Liquid Handling Validation for High Throughput Screening using Artel's MVS® Technology

Jason Haelewyn
Celgene, 4550 Towne Centre Ct., San Diego CA 92121

Abstract

There are a number of factors that determine the quality of data generated in any experiment. One factor, that most scientists take for granted, is the ability of liquid handling devices to deliver small volumes with both precision and accuracy. This is especially true in an environment utilizing automated liquid handling devices such as in a High Throughput Screening (HTS) laboratory. In these environments the job of validating the precision and accuracy of, possibly many different, automated liquid handling devices can be tedious work. It is often left to dedicated technicians who utilize a variety of absorbance and fluorescence techniques. However, not all labs have the resources to keep individuals dedicated to this task. Many small biotech companies, and in fact many larger ones, rely on the instrument manufacturers to validate liquid handling at the factory and possibly after on-site installation. After the installation period, however, these services may only occur once annually. Artel has developed a complete dual-dye, dual-wavelength, ratio-metric validation system that is both easy to use and with measurement results that are traceable to National Institute of Standards and Technology (NIST). Celgene has been able to utilize the Artel MVS® system to validate several liquid handling platforms used in its lead discovery process. This system has not only allowed Celgene to easily verify the original manufacturer's specifications, but has also allowed for routine weekly/monthly validations of the instruments without the need for hiring dedicated personnel.

Introduction

Multi-channel liquid handling devices are an integral part of drug discovery laboratories today. The accuracy and precision of these devices are essential to producing high quality data reproducibly. For hand-held pipettes this typically means sending the devices out to a metrology lab, which specializes in cleaning, repairing, and calibrating the devices. However for large, fully automated liquid handling systems commonly found in High Throughput Screening laboratories, this cannot be done due to the size of the instruments. Validation of liquid handling for these instruments must be done on-site. This can present a problem for many labs, which may not be equipped or staffed with their own metrology departments. In these cases, liquid handling validation can become a nagging problem because of the costs or time involved in properly calibrating an instrument and routinely validating its performance. Typical solutions to on-site calibration of large liquid handling instruments usually involve various fluorescent or absorbent dyes, gravimetric testing, or customized contracts with manufacturers for annual validations. Use of commercial dyes is common, but good results are largely dependent on the sensitivity of the dye, the volume of dilution solvent in the well, and the quality of the calibration curve. Because some dyes degrade over time it may be necessary to produce calibration curves prior to every validation. Gravimetric analysis of liquid handling can be problematic due to the effects evaporation, especially when dealing with the very small volumes associated with HTS. Customized contracts can become very expensive and most manufacturers do not have field service personnel who have been trained for volume validations. For these reasons, Artel has developed the Multi-channel Verification System (MVS®) (Fig.1) to accurately, precisely, and easily measure dispensed volumes from 0.030µl to 200µl on a range of systems from 1 to 384 channels. The MVS system utilizes dual-dye, dual-wavelength, ratiometric photometry to determine the delivered volume to a microtiter plate. This colorimetric method is fully traceable to NIST and has been approved by the International Organization of Standardization (ISO), especially for small volumes. The system also includes a Data Manager, which makes evaluating data and summary statistics easy and manageable for larger multi-plate validation sets.

Celgene has recently begun using an MVS system for liquid handling validation of several multi-channel instruments in its Biochemistry department. Several aspects of HTS operations were examined including comparisons of initial system performance vs. optimized settings, precision and accuracy of repeated use cycles on disposable tips, and establishing regular maintenance schedules.

Figure 1. The Artel MVS® system.

Using the software is intuitive and easy, utilizing fill-in style forms or pull-down menus. The first step is to set up a device to be tested (Fig.2). Next, configure a layout for the dispensing that will be analyzed (Fig.3).

Figure 2. Setting up a new device using the Artel MVS software.

Figure 3. Setting up a new layout using the Artel MVS software.

Starting a Verification is as easy as clicking the large green button on the main screen (Fig.4). After selecting the desired parameters, the Artel software will take you through the verification process step by step (Fig.5).

Figure 4. Starting a validation using the Artel MVS software.

Figure 5. The validation process using the Artel MVS software.

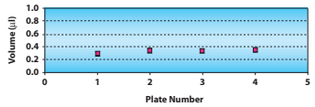


Figure 6. A baseline analysis of the performance of a SciClone ALH3000 (Caliper Life Sciences, 68 Elm Street, Hopkinton, MA 01748) delivering 0.5µl into solution. The delivery was made using a 384 low volume head with disposable tips utilizing a final mix in the destination plate post dispense. The settings for the 0.5µl delivery were previously validated after the on-site installation of the instrument. The original validation was made by an absorbance measurement of tartrazine dye compared to a calibration curve.

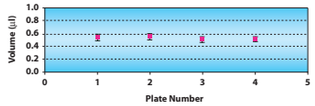


Figure 7. Performance of the SciClone ALH300 after optimizing the 0.5µl delivery. The optimization was made by analyzing several dispenses, at different volumes at or near 0.5µl, with the Artel MVS® system. Those data were used to calibrate a Liquid Class definition in the SciClone software. This optimized Liquid Class was then used to deliver 0.5µl.

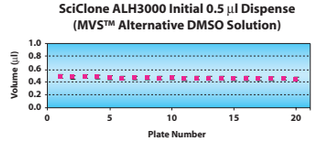


Figure 8. Multiple low volume dispense and wash cycles with disposable tips on the SciClone ALH3000. The dispense parameters for the SciClone ALH3000 were optimized for 0.5µl dispense of Artel's MVS Alternate Solution at 75% DMSO. Tips were loaded new and used to transfer 0.5µl of the Alternate Solution to the destination plate. A mix step was performed in the destination plate. The tips were then washed in a circulating water bath prior to the next transfer.

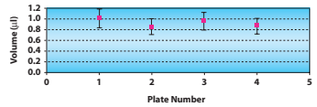


Figure 9. A baseline analysis of the performance of an EP3 (Perkin Elmer, 549 Albany Street, Boston, MA 02118) delivering 0.5µl into solution. The delivery was made using a 384 P30 head with disposable tips utilizing a final mix in the destination plate post dispense. Perkin Elmer does not quote specifications for the EP3 P30 head delivering 0.5µl, thus factory settings for 1µl transfer were used for the 0.5µl transfer.

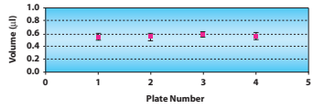


Figure 10. Performance of the EP3 after optimizing the 0.5µl delivery. The optimization was made by using the Artel MVS system to carefully analyze the residual volume remaining in the tips after a mix cycle in the source plate. A final aspiration using an optimized Performance File was added prior to dispensing. The transfer into the destination plate was followed by a mix cycle in the destination plate.

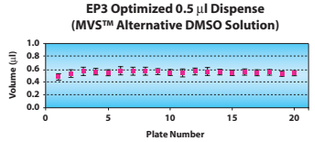
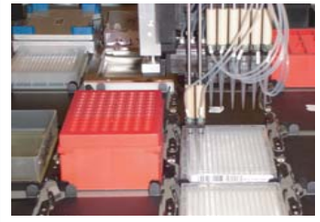
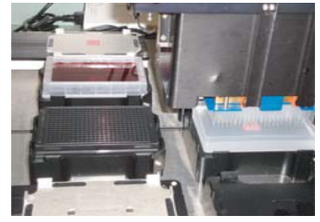


Figure 11. Multiple low volume dispense and wash cycles with disposable tips on the EP3. The dispense parameters for the EP3 were optimized for 0.5µl dispense of Artel's MVS Alternate Solution at 75% DMSO. Tips were loaded new and used to transfer 0.5µl of the Alternate Solution to the destination plate. A mix step was performed in the Alternate Solution prior to an aspiration of 0.1µl. After the transfer, a mix step was performed in the destination plate. The tips were then washed in a series of two water baths prior to the next transfer sequence.





Conclusions

- Factory dispense settings were easily analyzed and performance was optimized on-site, in the laboratory, using MVS results.
- The precision and accuracy of several automated liquid handling systems were validated when using disposable tips through multiple dispense and wash cycles similar to those used in an HTS setting.
- Disposable tips can be used to deliver reliable performance through multiple dispense and wash cycles in an HTS environment.
- Optimization and validation of an reliable 0.5µl dispense on the Perkin Elmer EP3, with standard P-30 384 channel head, was achieved which exceeds the manufacturer's specifications.
- Delivery of specific volumes were standardized across multiple liquid handling instruments from different vendors, allowing a uniform dispense of reagents regardless of which instrument was being used.
- The Artel MVS system is a fast and easy method for in-house calibration of liquid handling devices requiring very little user training.
- The Artel MVS system's Data Manager software produces useable and easy to understand reports well suited for monthly/weekly validation tests.
- The Artel MVS system cannot perform validations for 100% DMSO solvents.
- The Artel MVS system requires large amounts of Diluent Solution (Blue Dye) when validating small volumes over large numbers of plates, such as those encountered in HTS.
- Constant barcode scanning of solutions becomes tedious when validating large numbers of plates.

Acknowledgements

Celgene would like to thank Keith Albert, Artel, for his consulting on the use of the Artel MVS system.

Celgene would also like to thank Artel for their generous support by providing reagents for the completion of data presented in this poster.