ADVANCED INSTRUMENTS

White Paper

A Dual-Dye Ratiometric Photometry System for Verification of Multichannel Liquid Delivery Devices

Technical Description, Operation, Selected Applications, and Method Comparison

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ABSTRACT

Verification and calibration of automated liquid handling instruments are important for applications that demand high levels of data integrity, such as drug discovery and development, proteomics, genomics, and molecular diagnostics. The lack of standardization in verification and calibration methods, however, makes it difficult to evaluate the volumetric performance of instruments over time and to compare results between laboratories.

The Artel portfolio is designed around a *dual-dye ratiometric photometry* system for verification of multichannel liquid delivery devices. Available as the Artel MVS® Multichannel Verification System,[1,2] it determines both the accuracy and precision of each individual channel of a multichannel liquid delivery device and delivers traceable volume measurements in the range from 10 nL to 350 µL.[3]

This report presents the technical and scientific application of dual-dye ratiometric photometry in microtiter plates, upon which the MVS is based. This report also documents the MVS components and describes its operating procedure. Examples of selected applications and comparisons to other calibration methods are also provided.

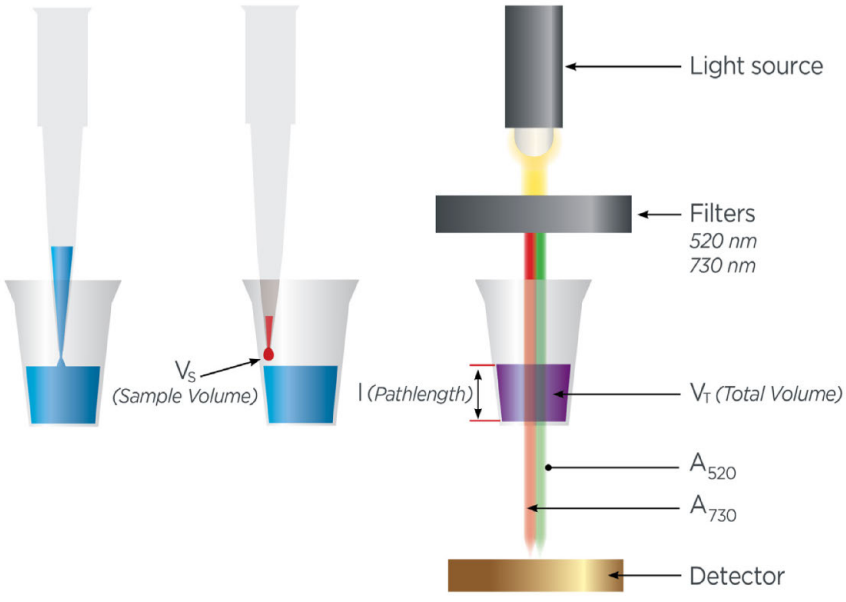


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LABORATORY AUTOMATION AND VOLUMETRIC PERFORMANCE

The emergence of laboratory automation has revolutionized the biotechnology and pharmaceutical industries. Automated liquid handling systems are highly effective at increasing throughput and decreasing labor expenditure in a number of applications such as drug discovery and development, proteomics, genomics, and molecular diagnostics. Because these applications demand a high level of data integrity, they rely on the intrinsic accuracy and precision of automated systems. In order to maintain confidence in the data generated from automated liquid handling systems, it is important that these systems be calibrated and their volumetric performance be verified frequently.

There are a number of parameters that must be considered for calibrating and verifying the performance of automated liquid handling systems in the laboratory environment.[4] For multichannel devices, validation must be performed for each individual channel to assure accuracy and precision across the entire system. The validation method must be able to measure the small volumes that are typically dispensed by automated liquid handling systems. It is important that measurement results produced by the validation method be traceable to the International System of Units (SI) in order to achieve consistent volumetric performance for all instruments and to allow inter-laboratory comparability. Further, it is important that the validation method be robust, rapid, and easy to use in the laboratory environment by in-house personnel so that the volumetric performance of automated liquid handling systems can be verified frequently without causing unnecessary delay in use of the instruments for their intended applications. Finally, the acquisition and storage of validation data needs to meet certain security standards to comply with various regulatory requirements.

This report describes dual-dye ratiometric photometry in microtiter plates and its application in the Artel MVS® Multichannel Verification System to calibrate multichannel liquid delivery equipment. The MVS measures volumes as low as 10 nL and generates measurement results that are traceable to the International System of Units (SI) through the national standards of NIST (National Institute of Standards and Technology, Bethesda, Maryland, USA).[3] The MVS software also allows laboratories to comply with 21 CFR Part 11, which includes security features, an audit trail, electronic records, and electronic signatures.[5]

DUAL-DYE RATIOMETRIC PHOTOMETRY

The MVS is an extension of the proprietary dual-dye ratiometric photometry method that has been used in the Artel PCS® Pipette Calibration System for a number of years. Dual-dye photometry is a recognized method for micropipette calibrations as described in ISO 8655-7, and for automated liquid handlers as described in the ISO IWA 15 agreement.[6] The dual-dye ratiometric photometry method employs two colorimetric dyes with distinct absorbance maxima at 520 nm (red dye) and 730 nm (blue dye). By measuring the absorbance of these dyes under defined conditions and applying the Beer-Lambert law, the MVS determines both the accuracy and precision of each channel in a multichannel liquid delivery device.

BEER-LAMBERT LAW

The concentration of a chromophore, or dye, in solution can be determined photometrically using the Beer-Lambert law. Simply stated, the law claims that when light is passed through a solution containing some concentration of chromophore, there is a linear relationship between the concentration of the chromophore and the amount of energy it absorbs. In mathematical terms, the law is written as:

$$A_\lambda = \varepsilon_\lambda l C$$

where A_λ is the absorbance of the chromophore at a specific wavelength λ, ε_λ is the molar extinction coefficient of the chromophore at wavelength λ, *l* is the path length of light passed through the solution, and C is the molar concentration of the chromophore in solution. This proportionality is most commonly used to determine an unknown concentration of a chromophore in solution, where the molar extinction coefficient at the measurement wavelength is known and the path length of light through the solution is known or fixed.[7,8]

Alternatively, the Beer-Lambert law can be used to determine an unknown path length traversed by a photometric light beam if both the molar extinction coefficient and concentration of the chromophore are known, which is the process employed with the MVS. With the dual-dye ratiometric photometry method, the concentration of each of the dyes is carefully controlled. Since the molar extinction coefficient for a given chromophore or dye is a physical constant, the absorbance per unit path length (*a*) for each dye in the MVS solutions can be determined. For example, the absorbance per unit path length of the blue dye at a specific concentration is calculated by the following equation:

$$\frac{A_{730}}{l} = \varepsilon_{730} C = a_b$$

Where A_{730} is the absorbance of the blue dye at a wavelength of 730 nm, ε_{730} is the molar extinction coefficient of the blue dye at that wavelength, *l* is the path length of light passed through the solution, and *C* is the molar concentration of blue dye. The final result, a_b, is the absorbance per unit path length of the blue dye. Similar calculations are performed to determine the absorbance per unit path length of the red dye (a_r).

$$\frac{A_{520}}{l} = \varepsilon_{520} C = a_r$$

In the MVS, the concentration of the red dye is varied while the concentration of the blue dye is kept constant. Since the absorbance per unit path length can be determined for the red and the blue dye for each MVS solution, the sample volumes can be calculated as a function of the path length of light passed through the sample.

MVS COMPONENTS

The MVS has been designed to provide accurate and precise photometric measurements and to achieve measurement traceability to the International System of Units through national standards. The components of the MVS include the following (shown in Figure 1):

1. Custom manufactured microtiter verification plates that are dimensionally characterized and barcoded with the lot number and a unique identifier. Characterized microtiter verification plates are available in ANSI/SBS 96-well and 384-well formats.

2. MVS QualAssure solutions, stock solutions, diluent, and baseline solution, all of which contain known concentrations of dyes and are characterized with absorbance measurements traceable to the SI.

3. A specialized calibrator plate consisting of sealed precision quartz cuvettes containing the same dyes used in the sample solutions and diluent. Traceable absorbance data for each cuvette is recorded within a barcode. Neutral density glass within each calibrator plate provides an internal control to ensure that each cuvette is performing properly.

4. A microtiter plate shaker that provides optimal mixing of sample solutions and diluent in the microtiter plate wells.

5. A customized microtiter plate reader that delivers reliable absorbance measurements at 520 nm and 730 nm.

6. A laptop computer with ArtelWare software.

7. A compatible barcode reader to ensure traceability and eliminate user error.

8. A mobile workstation, which accommodates all MVS components and allows the MVS to be moved from lab to lab, enabling its use in close proximity to the automated liquid handling devices.



Figure 1. *MVS Components*

VERIFICATION PLATES

Verification Plates are manufactured in lots, under conditions that minimize well-to-well and plate-to-plate variations. A sample of each lot is collected and all wells in the sample are characterized by a combination of dimensional, gravimetric, and photometric methods. Data are processed to develop corrections that compensate for well-to-well deviations and deviations from ideal well geometry as well as meniscus behavior. The plate and lot specific information is embedded in a barcode, which is used in the volume calculations for each well.

These dimensionally characterized verification plates are available in both 96-well and 384-well formats, and conform to ANSI/SBS dimensional requirements for footprint, height, flange and well spacing dimensions. These plates provide the best traceability and measurement uncertainty in the range from 10 nL to 350 µL.

SOLUTIONS

MVS solutions are designed to work together. The absorbance per unit path length of the red and blue dyes is determined for each MVS solution on a manufacturing lot basis and this information is embedded in barcodes on each bottle. This further reduces any source of variability in measurement and allows solutions from different manufacturing lots to be used interchangeably.

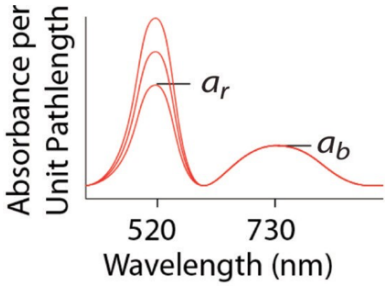
QualAssure Solutions

MVS QualAssure solutions contain two dyes with distinct absorbance maxima at 520 nm (red dye) and 730 nm (blue dye). Six QualAssure solutions containing different concentrations of the red dye are used for testing the performance of instruments over different volume ranges, which are summarized in Table 1 for standard profile 96-well and 384-well verification plates. The concentration of red dye in each of these solutions has been optimized to ensure that the absorbance readings at 520 nm are within the linear range of the detector. The concentration of the blue dye is constant in all sample solutions across all volume ranges (Figure 2).

	QualAssure HV (Aqueous)	QualAssure A (PlasProxy and SerumSub)	QualAssure B (Aqueous, PCRMix, PlasProxy and SerumSub)	QualAssure C (Aqueous, DMSO and PCRMix)	QualAssure D (Aqueous and DMSO)	QualAssure E (Aqueous and DMSO)
96-well std. profile	200.1 to 350.0 µL	50.00 to 200.0 µL	10.00 to 49.99 µL	2.000 to 9.999 µL	1.000 to 1.999 µL	0.1000 to 0.9999 µL
384-well std. profile	n/a	10.00 to 55.00 µL	2.500 to 9.999 µL	0.500 to 2.4999 µL	0.3000 to 0.4999 µL	0.0100 to 0.2999 µL

Table 1. *Traceable volume ranges that can be measured with each QualAssure solution, which is dependent on type of microtiter plate used.*

Figure 2. *The MVS QualAssure solutions contain two dyes with distinct absorbance maxima at 520 nm (red dye) and 730 nm (blue dye). The absorbance per unit path length of the red dye (a_r) varies according to the concentration of red dye, while the absorbance per unit path length of the blue dye remains constant for all solutions. Note: each line in the schematic represents a different MVS QualAssure solution.*



Diluent

The MVS Diluent is a buffered solution that contains blue dye, the concentration of which is the same as that present in the QualAssure solutions. This makes it possible to calculate the total volume independent of the ratio of sample solution to diluent simply by measuring the absorbance of the blue dye. Since the concentration of the blue dye is the same in both the sample solution and diluent, the amount of diluent added is not critical. To allow accurate absorbance measurements, the diluent is used to bring the total working volume per well to 200 µL in a 96-well verification plate and to 55 µL per well in a 384-well standard profile verification plate. Volume verifications between 200.1 µL and 350 µL use only one premixed dye solution (Range HV), and no diluent is required in this volume range.

Baseline Solution

MVS Baseline solution contains no dye and is used for establishing a baseline measurement for the microtiter plate. This eliminates the contribution of light scatter from the plate and aqueous solutions to the measured absorbance.

PLATE READER

The MVS Plate Reader is designed for accurate and precise absorbance measurements at 520 nm and 730 nm, with known uncertainty of measurement. Coupled with the MVS Calibrator Plate (see below), the MVS Plate Reader produces traceable absorbance readings over the working absorbance range of the sample solutions. Each reader is tested using a detailed release test procedure in the controlled environment of the Artel Lab and must earn the “MVS Plate Reader” designation before it can become part of an MVS.

CALIBRATOR PLATE

The Calibrator Plate provides a stable artifact which is used to determine local drift in the daily plate reader performance. This reader drift is removed from the MVS calculations by adjusting the output absorbance to the barcoded reference values determined by the factory reference spectrophotometer. This allows all plate readers in the field to generate comparable MVS results within the system performance specifications, as they are all held in reference to the Artel factory reference spectrophotometer. This means that MVS systems with either reader type, at any location in the world will produce standardized, traceable results.

Sealed cuvettes containing MVS dye solutions are used in the calibrator plate instead of neutral density glass or wavelength specific interference filters. Using solutions containing the very dyes used for MVS testing allows for more accurate wavelength specific corrections of the plate reader. Using solution-filled cuvettes also accommodates for daily temperature differences assuming that the calibrator plate and the sample solutions are at the same temperature.

The integrity of each cuvette in the calibrator plate is ensured by comparison against a neutral density (ND) glass standard held within the plate. Long term integrity is also maintained by periodic recertification, which includes cleaning of all components, exchanging solutions, as well as acquiring new absorbance measurement values specific to each cuvette, and generating a new barcode for the plate.

PLATE SHAKER

The MVS plate shaker provides optimal mixing of solutions to ensure homogeneous mixing of the red and blue dyes and flattening of the meniscus in the microtiter wells of the Verification Plate. Specific speed, duration and shaking cycles have been determined for each plate type supported.

MOBILE WORKSTATION

The mobile workstation accommodates all of the MVS components and can be moved directly into the laboratories where the automated liquid handling devices are located. This allows the MVS to be used in close proximity to those devices and enables one MVS to service multiple laboratories.

SYSTEM SOFTWARE AND BARCODE READER

ArtelWare software integrates the absorbance readings at 520 nm and 730 nm from the plate reader with the barcoded information from the verification plate, calibrator plate, and MVS QualAssure solutions to create a comprehensive report of the volumetric performance of each channel of a multichannel liquid handling device. The data is securely stored in a SQL database, locally or networked, for easy viewing and analysis. ArtelWare contains a full set of security features such as Windows Authentication or allows local SQL based logins, multiple access control levels, password protection, electronic signatures, inventory and summary reporting, and an audit trail, facilitating the laboratory's compliance with 21 CFR Part 11 requirements.

EXAMPLE OF MVS VOLUME CALCULATION

The following example in this report describes the calculation of volumes in wells of 96-well plates. Different calculations are performed for 384-well plates,[9] alternative solutions,[10] and serial dilution[11] applications, which are described in the respective references.

Volume calculations are performed in three steps:

1. Calculation of the liquid depth (based on the Beer-Lambert law) in each well of a microplate,
2. Calculation of the total volume of liquid in each well, and
3. Calculation of the sample volume in each well.

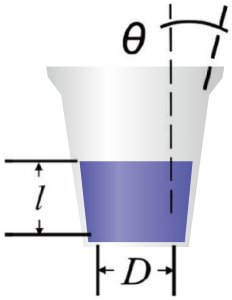
As the concentration of the blue dye is the same in all QualAssure solutions and in the diluent, it is possible to determine the liquid depth (l) in each well (which is equivalent to the path length of light passed through the sample) simply by measuring the absorbance of the blue dye at 730 nm (A_{730}) and using the absorbance per unit path length of the blue dye at 730 nm (a_b), determined by Artel, as shown in Equation 1:

Equation 1. *Liquid Depth Calcuation*

$$l = \frac{A_{730}}{a_b}$$

Wells of a standard profile 96-well plate have the geometrical shape of a truncated cone, as illustrated in Figure 3. Once the liquid depth is known, the geometrical equation for the volume of a truncated cone can be used to determine the total volume of liquid in the well. The calculation of the total volume (V_T) is based on the liquid depth (l), taper angle ($\emptyset$) and diameter (D) of the microtiter plate wells, as shown in Equation 2.

Figure 3. *The microtiter plate well bottom diameter (D), the taper angle (∅), and the path length of light passed through a sample (l) are critical to volume calculations.*



Equation 2. *Total Volume Calculation*

$$V_T = \pi l \frac{D^2}{4} + \pi D l^2 \frac{\tan(\theta)}{2} + \pi l^3 \frac{\tan^2(\theta)}{3}$$

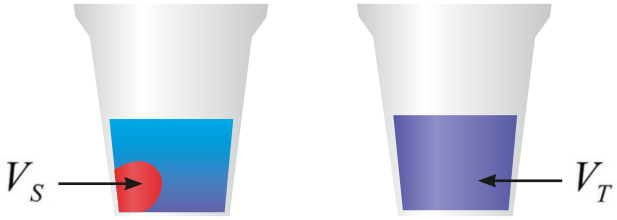
The calculation of the sample volume (V_S) is based on the total volume (V_T), the measured absorbance of the blue dye at 730 nm (A_{730}), the measured absorbance of the red dye at 520 nm (A_{520}), the absorbance per unit path length of the blue dye at 730 nm (a_b), and the absorbance per unit path length of the red dye at 520 nm(a_r), as shown in Equation 3 (see also Figure 4).

The calculated sample volume is then adjusted based on the deviation from ideal performance (described in the verification plates section above) within the plate lot for a specific volume range.

Equation 3. *Sample Volume Calculation*

$$V_S = V_T \left(\frac{a_b}{a_r} \right) \left(\frac{A_{520}}{A_{730}} \right)$$

Figure 4. *The calculation of the sample volume (V_S) is based on the total volume (V_T), the ratio of measured absorbance, and the ratio of the absorbances per unit path length of the red and blue dyes.*



OPERATING PROCEDURE

The operating procedure for the MVS enables the user to verify the performance of each channel of a multichannel liquid delivery device quickly and conveniently, via the following steps:

1. **Read the MVS Calibrator Plate**
   The calibration step typically is performed only once daily to determine the reader drift and requires only a few minutes. Recalibration of the MVS during one day's use is only necessary if the operating conditions of the MVS have changed (i.e., different location or a significant change in temperature).

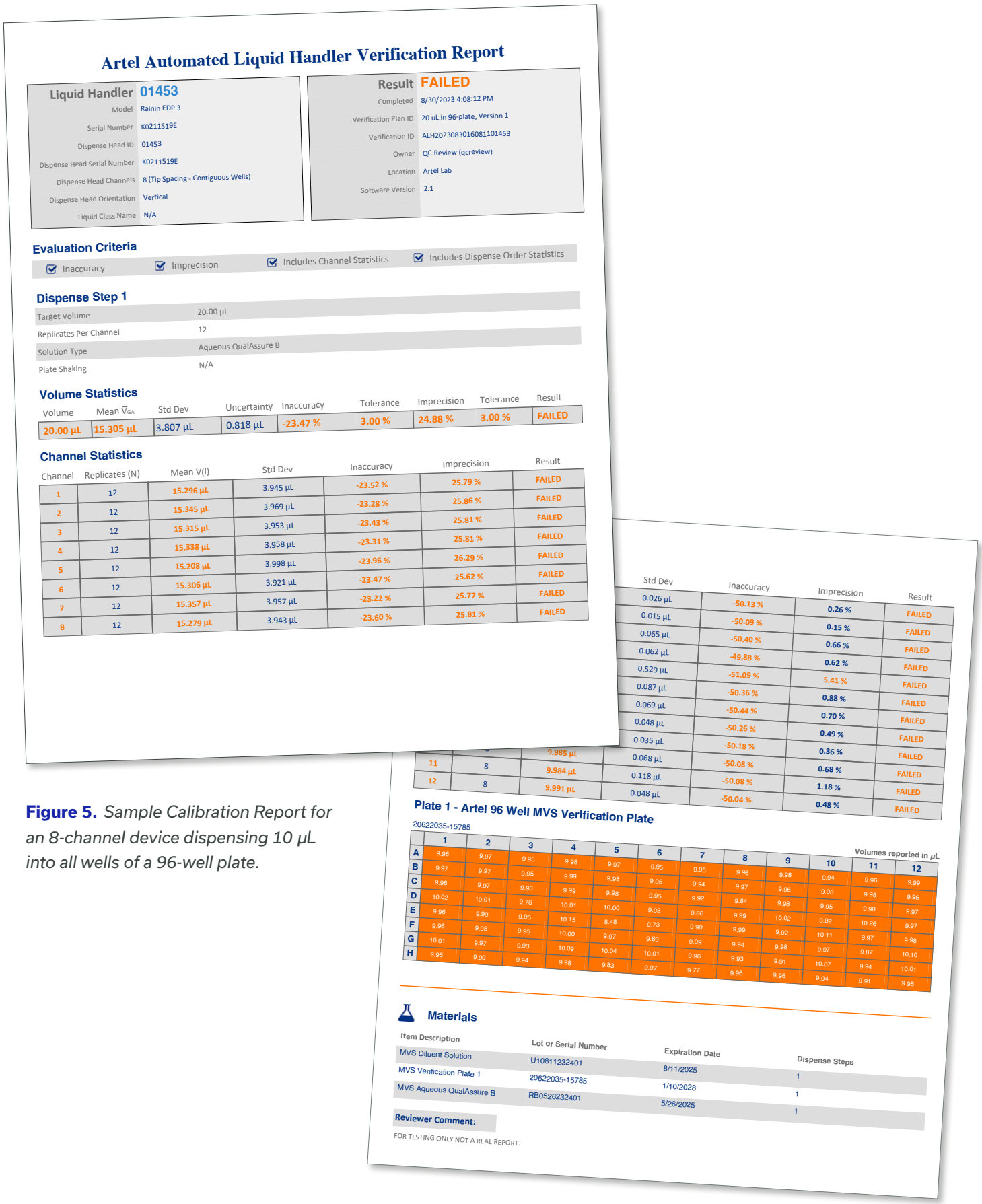
2. **Take a baseline measurement**
   After reading the Calibrator Plate, a baseline ("zero") measurement is made using the MVS baseline solution, which contains no dye. Each time the MVS is calibrated with the Calibrator Plate, a new baseline measurement is required.

3. **Dispense MVS QualAssure solution using the multichannel device under test**
   The multichannel liquid handling device under test is used to dispense the MVS QualAssure solution containing the red and blue dyes into the microtiter plate wells. For volume verifications in 96-well standard profile plates, Diluent is added for a total working volume of 200 µL, and in 384-well standard profile plates for a total working volume of 55 µL. These working volumes will ensure an optimal optical path length for the absorbance measurements.

4. **Shake the Verification Plate**
   After the appropriate amounts of QualAssure solution and diluent have been dispensed, the Verification Plate is placed on a plate shaker to mix both dye solutions and to flatten the meniscus inside the wells.

5. **Begin photometric measurements**
   The MVS plate reader measures the absorbance of the dyes at 520 nm and 730 nm for each of the 96 or 384 wells in the Verification Plate. The sample volume is calculated from the measured absorbances of the red and blue dyes and the barcoded information from each system component used in the particular verification.

6. **Generate report**
   A comprehensive report of the calculated verification data is displayed on screen and stored in a secured database. Individual channels that fail to meet established tolerances are indicated in color as shown in Figure 5 (this example shows 12 dispenses of an 8-channel device into a 96-well plate).



Artel Automated Liquid Handler Verification Report

Liquid Handler	01453
Model	Rainin EDP 3
Serial Number	K0211519E
Dispense Head ID	01453
Dispense Head Serial Number	K0211519E
Dispense Head Channels	8 (Tip Spacing - Contiguous Wells)
Dispense Head Orientation	Vertical
Liquid Class Name	N/A

Result	FAILED
Completed	8/30/2023 4:08:12 PM
Verification Plan ID	20 µL in 96-plate, Version 1
Verification ID	ALH20230830160811O1453
Owner	QC Review (qcreview)
Location	Artel Lab
Software Version	2.1

Evaluation Criteria

☑ Inaccuracy ☑ Imprecision ☑ Includes Channel Statistics ☑ Includes Dispense Order Statistics

Dispense Step 1

Target Volume	20.00 µL
Replicates Per Channel	12
Solution Type	Aqueous QualAssure B
Plate Shaking	N/A

Volume Statistics

Volume	Mean $\bar{V}_{GA}$	Std Dev	Uncertainty	Inaccuracy	Tolerance	Imprecision	Tolerance	Result
20.00 µL	15.305 µL	3.807 µL	0.818 µL	-23.47 %	3.00 %	24.88 %	3.00 %	FAILED

Channel Statistics

Channel	Replicates (N)	Mean $\bar{V}$(I)	Std Dev	Inaccuracy	Imprecision	Result
1	12	15.296 µL	3.945 µL	-23.52 %	25.79 %	FAILED
2	12	15.345 µL	3.969 µL	-23.28 %	25.86 %	FAILED
3	12	15.315 µL	3.953 µL	-23.43 %	25.81 %	FAILED
4	12	15.338 µL	3.958 µL	-23.31 %	25.81 %	FAILED
5	12	15.208 µL	3.998 µL	-23.96 %	26.29 %	FAILED
6	12	15.306 µL	3.921 µL	-23.47 %	25.62 %	FAILED
7	12	15.357 µL	3.957 µL	-23.22 %	25.77 %	FAILED
8	12	15.279 µL	3.943 µL	-23.60 %	25.81 %	FAILED

Std Dev	Inaccuracy	Imprecision	Result
0.026 µL	-50.13 %	0.26 %	FAILED
0.015 µL	-50.09 %	0.15 %	FAILED
0.065 µL	-50.40 %	0.66 %	FAILED
0.062 µL	-49.88 %	0.62 %	FAILED
0.529 µL	-51.09 %	5.41 %	FAILED
0.087 µL	-50.36 %	0.88 %	FAILED
0.069 µL	-50.44 %	0.70 %	FAILED
0.048 µL	-50.26 %	0.49 %	FAILED
0.035 µL	-50.18 %	0.36 %	FAILED
0.068 µL	-50.08 %	0.68 %	FAILED
0.118 µL	-50.08 %	1.18 %	FAILED
0.048 µL	-50.04 %	0.48 %	FAILED

11	8	9.985 µL
12	8	9.984 µL
	8	9.991 µL

Plate 1 - Artel 96 Well MVS Verification Plate

20622035-15785

Volumes reported in µL

	1	2	3	4	5	6	7	8	9	10	11	12
A	9.96	9.97	9.95	9.98	9.97	9.95	9.95	9.96	9.98	9.94	9.96	9.99
B	9.97	9.97	9.95	9.99	9.98	9.95	9.94	9.97	9.96	9.98	9.98	9.96
C	9.96	9.97	9.93	9.99	9.98	9.95	9.92	9.84	9.98	9.95	9.98	9.97
D	10.02	10.01	9.76	10.01	10.00	9.98	9.86	9.99	10.02	9.92	10.26	9.97
E	9.96	9.99	9.95	10.15	8.48	9.73	9.90	9.99	9.92	10.11	9.97	9.98
F	9.96	9.98	9.95	10.00	9.97	9.89	9.99	9.94	9.98	9.97	9.87	10.10
G	10.01	9.97	9.93	10.09	10.04	10.01	9.96	9.93	9.91	10.07	9.94	10.01
H	9.95	9.99	9.94	9.98	9.83	9.97	9.77	9.96	9.96	9.94	9.91	9.95

Materials

Item Description	Lot or Serial Number	Expiration Date	Dispense Steps
MVS Diluent Solution	U10811232401	8/11/2025	1
MVS Verification Plate 1	20622035-15785	1/10/2028	1
MVS Aqueous QualAssure B	RB0526232401	5/26/2025	1

Reviewer Comment:

FOR TESTING ONLY NOT A REAL REPORT.

Figure 5. *Sample Calibration Report for an 8-channel device dispensing 10 µL into all wells of a 96-well plate.*

ACCURACY AND PRECISION[i]

The MVS measures the sample volume dispensed by every channel of a multichannel liquid handling instrument. Thus, MVS measurement results can be used to evaluate the accuracy and precision of the instrument under test.

For liquid handling devices, accuracy refers to the concept of how near a dispensed volume is to the desired target volume, which is usually set or displayed digitally. Precision refers to how near the volumes of a set of dispensed aliquots are to one another, regardless of how close this set of volumes is in relation to the target volume.

It is possible for an instrument to be precise but not accurate, due to the instrument's systematic error (Figure 6). In other words, an instrument may consistently deliver volumes within a narrow range, but the dispensed volumes may be systematically above or below the desired target volume. Conversely, it is also possible for an instrument to have little systematic error, but not be precise. This is the case when an instrument dispenses volumes that are widespread both above and below the target volume, but the average of these volumes is close to the target volume.

The MVS verification report includes two types of descriptive statistics related to accuracy and precision, expressed as relative inaccuracy and coefficient of variation, respectively.

The coefficient of variation (CV) is the standard deviation of the volume dispensed divided by the mean volume, and is expressed as a percentage (CV = 100% x standard deviation / mean volume). The CV is a measure of the imprecision (lack of precision) in a series of replicate dispenses, and this statistic can be used as an estimate of the random error of the liquid handling device.

The relative inaccuracy (RI) is calculated as the percent difference between the mean volume and the target volume, divided by the target volume (RI = 100% x (mean volume – target volume) / target volume). This statistic is often used as an estimate of the systematic error of the liquid handling device. It can also be correctly called the "inaccuracy of the mean volume."

[i]Concepts and terms related to measurement error such as accuracy, precision, systematic error and random error are carefully defined in the third edition of the VIM.[12] There is some inconsistency in use of these terms within the liquid handling industry, and this paper attempts to bridge the gap by using terms that are familiar to the industry, while also being reasonably consistent with the VIM.

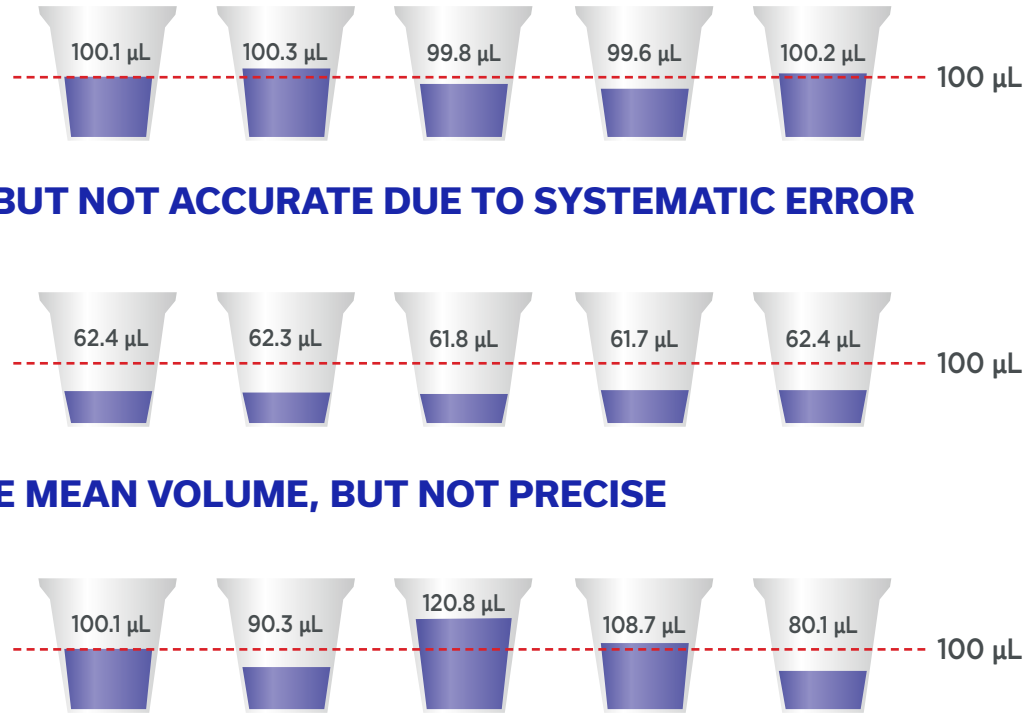


Figure 6. *Accuracy and precision are important performance features of multichannel liquid handling devices.*

The data presented in the following examples illustrates the accuracy and precision of several liquid handling instruments over a range of target volumes. The expected accuracy and precision of each instrument should be based on the specifications of each instrument.

As an example: An 8-channel liquid handling device shows very good precision but poor accuracy (Figure 7). The data collected at each volume corresponds to 36 dispenses (288 microplate wells filled with MVS sample solution). At each target volume tested, the dispensed volume is consistently low.

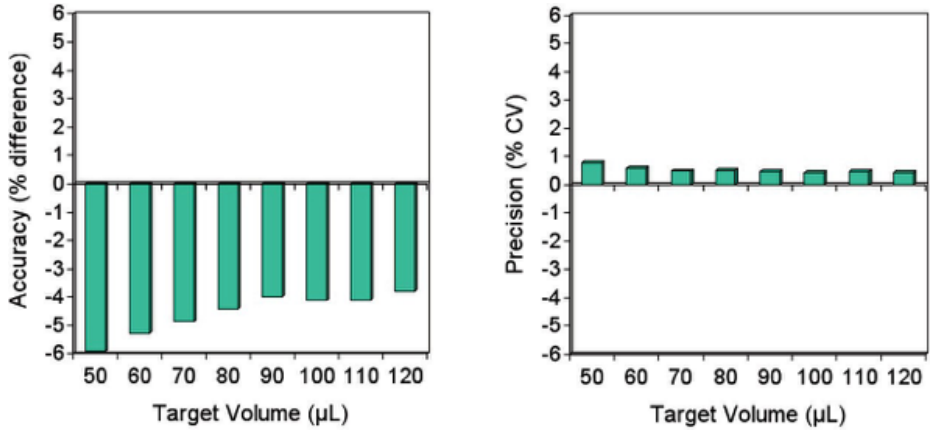


Figure 7. *An 8-channel liquid handling device shows very good precision but poor accuracy due to systematic error; dispensed volumes are consistently lower than target volumes.*

The performance of an 8-channel liquid handling device that is both precise and accurate over the range of volumes tested is illustrated in Figure 8. In this experiment, the data for each target volume correspond to a 96-well microtiter plate filled with MVS QualAssure solution (i.e., 12 replicates of an 8-channel dispense). At every target volume tested, the mean dispensed volumes are accurate. Although precision is reasonably good across the range of volumes tested, the instrument is more precise at volumes of 50 µL and above.

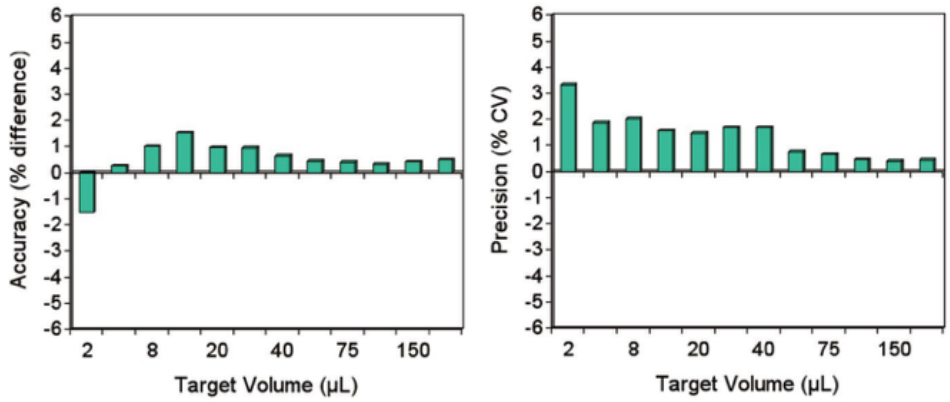


Figure 8. *This 8-channel liquid handling device shows both good precision and accuracy over the range of target volumes tested.*

Figure 9 illustrates the performance of a 96-channel automated liquid handling instrument across a range of target volumes. For each volume tested, three replicate 96-well microtiter plates were employed to obtain three data points per channel. Although the manufacturer rated this instrument for volumes from 25 µL to 200 µL, the MVS test results indicate that the accuracy of the instrument performance falters at lower volumes. The instrument shows good precision over the manufacturer's recommended range of 25 µL to 200 µL. Large systematic error is observed when target volumes are set below 50 µL

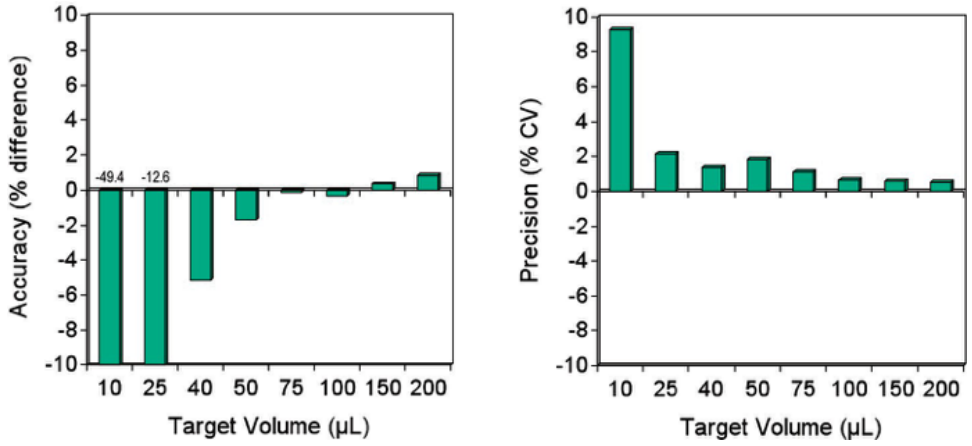


Figure 9. *A 96-channel automated liquid handling instrument shows good precision over the manufacturer's recommended range of 25 µL to 200 µL, but is systematically low at target volumes below 50 µL.*

COMPARISON WITH OTHER VERIFICATION METHODS

Since prior to the development of the MVS, alternative performance measurement methods based on fluorometry, single-dye photometry, or gravimetry have been often employed. Each of these methods has inherent limitations that have constrained their usefulness for verification and calibration of multichannel automated liquid handling devices.

Fluorometry

Fluorometric methods for evaluating the volumetric performance of liquid handling devices are based on the assumption that there is a linear relationship between the fluorescence measured at a specific wavelength and the concentration of the fluorophore in solution. This linear relationship exists only for certain fluorescent dyes under specific conditions, as fluorophores are subject to quenching and photobleaching, and no national standards for fluorescent dyes currently exist. Because of these limitations and the limited stability of most fluorescent dyes, fluorometric methods require a standard curve to be generated for every solution used for volumetric calibrations, at each time this solution is being used. The labor-intensive nature of fluorometric methods together with the lack of stable fluorescence standards limits the utility of these methods in verifying and calibrating multichannel liquid handling devices. Additional measurement errors may be introduced by non-compensated performance fluctuations of the fluorescence reader, and inaccurate and/or imprecise additions of diluent volumes to the fluorescent samples. Finally, there may be considerable variability induced through the manually established standard curves, depending on the pipetting technique of the individual technician.

Single-Dye Photometry

Single dye photometric methods involve measuring the absorbance of one dye at a specific wavelength and calculating the volume based on a calibration curve that converts absorbance to volume. When measuring small volumes, a clear diluent must be added to the single dye sample in order to fill the well. Since volumetric measurements are based on the absorbance of only one dye, variability in delivery of the clear diluent will create errors in volume calculations.

Other sources of error are also present. Many “in-house” single dye photometry methods use dye solutions that are not fully characterized and traceable. Non-compensated drift of the absorbance plate reader and potential inconsistencies in the manual generation of standard curves (as outlined in the fluorometry discussion) may contribute to measurement errors. Reproducible, standardized measurements are thus difficult to obtain with single dye photometric methods.

Gravimetry

Gravimetry is a common approach for evaluating the performance of liquid handling devices, and typically involves measuring a volume of solution (usually water) into a container whose weight is measured before and after dispensing the solution. Working conditions (e.g. temperature, pressure, humidity, etc.) must be carefully controlled and monitored to obtain accurate results with gravimetric methods; control of the working conditions is particularly important when measuring small volumes in order to reduce the uncertainty introduced by electrostatic effects and evaporation.

For multichannel devices, gravimetry is problematic since it involves either a very cumbersome and timeconsuming process, whereby the volumes measured by each channel of a multichannel device are weighed separately, or a simplified process in which volumes measured by all channels are dispensed into a single container to obtain an average weight. Because gravimetric methods for evaluation of individual channels so much technician time, they are impractical for the frequent verification and calibration needed to assure optimal performance of multichannel devices. On the other hand, simplified gravimetric methods that measure the cumulative weight of liquid dispensed from all tips often do not detect the performance problems of individual channels because the volumes are pooled and averaged.

Gravimetry has been used under the well-defined and highly controlled conditions of the Artel Laboratory,[13] as a comparator for verifying the volumes measured by the MVS. Figure 10 shows the correlation between volumetric measurements verified with the MVS and those verified with a gravimetric balance for an 8-channel liquid handling device. In this experiment, the data collected at each volume corresponds to a single 96-well microtiter plate filled with MVS QualAssure solution (12 repetitive dispenses of the 8-channel device).

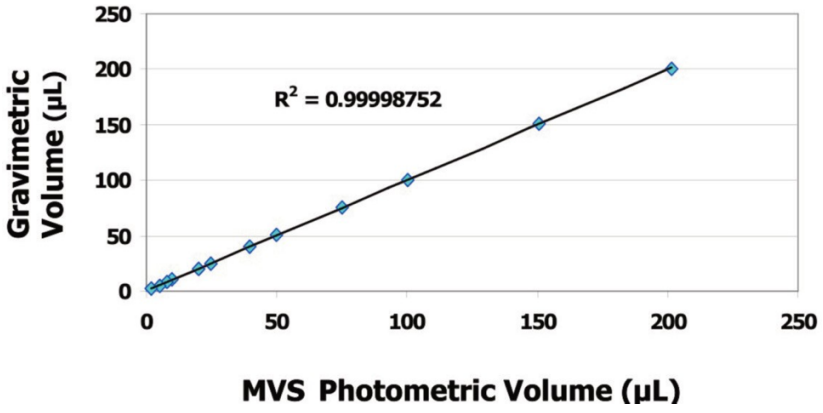


Figure 10. *Volumes verified by gravimetry and by the MVS are highly correlated.*

The results demonstrate that the volumes verified by the MVS are highly correlated with volumes verified by gravimetric methods. It is important to note that since the MVS is designed to test individual channels, the data from this experiment consists of 12 replicate measurements for each of the 8 channels for every target volume tested. By contrast, the entire microtiter plate was weighed in the gravimetric method and thus the results of gravimetry reflect the average volume dispensed by all channels.

A similar comparison was performed for a 96-channel automated liquid handling device tested with the MVS and gravimetry. Figure 11 shows the percent difference between volumes verified by the MVS and gravimetric determinations. In this experiment, three 96-well microtiter plates were dispensed to yield a total of three replicate measurements for each of the 96-channels for every target volume tested with the MVS. Again, since the entire microtiter plate was weighed, gravimetric measurements reflect the average volume dispensed by the 96 channels. Results indicate a high degree of correlation between the MVS and the carefully controlled gravimetric methods.

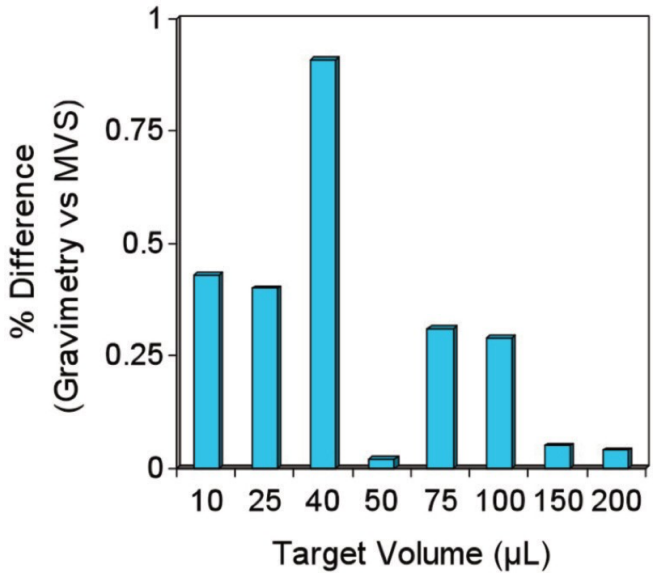


Figure 11. *The MVS demonstrates a high degree of accuracy as compared to gravimetry.*

CONCLUSIONS

The Artel MVS by Advanced Instruments is designed to verify both the accuracy and precision of any multichannel liquid delivery device. The integrated components provide the user with the ability to test the performance of a liquid delivery device in a matter of minutes; allowing for periodic full device calibration, more frequent interim verifications, or routine protocol improvement or optimization. The ability to report both the accuracy and precision of liquid delivery processes provides the user with a full assessment of liquid delivery device performance, thus ensuring the integrity of data produced by that device. Because MVS measurement results are traceable to the SI, inter-laboratory comparisons can be made, further ensuring data integrity within a multi-laboratory environment. With the Compliance Suite add-on, ArtelWare includes security features which facilitate compliance with 21 CFR Part 11 requirements. The MVS represents a truly standardized platform for direct performance comparisons between liquid delivery devices across multiple laboratories, greatly facilitating method transfers and allowing improved quality assurance protocols.

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13. Accredited for compliance with ISO 17025:2005 by the American Association for Laboratory Accreditation (A2LA). Certificate numbers 2093.01 for chemical testing and 2093.03 for calibration. www.a2la.org.

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Artel designs, develops, manufactures, distributes and supports analytical systems including instruments, consumables and software for quality assurance applications in laboratory liquid handling. The Company also provides related training and on-site services.

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