

introduction

Assay-ready plates are becoming key to maximising 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 nanolitre quantities of DMSO-based stock solution are required to keep concentrations low when preparing assay-ready plates.

Sub-microlitre liquid handling is technically challenging and requires accurate verification to within a few nanolitres. Sub-microlitre 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. TTP Labtech's mosquito[®] uses 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 optimise the use of our liquid handling products: mosquito[®] HTS, mosquito[®] HV and dragonfly[®]. The Artel system used has helped with understanding the reliability and reproducibility of our liquid handlers and has ensured standardisation of the product portfolio.

1. mosquito HTS and HV



Table 1: Specifications of the mosquito HTS and HV.

	HTS	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 utilises 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 verification



Table 2: mosquito HTS 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 HTS 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 wet 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: digital liquid handler



dragonfly 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 offers digital microlitre dispensing for automation of a wide range of high throughput chemical and biological screening assays.

Assay optimisation entails the study of multiple parameters to maximise 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 analyse each individual parameter. Often, such studies are limited by manual pipetting when performed in microplates.

Table 6: dragonfly 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, mosquito HV and dragonfly 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 delivered accuracy and precision of better than 3%.

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

