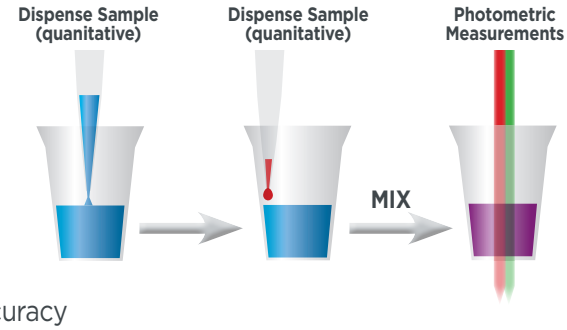
Understanding liquid handler performance behavior

Managing a Volume Dispensing Device: **MVS**® Uses and Applications for Understanding and Optimizing Liquid Handler Performance

All assays performed within a microtiter plate are volume-dependent. All concentrations of biological and chemical components in these assays, as well as the associated dilution protocols, are volume-dependent. Therefore, the accuracy and precision of individual, or step-wise, volume aspirations and dispenses directly impacts assay results. Inaccurate and imprecise delivery could mean the difference between a false-positive, false-negative, true positive or true-negative result. **Knowing an assay's exact volume and component concentrations is critical to interpreting the results.**

The **MVS**® (Multichannel Verification System) enables accurate and precise measurement of liquid volumes, as well as measurement of dilution step accuracy, in microtiter plates. This fast, easy and reliable system is used to assess liquid handler performance without the need of preparing dye solutions, standard curves, or having rigorous environmental controls as required with other verification methods. This measurement platform allows a user to understand device behavior and creates an environment where an individual assay's performance and methodology can be optimized and validated. The MVS can measure the performance of any handheld multichannel pipettor or any robotic automated liquid handler incorporating 1 – 384 channels for target volumes as low as 10 nL.

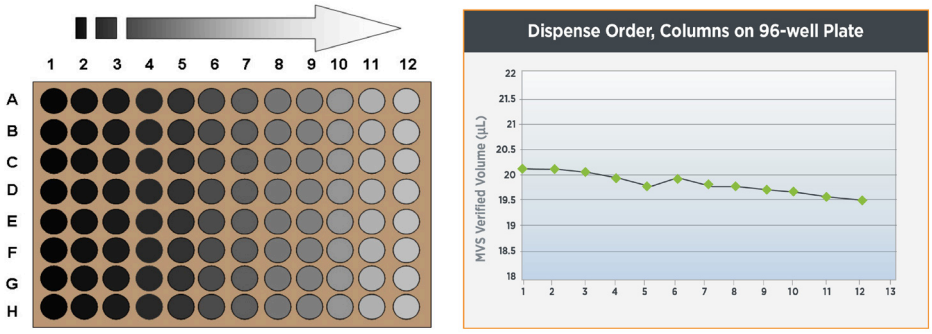


Standardized MVS QualAssure™ solutions manufactured by Artel are dispensed into a 96-well or 384-well microtiter plate using the liquid handler of interest. The target volume in each well is measured via a proprietary dual-dye, dual-wavelength ratiometric method and the MVS instantly reports the transferred target volumes per tip and per well (including statistics). When employing a 96-well microplate, MVS specifications for inaccuracy and imprecision are less than 3% and less than 1.5%, respectively. With a 384-well plate, MVS specifications for inaccuracy and imprecision are less than 10% and less than 3%, respectively.

The following list identifies several ways life science laboratories integrate MVS into their QC processes. This list is not comprehensive, but is intended to educate the user on the versatility and usefulness of the MVS platform.

(1) Dispense order and trending

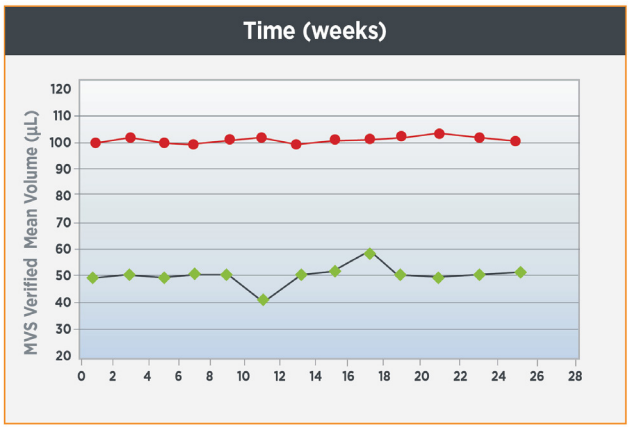
It is quite common to aspirate a large volume followed by sequential, smaller volume dispenses to multiple wells, rows, columns, or even multiple plates. The MVS can be used to identify statistical relationships between sequential dispenses and can be employed to characterize dispense order trending, which allows the user to manipulate an aspirate/dispense protocol for optimal device performance.



A schematic representation of a 96-well microtiter plate showing volume dispense trending. In this representation, an 8-channel device was used to dispense 20 µL across the plate from column 1 to column 12 (left). Although the volumes in each column should be identical, the actual volume is sequentially lowering (drifting downward) with each dispense (right).

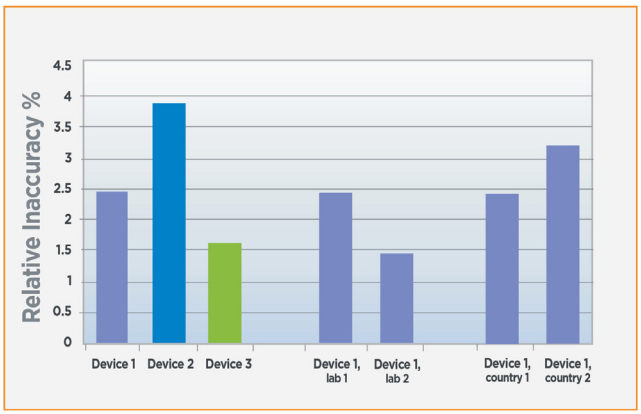
(2) Dispense trending vs. time

The MVS measurements can be employed to create offline trending plots of performance for each volume dispensing device over time. Regardless of the calibration interval for each device, the MVS can be used between intervals to learn about device drifting as a function of time. Within minutes, the MVS allows the user to 'spot check' and verify target volumes that are critical to an assay. Knowing if and when devices fail between calibration intervals saves time, labor, and reagent costs, and will also prevent loss of time and capital in the future.



(3) Multiple device comparison checking

Because the MVS results are traceable to the SI (International System of Units) through standards maintained by NIST (USA) and NPL (UK) the user can directly compare the accuracy and precision of different multichannel dispensing devices. The MVS allows any device with 1, 2, 4, 8, 12, 96 or 384 dispensing channels to be compared, regardless of make, manufacturer, or location.

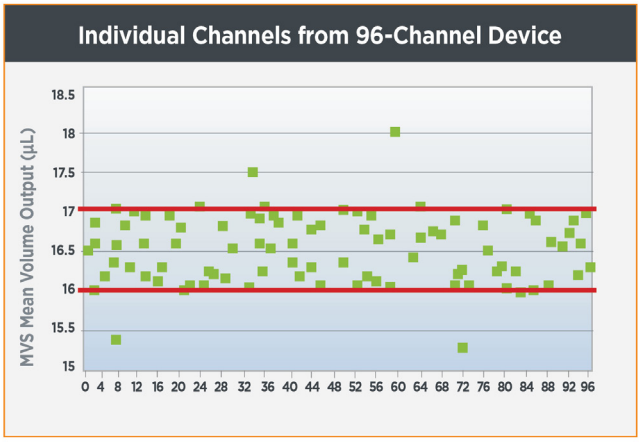


(4) Method transfer and confirmation of data consistency

The MVS can also be used to facilitate method and assay transfer. Before, during or after an assay is transferred to a different operator, laboratory, department, or facility, the working assay's critical volume transfers can be measured and used as a benchmark for subsequent performance checks. Understanding the dispense dynamics and behaviors for each device during these transitions will ensure a smooth transition and reduce troubleshooting, assay/device downtime, and downstream economic loss.

(5) Tip-to-tip reproducibility

For multichannel dispensing devices, such as an 8-, 12-, 96-, and 384-tip systems, the MVS can be used to compare channel-to-channel (tip-by-tip) performance. Each individual tip within the delivery device might perform with some variability and defining this variability, or determining which channels "misbehave", allows assay results to be properly interpreted.



6) Reproducible sample preparation

If a multichannel device is used to repeatedly prepare critical sample solutions and/or assay plates, the MVS can be a useful tool to track device performance as it relates to sample-to-sample or lot-to-lot reproducibility. As a specific example, consider a compound storage facility that dispenses characterized compounds into "mother" plates, which are used for subsequent aspirate/dispense "hit-picking" protocols and also used to create "daughter" plates. It is very important that the daughter plates are prepared and in a reproducible fashion.

(7) Rare and expensive reagent testing

If a rare and/or expensive reagent is dispensed by the liquid handler, it is a good idea to "spot check" the liquid handler prior to performing the assay. The MVS is fast and easy to use, so the user can quickly verify the performance of any liquid handler system prior to running critical assays. This process allows the user to have confidence in the protocol and potentially avoids the initial loss, or unnecessary destruction, of rare and expensive reagents.

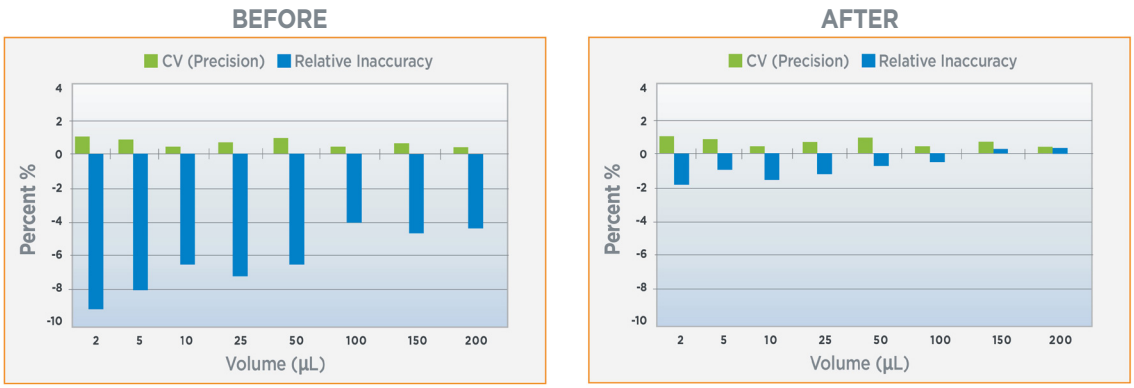
(8) Traceable GLP/GMP compliance

With its traceable measurements, the MVS allows for preparing standard operating procedures for liquid dispensing devices in GMP and cGLP laboratories. In a specific case, one company implemented MVS into their standard operating procedures to directly compare results from three different laboratories located in different parts of the country. Because MVS facilitates 21 CFR Part11 software compliance and provides traceable measurements, the company is able to use the MVS to standardize procedures, paperwork, and interpretation of laboratory results.

(9) Operational Qualification and protocol optimizations

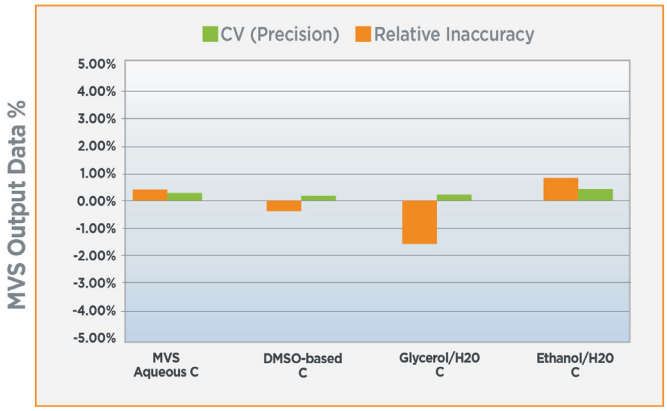
Developing a Operational Qualification (OQ) protocol for a particular liquid handler, including the target volumes required by the assay development team can be a straight-forward procedure if utilizing the MVS. The MVS can be used to help optimize each adjustable parameter within a volume dispensing protocol or can be used to validate an entire method. Adjustable liquid handler parameters include, but are not limited to: (1) pre- and post- air gaps; (2) blow-out volumes; (3) off-set volumes; (4) dry tip vs. wet tip; (5) tip type, tip quality, and volume capacity; (6) new tip vs. used tip; (7) aspirate/dispense rates; (8) aspirate/dispense heights; (9) tip-touches; (10) reservoir location; (11) on-board mixing; (12) wash steps.

Performance for a multichannel device before (top) and after (bottom) optimizing the aspirate/dispense protocols to fall within a tighter tolerance window.



(10) Non-aqueous and complex solutions

While liquid handlers are certainly capable of handling a wide array of reagent types, it is commonly known that performance parameters can vary significantly when the reagents are complex in nature. When liquid handlers are employed to aspirate/dispense aqueous-based reagents, there are many methodologies used to calibrate/verify the system's ability to properly perform within a assay's tolerances (including photometric and gravimetric methods). In other situations, however, liquid handlers are also employed to dispense complex and/or non-aqueous reagents (such as dimethyl sulfoxide, serum, aqueous-based mixtures with detergents, etc.) for which there are fewer accepted methodologies to verify system performance. MVS allows the user to create their own test liquids, known as Alternative Solutions, to test liquid handler performance for liquid types that are similar to those used in the assays. Using easy-to-follow directions via a software helper, MVS reagents are gravimetrically combined with a user's specific solution (such as DMSO), to create a new dye-based test liquid that can be used to simulate the operator's solution. This Alternative Solution functionality allows the user to learn device behavior for specific solution types. Trial and error experimentation might be required.



Alternative Solution Test Liquid

The plot shows relative inaccuracy % and CV % for a calibrated syringe used to dispense 8 µL of MVS Range C and three different Alternative Solutions. The three Alternative Solutions consist of, by volume in water, (a) 90% DMSO; (b) 20% glycerol, and (c) 50% ethanol.

(11) Operator assessment

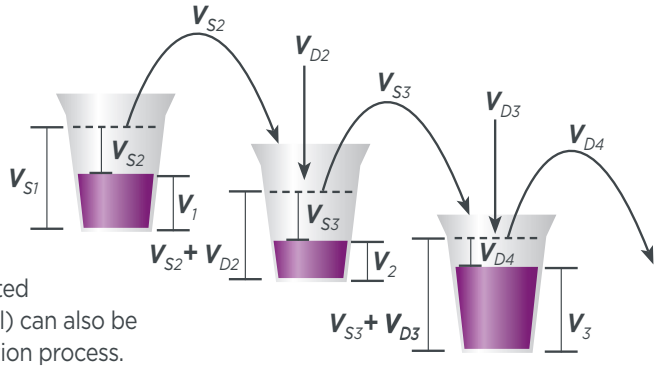
The MVS is also used to compare pipetting performance from operator-to-operator as well as a tool to compare liquid handler methods (software scripts) written by operators at different locations. Use of MVS can help reduce potential errors in assay development when identical processes should be running side-by-side or day-to-day in different laboratories with different operators. The MVS can help train operators on using proper pipetting techniques to dispense volumes within specific tolerance windows.

(12) Site acceptance testing and pre- and post-preventative maintenance testing

The MVS can be utilized during/after installation of a new liquid handler to validate system performance immediately. In addition, it can be used before and after field service to document and validate changes and adjustments to performance.

(13) Step-wise dilution accuracy

The MVS can be used to assess a liquid handler for dilution step accuracy. In either a 96-well or 384-well plate, dilution steps can be measured up to up to 1/2048 of the original starting material. User-defined tolerance limits for accuracy can be defined at each step.



(14) MVS integration for in-process QC

MVS can be integrated onto the deck of a liquid handler for a more automated approach to volume verification. The performance data (per tip and per well) can also be exported as XML to a company's LIMS system for full control of the verification process.

(15) Testing plates in groups (Batch function)

Users can streamline testing groups of volume verification tests into one method by storing bar codes for plates and solutions. The need to continuously scan bar codes between different verification events is eliminated.

(16) Determining your assay's tolerances

With the MVS, you can start to understand how liquid handling variability can affect your important assays as well as the amount of liquid handling variability that can still produce an acceptable assay result. By tweaking and modifying dispense parameters (followed by volume measurement with the MVS), a direct correlation between a passing and failing assay can be simply based on the accuracy of the volume transferred (remember, analyte concentration is volume-dependent).

For more information:

(a) Dong et al. The Use of a Dual Dye Photometric Calibration Method to Identify Possible Sample Dilution from an Automated Multichannel Liquid-Handling System. *J. Assoc. Lab. Autom.*, **2006**, *11*, 60-64.

(b) Bradshaw et al. 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.

(c) Albert et al. Verifying Liquid Handler Performance for Complex or Non-Aqueous Reagents: A New Approach. *J. Assoc. Lab. Autom.*, 2006, *11*, 172-180.

(d) Knaide et al. Rapid Volume Verification in High-Density Microtiter Plates Using Dual-Dye Photometry, J. *Assoc. Lab. Autom.*, 2006, *11*, 319-322.

(e) Albert and Bradshaw. Importance of Integrating a Volume Verification Method for Liquid Handlers: Applications in Learning Performance Behavior, *J. Assoc. Lab. Autom.*, **2007**, *12*, 172-180.

(f) Bradshaw et al. Determining Dilution Accuracy in Microtiter Plate Assays Using Quantitative Dual-Wavelength Absorbance Method, *J. Assoc. Lab. Autom.*, **2007**, *12*, 260-266.

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