Verifying the accuracy and precision of liquid handlers is essential for assay reliability

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Introduction

Assay-ready plates are becoming key to maximizing throughput and assay robustness for many HTS departments. These plates contain test compounds prepared at specific concentrations from library stocks ahead of time. DMSO is routinely used for compound dissolution, but its presence at concentrations above 1% can markedly affect assay performance. Thus, accurate volume transfer of nanoliter quantities of DMSO-based stock solution are required to keep concentrations low when preparing assay-ready plates.

Sub-microliter liquid handling is technically challenging and requires accurate verification to within a few nanoliters. Sub-microliter dispensing needs have led many laboratories to automate their processes. With the pipetting of such low volumes, liquid handling accuracy and precision can impact assay variability significantly.

SPT Labtech's mosquito® uses true positive displacement, disposable tips to aspirate and dispense volumes down to 50 nL. In this study, the Artel MVS® was used to verify mosquito's precision and accuracy across a range of aqueous and DMSO target volumes for both wet and dry dispensing.

In this poster we will present data using the Artel MVS® to validate and optimize the use of our liquid handling products: mosquito LV, mosquito HV and dragonfly® crystal. The Artel system used has helped with understanding the reliability and reproducibility of our liquid handlers and has ensured standardization of the product portfolio.

1. mosquito LV and HV



Table 1: Specifications of mosquito LV and HV.

	LV	HV
volume range	25 nL – 1.2 µL	500 nL – 5 µL
plate capacity	2 or 5	
column format	8 or 16	
throughput	2 min / 96 well plate 3 min / 384 plate copy 5 min / 4 x 384 plate stamp out	

2. Artel MVS system



Image courtesy of Artel, Westbrook, Maine, USA

The Artel MVS reliably verifies the accuracy and precision performance of automated liquid handlers in minutes. The system supports an unbroken chain of traceability to national and international standards.

The dual-dye ratiometric photometry method employed utilizes two dyes with distinct absorbance maxima at 520 nm (red) and 730 nm (blue). The concentration of blue dye is constant in all the sample solutions across the volume range and is equal to that of the diluent buffer. The blue dye is therefore used as an internal standard to calculate solution depth in each well. The automated liquid handler to be verified dispenses Artel sample solution and the diluent is added independently into a microplate. The absorbance of both wavelengths is measured for every well.

3. mosquito verifcation

Table 2: mosquito LV dry dispensation.

target volume	actual volume	relative inaccuracy	precision (%CV)
50 nL	49.4 nL	-1.3%	7.5%
100 nL	96.2 nL	-3.7%	4.0%
600 nL	631 nL	5.1%	0.9%
1,200 nL	1250 nL	4.4%	0.9%

Table 3: mosquito LV wet dispensation.

target volume	actual volume	relative inaccuracy	precision (%CV)
50 nL	51.4 nL	0.8%	6.1%
100 nL	101 nL	1.0%	7.6%
600 nL	587 nL	-2.1%	0.9%
1,200 nL	1170 nL	-2.5%	0.6%

Table 4: mosquito HV dry dispensation.

target volume	actual volume	relative inaccuracy	precision (%CV)
0.5 µL	0.45 µL	-9.3%	7.8%
1 µL	0.971 µL	-2.9%	4.0%
2 µL	1.99 µL	-0.5%	2.5%
4 µL	4.04 µL	1.0%	0.7%

Table 5: mosquito HV wet dispensation.

target volume	actual volume	relative inaccuracy	precision (%CV)
0.5 µL	0.54 µL	8.0%	2.3%
1 µL	1.01 µL	0.8%	1.4%
2 µL	1.97 µL	-1.5%	0.6%
4 µL	3.95 µL	-1.3%	0.6%

4. dragonfly crystal: digital liquid handler



dragonfly crystal provides automated non-contact, positive displacement dispensing enabling the rapid generation of microplates containing multiple reagents. With a minimum dispense volume of 0.5 µL and resolution of 0.1 µL, dragonfly crystal offers digital microliter dispensing for automation of a wide range of high throughput chemical and biological screening assays.

Assay optimization entails the study of multiple parameters to maximize screening performance. As a result, the determination of the optimal conditions can be a costly, time consuming process requiring the set-up of multiple studies to analyze each individual parameter. Often, such studies are limited by manual pipetting when performed in microplates.

Table 6: dragonfly crystal dry dispensation.

target volume	actual volume	relative inaccuracy	precision (%CV)
5.4 µL	5.31 µL	-1.7%	1.3%
13 µL	13.1 µL	0.5%	5.2%
20.8 µL	20.4 µL	-1.7%	0.44%
32.4 µL	31.7 µL	-2.1%	2.1%
40.1 µL	39.0 µL	-2.7%	0.93%
50 µL	48.8 µL	-2.4%	0.95%

Conclusion

The accuracy and precision performance of mosquito LV, mosquito HV and dragonfly crystal using DMSO were all shown to be within expected specification. mosquito products gave accuracies better than 4% and precision better than 7% across the volume ranges. dragonfly crystal delivered accuracy and precision of better than 3%.

Utilization of the Artel MVS for performance verification can prevent problems, bolster laboratory productivity, and provide confidence in the integrity of data.