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White Paper

The Multichannel Verification System (Artel MVS) by Advanced Instruments enables on-site liquid handler evaluation, saving time while delivering equivalent volumetric performance measurement results to the fluorescence test method used to evaluate Echo Acoustic Liquid Handlers

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Abstract

Echo acoustic liquid handlers are automated liquid handlers (ALHs) that use acoustic technology to provide contact-free transfer of liquids in increments of 2.5 nL or 25 nL depending on the model/series. Echo acoustic liquid handlers are compatible with common solvents for biopharmaceutical applications including dimethyl sulfoxide (DMSO) and a variety of aqueous-based solutions. Beckman Coulter Life Sciences currently uses a fluorescence method to evaluate the accuracy and precision of systems, but customers would like an alternative method to validate the performance of the Echo acoustic liquid handlers. An alternative method would minimize downtime and provide immediate performance feedback. Artel by Advanced Instruments provides a complete system, the Multichannel Verification System (Artel MVS), that can be used for liquid handler evaluation. The Artel MVS supports compliance to ISO 23783-2: Measurement procedures for the determination of volumetric performance. This study was designed to evaluate whether the Artel MVS and Beckman Coulter Life Sciences fluorescence test methods provide equivalent volume verification results at 25 nL and 250 nL on Echo acoustic liquid handler-525 and Echo acoustic liquid handler-655 for select aqueous and DMSO solutions.

Introduction

Echo acoustic liquid handlers use acoustic technology to dispense solutions commonly used in the biopharmaceutical industry, including aqueous-based solutions and DMSO solutions with varying hydration levels. The dispensing process from acoustic source plates and/or vials is contactless, with volumes as low as 2.5 nL or 25 nL depending on the model/series. The low nanoliter incremental volume delivery approach allows the direct preparation of assay plates at varying concentrations of reagent, by simply adjusting the number of increments dispensed per well. The time requirement per dispense causes a practical limitation of this approach for larger volumes, but volumes up to 1,000 nL are commonplace. The contactless dispense minimizes the potential for contamination of stock solutions, an important consideration for compound management libraries, and as such the pharmaceutical industry is a key customer. Furthermore, with miniaturization of assays on the rise, Echo acoustic liquid handlers are being used in a variety of laboratories and industries.

Evaluating the performance of ALHs is important to the quality of data collected and conclusions drawn from scientific studies and can be a regulatory requirement. ISO 23783-2: Measurement procedures for the determination of volumetric performance provides an overview of a variety of methods and is a useful reference. When selecting a test method, consideration must be given to accuracy and precision specifications of the ALH under test. The performance specifications for Echo acoustic liquid handlers are 8% CV and 10% Inaccuracy. A good rule of thumb for selecting a test method to evaluate precision of an ALH is to exceed the limits of the method by at least a factor of 1.5. For example, the Echo acoustic liquid handler imprecision of 8% CV, divided by 1.5 would require a test method to have a % CV specification of 5.3%. When selecting a method for volume verification, the true volume is defined as the True Volume = Reported Volume ± Measurement Uncertainty. When the Method Uncertainty uses a coverage factor (k) equal to 2, that corresponds to two-standard deviations or a two-sigma confidence interval. For normally distributed data, this means the True Volume may be expressed as above with a confidence of 95%. An acceptable uncertainty is ½ the inaccuracy specification of the ALH under test. For Echo acoustic liquid handlers this would correspond ± 5% of the test volume. In summary, the test method should have a % CV of less than 5.3% and an inaccuracy of less than 5% to be used to evaluate Echo instruments with the volume delivery specifications noted above.

The Artel MVS and Beckman Coulter Life Sciences fluorescence test methods provide equivalent volume delivery results at 25 nL and 250 nL

Beckman Coulter Life Sciences uses a fluorescence test method to analyze low nanoliter test volumes [1]. In-house laboratory services prepare the calibration curves and use controls to ensure acceptable accuracy and precision criteria are achieved. Beckman Coulter Life Sciences customers, such as those in the pharmaceutical industry, would like an alternative test method for verifying the performance of the ALH for their specific protocols at test conditions defined by their workflows and processes. An on-site test method will provide faster results enabling repair and optimization of the system with specific protocols and procedures. Artel by Advanced Instruments provides a complete system, the Artel MVS, that is robust and can be used on-site for liquid handler evaluation. A 384-well plate can be analyzed in under 10 minutes with the Artel MVS system. The dual-dye system also supports compliance to ISO 23783-2: Measurement procedures for the determination of volumetric performance [2]. This study was designed to evaluate whether the Artel MVS and Beckman Coulter Life Sciences fluorescence test methods provide equivalent volume delivery results at 25 nL and 250 nL on Echo acoustic liquid handler-525 and Echo acoustic liquid handler-655 for specific aqueous and DMSO solutions.

Materials and Methods

In this work, Beckman Coulter Life Sciences and Artel by Advanced Instruments collaborated to test an Echo acoustic liquid handler-525 and an Echo acoustic liquid handler-655 using the Beckman Coulter Life Sciences fluorescence method and the Artel MVS, which is a mobile system that can be used in any laboratory. Detailed method information for both methods can be found in the references. Briefly, Beckman Coulter Life Sciences fluids contain 0.15 mM fluorescein that is transferred into 384-well Greiner Bio-One 384-well µClear™ Bottom Polystyrene Microplates (Supplier: Greiner Bio-One 781096) and analyzed by laboratory personnel. The Artel MVS system is a commercial system that comes complete with aqueous or DMSO-based dye solutions, 384-well verification plates (black, optical-bottom microplates ARTEL MVS Part Number ARTEL MVS-245), shaker, an instrument with accompanying calibration plate, and software that is 21 CFR Part 11 compliance ready. Of note, while the Echo system can accurately transfer 70% and 85% DMSO solutions, and the Artel system can accurately measure dual dye fluids that are intentionally made at 70% or 85% DMSO, if one were to allow a dual dye fluid to hydrate from 100% DMSO to 70% DMSO the measured response would be 30% lower than nominally expected. **It should be noted that since Artel provides 100% DMSO solutions, care should be taken to prevent uncontrolled hydration.** Artel provides 100% DMSO solutions. Customized 70% and 85% DMSO solutions were manufactured to support this work. Table 1 provides an outline of the testing plan.

Table 1. Testing plan for Echo acoustic liquid handlers. Source plates were polypropylene unless otherwise indicated.

Echo acoustic liquid handler	Aqueous calibrations	DMSO calibrations	Target test volumes achieved with incremental dispenses
E-525 (25 nL increments)	384PP_AQ_BP	Not applicable as DMSO is not supported for E525.	25 nL and 250 nL
E-655 (2.5 nL increments)	384PP_AQ_GP3	384PP_DMSO2	25 nL and 250 nL

A comparison of the two method specifications and calculated measurement uncertainty for each test volume evaluated in this study is provided in Table 2 (ISO 23783-2: Measurement procedures for the determination of volumetric performance was used as a reference). A preliminary evaluation was performed to determine whether the performance of the Echo acoustic liquid handlers was adversely affected by the concentrations of Artel MVS test solutions compared to fluorescein test solutions. It was found that the Echo instrument's performance was independent across the concentrations of solutions evaluated in this work (data not shown). It was also determined that mixing is an important parameter, and destination plates should be mixed before analysis as recommended in the Artel MVS software.

Table 2. Specifications for Artel MVS method and Fluorescein [1] method in 384-well plates.

Method	ARTEL MVS	Fluorescein
Detection technique	Dual-dye absorbance spectroscopy	Single-dye fluorescence spectroscopy
Imprecision (% CV)	25 nL: 0.47% 250 nL: 0.44%	25 nL: 2% 250 nL: 2%
Uncertainty (% Inaccuracy)	25 nL: 3.64% 250 nL: 2.04%	25 nL: 5% 250 nL: 5%

As the purpose of this collaboration between Beckman Coulter Life Sciences and Artel by Advanced Instruments was to evaluate whether the Beckman Coulter Life Sciences fluorescence test method and the Artel MVS method provide equivalent volume results for Echo acoustic liquid handlers, the % differences in volumes delivered were determined by Equation 1. Considering the uncertainty (% inaccuracy) of each test method, the largest acceptable % difference at 25 nL would be 8.6% and at 250 nL would be 7.0%.

$$\%\ difference = \frac{(X1-X2)}{V} \qquad \textbf{Equation 1}$$

where V equals the average test volume, X1 is the volume determined by the fluorescein test method and X2 is the volume determined by the MVS.

Results and Discussion

In all tests, the % CV was less than 5.0% for 384-well plate reads, demonstrating acceptable precision of the Echo acoustic liquid handler systems with various solutions and plate types at both 25 nL and 250 nL (Figure 1). In addition, the test volumes were within specification when testing with either method for both 25 nL and 250 nL transfers (Figure 2). The % difference for 25 nL test volumes was less than 7.5% and for the 250 nL test volumes less than 6.3%, demonstrating agreement in the volumes determined by either method within the uncertainty of the respective methods.

Figure 1. Precision Fluorescein method and Artel MVS method for aqueous and DMSO liquids on Echo acoustic liquid handler-525 and Echo acoustic liquid handler-655. BP=buffer protein and GP=glycerol protein. 70, 85, and 100 refer to percent DMSO with water being the balance. Replicates (1, 2, 3) refer to the same test conducted on different Echo instrument fixtures. In the case of BP,525-1 repeat, the same Echo instrument was tested as BP,525-1.

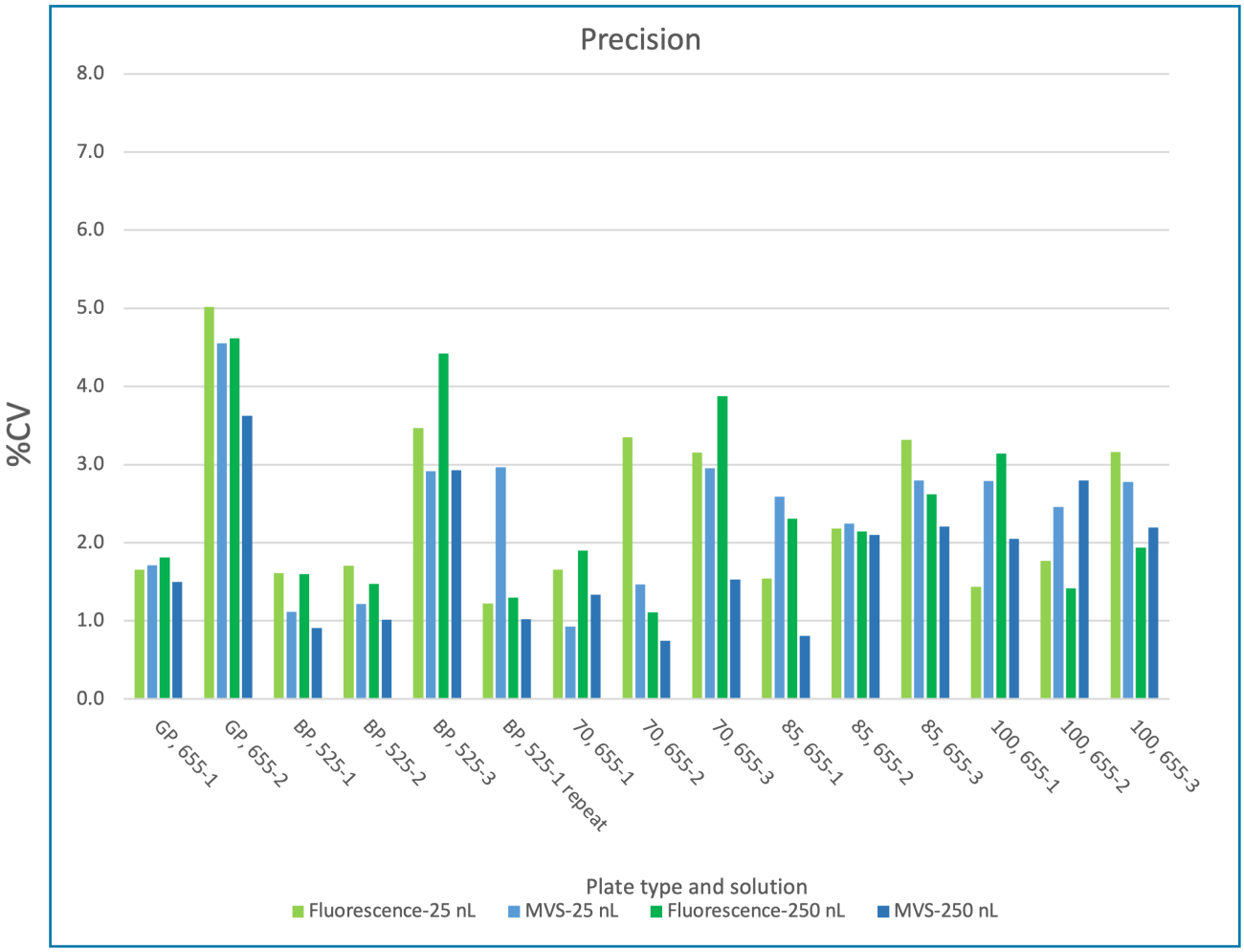
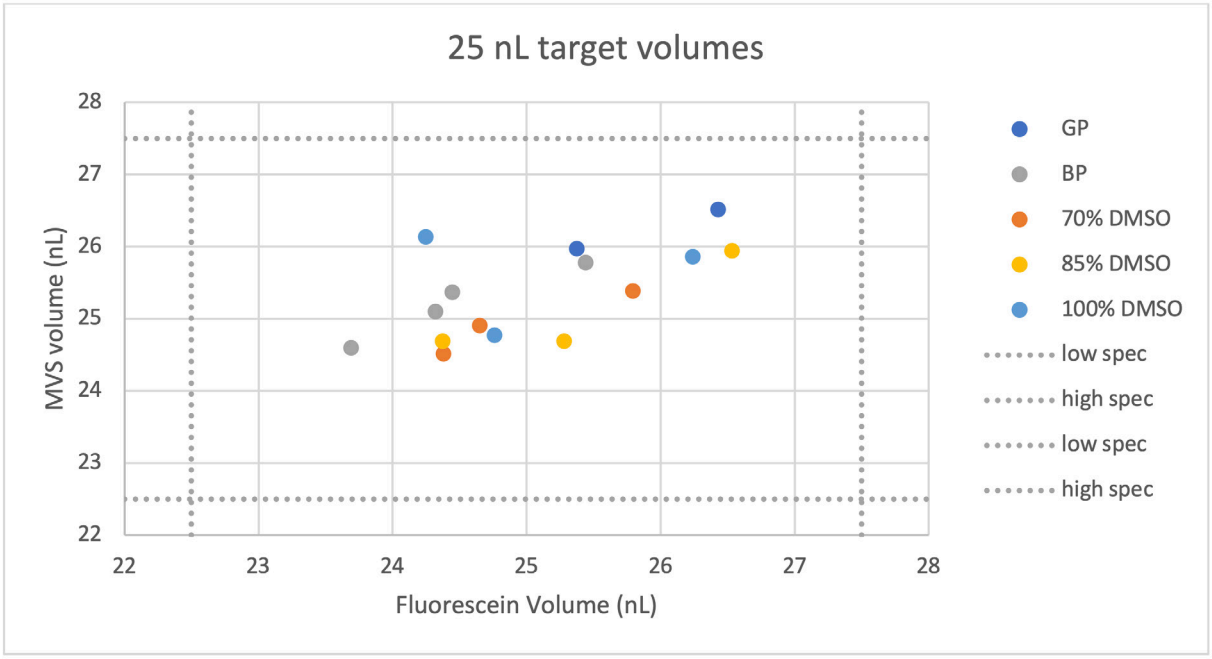
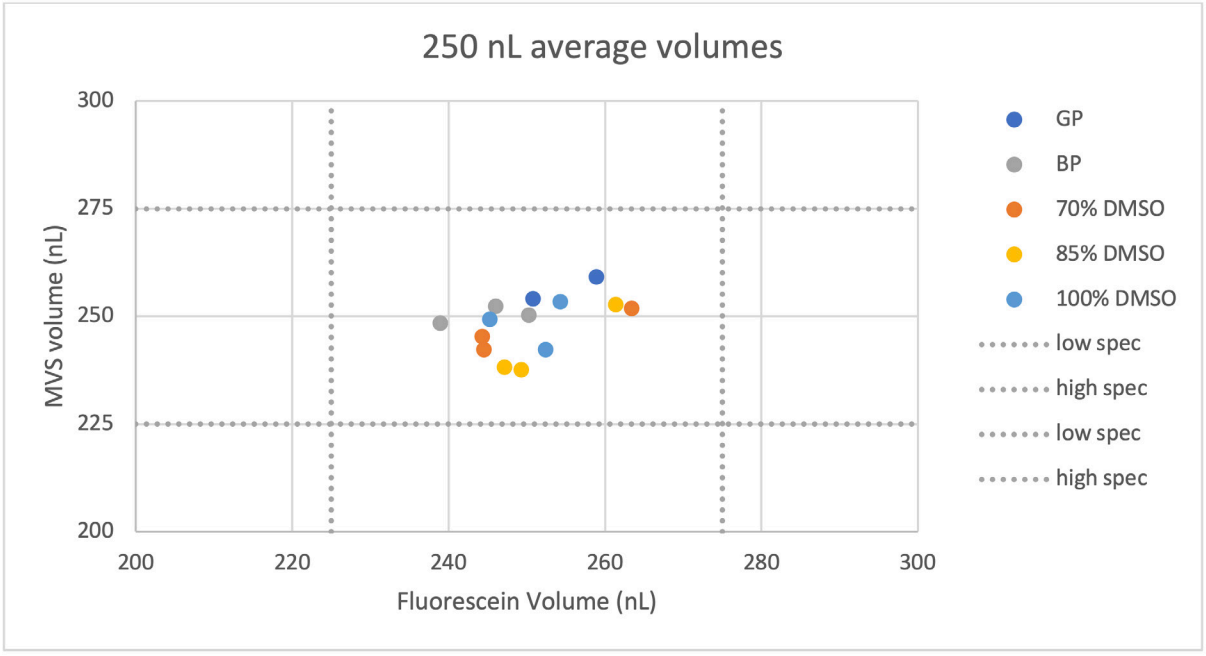


Figure 2. Accuracy of Fluorescein method and Artel MVS method for aqueous and DMSO liquids on Echo acoustic liquid handler-525 and Echo acoustic liquid handler-655. BP=buffer protein and GP=glycerol protein. 70, 80, and 100 refer to percent DMSO with water being the balance.

A. 25 nL



B. 250 nL



Conclusion

Echo acoustic liquid handler customers and engineers would like an alternative test method for validating nanoliter volume delivery The Artel MVS is a complete system for volume verification that is mobile, robust, and delivers results for a 384-well plate in under 10 minutes, enabling on-site repair and optimization of ALHs, reducing downtime and time for optimization. This work has demonstrated the equivalence of Artel MVS test method compared to the fluorescence test method employed by Beckman Coulter Life Sciences, for the selected plate types described [3]. (Note that certain Echo instrument calibrations are not compatible with the Artel MVS system, and must be evaluated by other methods, if necessary.) Thus, both test methods are acceptable for the evaluation of Echo acoustic liquid handler accuracy and precision for select calibrations. The Artel MVS system also supports compliance to ISO 23783-2: Measurement procedures for the determination of volumetric performance. Contact Artel by Advanced Instruments for more information on the Artel MVS technology.

References

1. Harris, D. et al. Debunking the Myth: Validation of Fluorescein for Testing the Precision of Nanoliter Dispensing. 2006 by The Association for Laboratory Automation. doi:10.1016/j.jala.2006.05.006.

2. ISO 23783-2: Measurement procedures for the determination of volumetric performance.

3. The concordance only applies to the Beckman 384PP source plate

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