6%	0.5%
25 µL	0.4%	0.4%	0.4%	0.4%

CF Chip*	$CV_{(1,1)}$	$CV_{(1,2)}$	$CV_{(1,3)}$	$CV_{(1)}$
25 µL	0.4%	0.3%	0.3%	0.3%
50 µL	0.3%	0.5%	0.3%	0.4%
100 µL	0.2%	0.3%	0.3%	0.3%
200µL	0.2%	0.2%	0.2%	0.2%

Table 2: Measured Performance of the FORMULATRIX® MANTIS® Liquid Handler.

Conclusion:

The reproducibility of experiments between genomics labs can be largely impacted by the imprecision of liquid transfers, especially at low nanoliter-scale volumes.

Evidenced by the data published in this application note, the MANTIS boasts CVs well under 2%, even at volumes as low as 0.1 µL, providing researchers with a solution that facilitates the generation of trustworthy results in highly sensitive methods such Next Generation Sequencing (NGS) and qPCR.

As more and more researchers move to sub-10 µL reaction volumes in their genomics workflows, it will become more critical than ever before to ensure highly precise liquid handling within these volume ranges. The MANTIS can be easily added to any traditional liquid handling workstation in order to obtain CV's under 2% across the entire pipetting range.

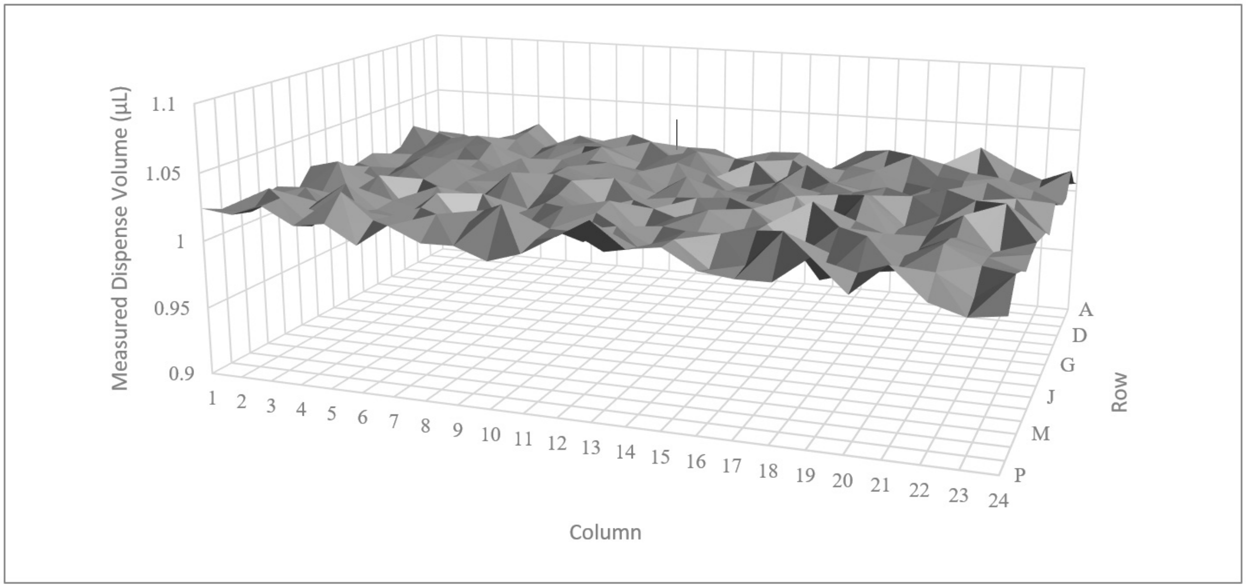

Figure 4: Measured dispense volumes for a single run with target volume of 1 µL (HV Chip CV(1,3))

Acknowledgements:

This work was carried out by Artel in collaboration with Formulatrix.

Artel is the global technology leader in low volume liquid delivery measurement and quality assurance. The company develops and manufactures highly accurate, precise, and easy-to-use systems available for ensuring liquid delivery data integrity and NIST-traceable results in life sciences laboratories. Artel innovations help laboratories improve processes and increase productivity, enhance product quality, and address compliance challenges.

References:

[1] ISO 8655-1:2002(en), Piston-operated volumetric apparatus - Part 1: Terminology, general requirements and user recommendations

[2] ISO IWA 15:2015, Specification and method for the determination of performance of automated liquid handling systems.