Verifying mosquito® nanoliter liquid dispensing using the Artel MVS®

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Abstract
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, which is not easily achieved using traditional approaches. mosquito uses positive displacement, disposable tips to aspirate and dispense volumes down to 50 nL. 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.

Introduction
For the purposes of this study the accuracy and precision performance of a mosquito liquid handler was evaluated using the Artel MVS. Aqueous MVS sample solutions were dispensed using mosquito into dry 384-well microplates. DMSO-based sample solutions were also dispensed by mosquito. These solutions were prepared using MVS stock (dye) solution in combination with DMSO solvent. MVS diluent or buffer was then dispensed in the wells using an independent bulk dispenser. Per the MVS, the measured absorbance values were used to calculate well-by-well volume values.

Conclusions
- mosquito performs better than its claimed specifications at virtually all volumes measured for both aqueous and DMSO-based sample dispensing.
- MVS is quick and easy to use for measuring the accuracy and precision performance of mosquito, allowing all presented data to be collected in approximately one day.
- Although accuracy performance of mosquito is not specified by the manufacturer, the performance measured by the MVS indicates inaccuracy of less than 5% when dispensing both aqueous and DMSO-based samples over the volume range tested without any adjustments or corrections.
- mosquito can reliably dispense different liquid types with equivalent precision performance.
- mosquito tips may be used multiple times without introducing variability.

1 Artel MVS®

Figure 1. Artel MVS

The Artel MVS® reliably verifies the accuracy and precision performance of automated liquid handlers in minutes.

The dual-dye ratiometric photometry method employed by the MVS utilizes two dyes with distinct absorbance maxima at 520nm (red) and 730nm (blue). Six sample solutions containing different concentrations of the red dye are used for testing the performance of instruments dispensing into 96- and 384-well plates over and entire volume range of 10 nL to 350 µL. The concentration of blue dye is constant in all sample solutions across the volume ranges and is equal to that of a diluent buffer. The blue dye is therefore used as an internal standard to calculate solution depth in each well.

An automated liquid handler is used to dispense sample solution and diluent into the wells of a microtiter plate, and the absorbance at both wavelengths is measured for every well. By applying the Beer-Lambert law, the MVS uses absorbance values and automatically calculates both the precision and accuracy of the volume delivered by each pipetting channel of the automated liquid handler.

2 mosquito® Instrument

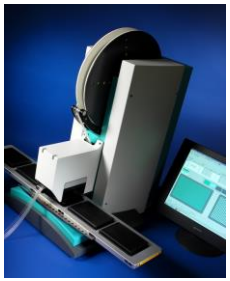

Figure 2. TTP LabTech mosquito

mosquito® is a low volume liquid handling instrument combining a low-cost disposable tip system with a positive displacement pipette to ensure zero cross-contamination.

mosquito is capable of pipetting volumes from 1.2µL down to 50nL with no washing required.

3 Accuracy and Precision Results

The accuracy and precision performance of mosquito was measured using the MVS using both DMSO- and aqueous-based sample solutions with minimal adjustment to optimize performance. The precision results were compared to published performance specifications for mosquito.

Results continued:

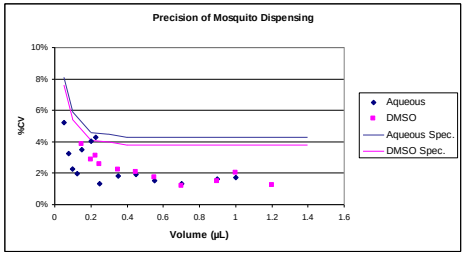

Figure 3. Precision performance of mosquito

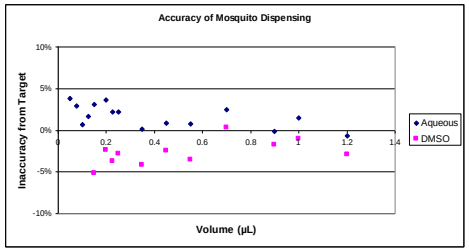

Figure 4. Accuracy performance of mosquito

mosquito's measured precision performance when dispensing aqueous and DMSO solutions into a dry 384-well plates is shown in **Figure 3**. For DMSO, unique tips were employed for each volume transfer. For aqueous solutions, unique tips were only used when the target volume was changed (for replicate dispenses of a specific target volume, the tips were re-used). The data collected show excellent comparability in precision performance for both the DMSO and aqueous sample solutions. mosquito performed within specification for all volumes measured, and in most cases, the precision performance was much improved compared to published specifications.

mosquito accuracy performance, as measured with the MVS, is shown in **Figure 4**. Using both aqueous and DMSO sample solutions, inaccuracy of mosquito dispensing is less than 5%. Agreement between the two sample types may be achieved by programming an offset into the liquid handler (not shown).

4 Tip-to-tip Variability for Aqueous Transfers

The aliquots transferred during this performance evaluation were dispensed from a group of 16 individual tips with 6 replicate dispenses (without changing the tips). The variability between the tips, per dispense, was measured by calculating the standard deviation of the volumes measured by the MVS for each tip. **Figure 5** shows that, although mosquito tips are disposable, the performance of the tip shows little change over multiple uses. Data collected for this presentation was limited to 6 replicates with the same tips.

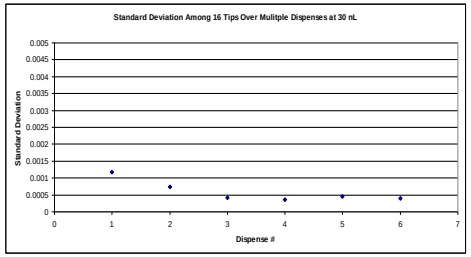

Figure 5. Standard deviation among 16 tips over multiple uses

5 Linearity

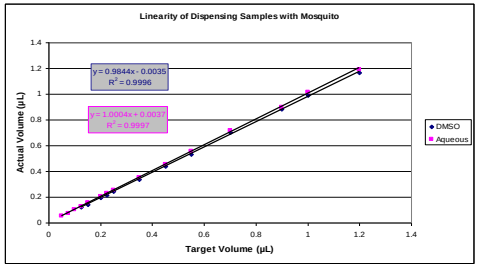

Figure 6. Volume Transfer Linearity of mosquito

Data collected shows a high level of correlation between the actual volumes dispensed and the expected volumes for the measured range when dispensing DMSO and aqueous sample solutions thereby demonstrating consistent performance of mosquito with these solvents (**Figure 6**).