ARTEL
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Accuracy and Precision in Low Volume Liquid Transfers

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EDC BIOSYSTEMS™

Introduction

Acoustic transfer systems operate by ejecting a rapid fire stream of tiny discrete droplets, with each droplet adding a few nanoliters to the total aliquot. This makes the system digital, in the sense that volumes can be changed by adjusting the number of droplets.

To perform accurately, the acoustically ejected droplets must be of uniform size, and free from dispense order bias. In other words, the first droplet in the series should be the same volume as all subsequent droplets. The assumption of constant droplet size is tested first in this study.

After confirming the absence of dispense order bias, it becomes possible to use the ATS-100 to test the sensitivity limits of the Artel MVS® and verify measurement system linearity in the range of 10 nL to 1000 nL. This test spans the full range covered by Artel claims of traceability to NIST for volume measurements made with DMSO or Aqueous solutions of Range E sample solutions.

Single droplets of 1 nL, 2 nL , 5 nL and 10 nL were also studied. These volumes are within the working range of the ATS-100, but extend below the 10 nL lower limit of current Artel performance claims. Extending NIST traceability to volume measurements in the single nanoliter domain is an active challenge.

Equipment and Methods

The liquid transfer system was an Acoustic Transfer System, ATS-100, from EDC Biosystems. This system can measure fluid properties[1], and adjust acoustic parameters such as energy and focal distance to change and control droplet size. Droplets from 1 nL to 10 nL size were studied.

Liquid volume measurements were made using the Artel MVS Multichannel Verification System[2] which was configured to test both aqueous and DMSO-based solutions with NIST-traceable results at volumes of 10 nL and greater. Fluorescein aqueous dyes were also used to test samples in the range of 1 to 10 nL. A study comparing the cost and convenience of these two methods has recently been published[3].

Acoustic transducer geometry is shown in Figure 1.

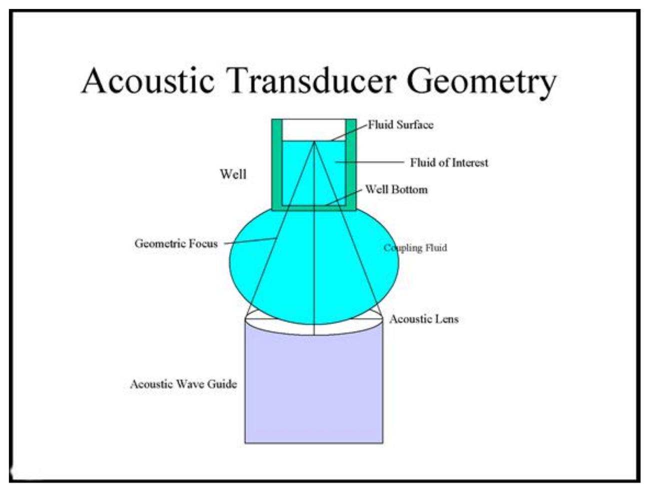

Figure 1: Acoustic energy is focused on the fluid surface to eject a droplet.

Acknowledgements

Equipment, consumable supplies and travel expenses were provided by Artel and EDC Biosystems. This work was performed at EDC Biosystems facilities in Fremont, California.

Results

Aqueous solutions of fluorescein and ponceau S dyes were transferred by the ATS-100 and measured by fluorescence and absorbance respectively. DMSO solutions containing ponceau S dye were also tested.

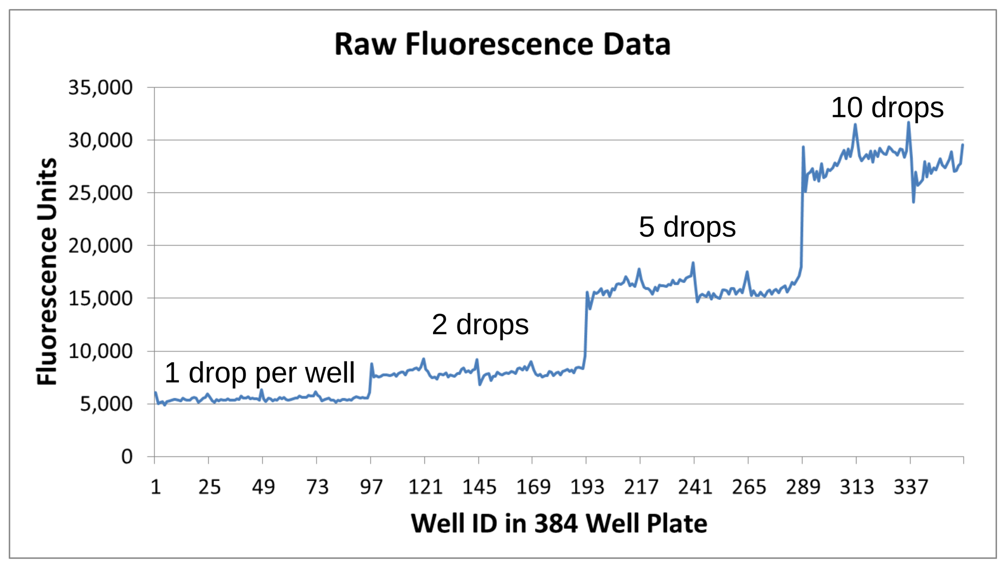

Figure 2: Raw fluorescence data are presented in sparkline format. ATS-100 droplet size was set to 1 nL and counts were varied from 1 to 10 drops per well. Some edge effect patterning was seen and is attributed to the fluorescence measurement method.

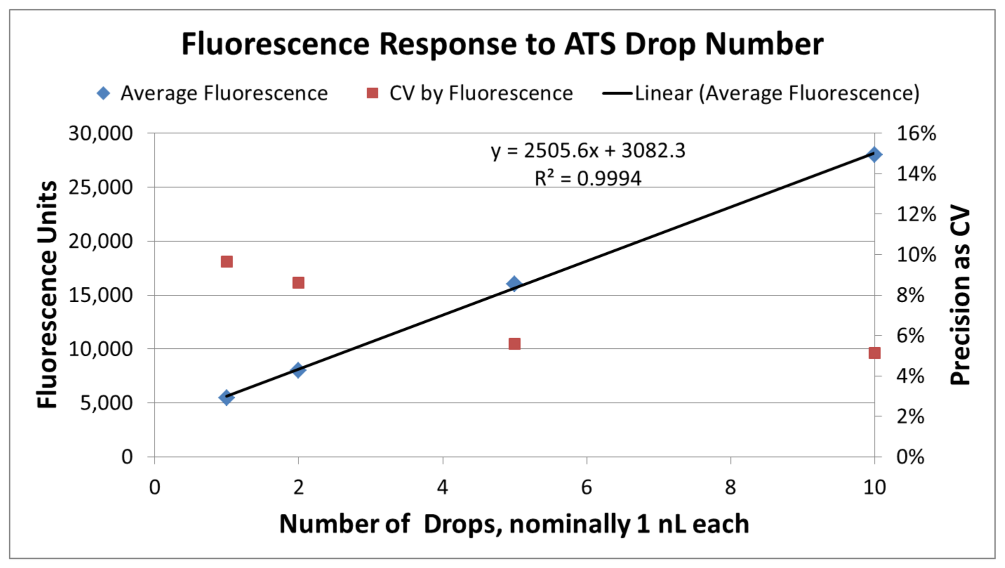

Figure 3: The average fluorescence response was linear over the range of 1 to 10 nL and show that ATS drop size is consistent and not dependent on dispense order. Precision data are plotted on the secondary axis.

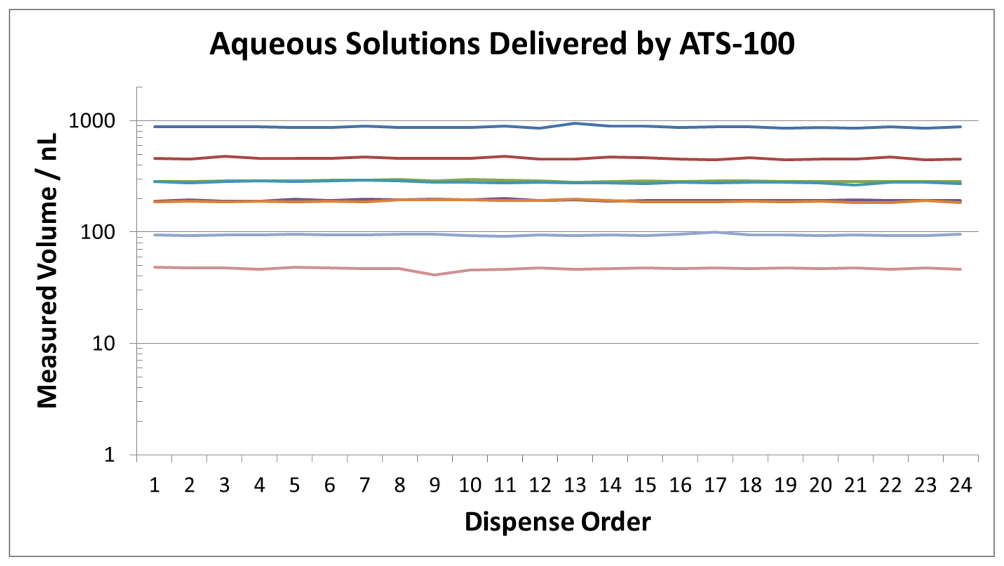

Figure 4: Aqueous MVS measurement results for each well are displayed in sparkline format. Data collected at volume settings from 50 nL to 1000 nL are displayed in this figure.

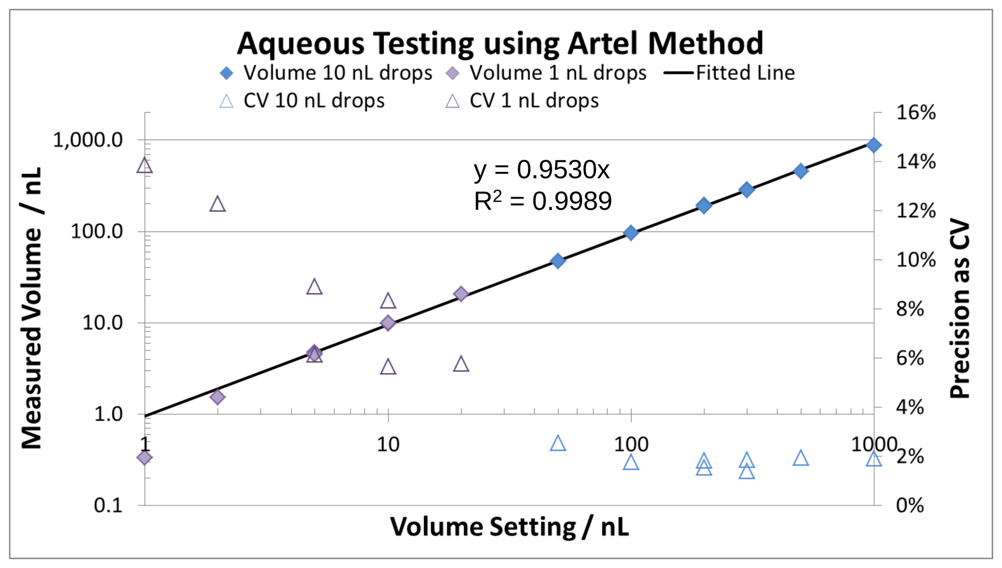

Figure 5: The MVS method delivers traceable results at 10 nL and above that the two systems (MVS and ATS-100) exhibited good co-linearity over the range of 10 nL to 1000 nL. ATS-100 data with droplet sizes of 1 nL and 10 nL are included in this figure.

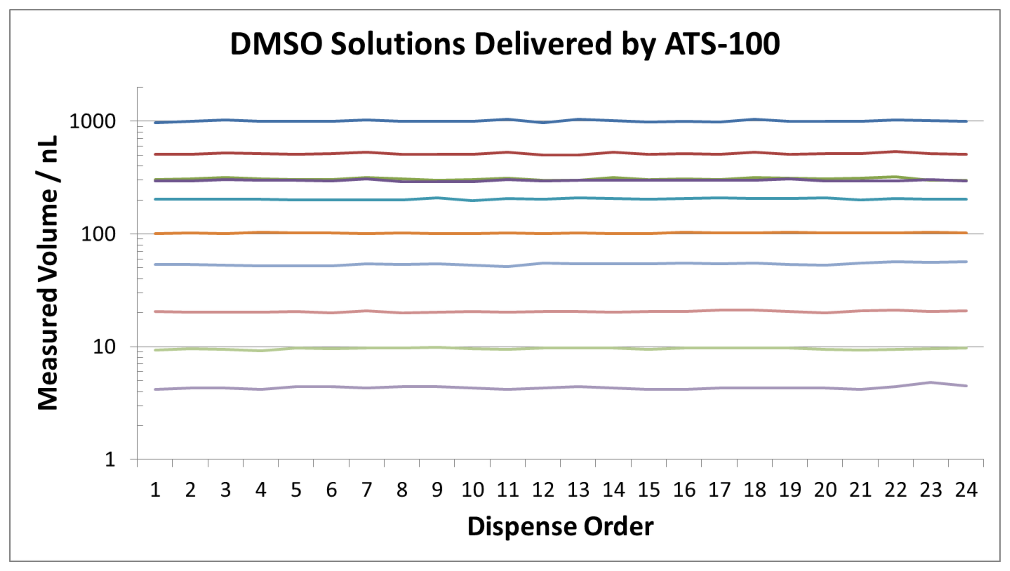

Figure 6: DMSO based MVS measurement results for each well are displayed in sparkline format. Data collected at volume settings from 5 nL to 1000 nL are shown in this figure.

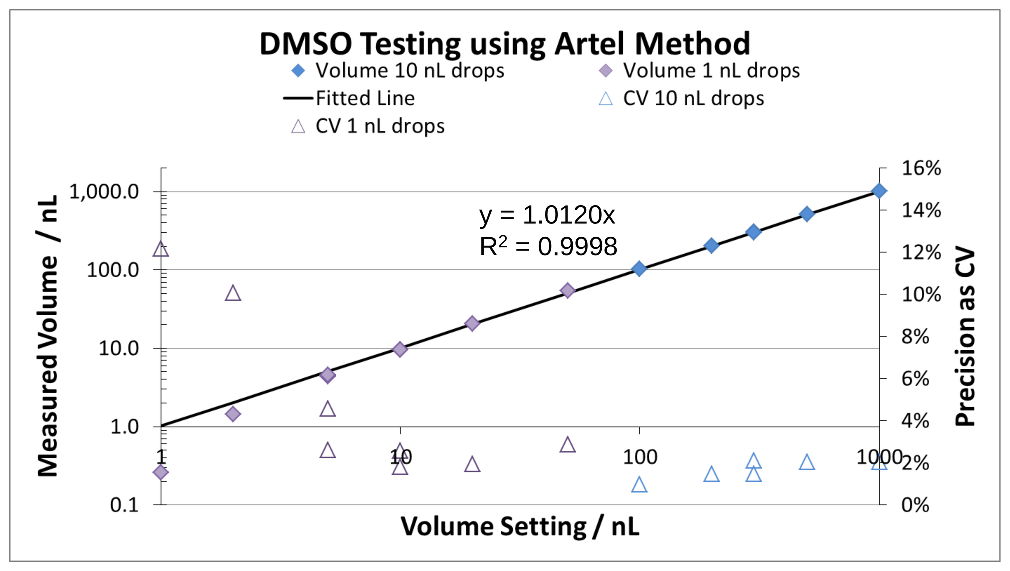

Figure 7: Accuracy and precision were confirmed to be excellent over the range of 10 to 1000 nL. At 1 nL and 2 nL the measurements show a departure from linearity which is attributed to the small absorbance signal obtained when using the Artel method far below the lower specified limit of 10 nL.

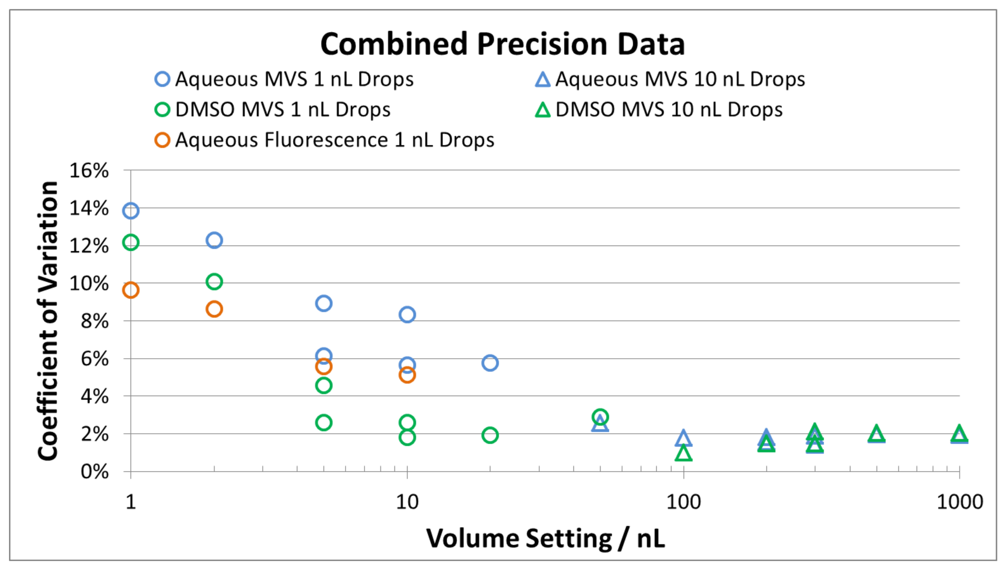

Figure 8 is a compilation of all CV results so that a visual comparison can be made among across the range of volumes, droplet sizes and solutions tested.

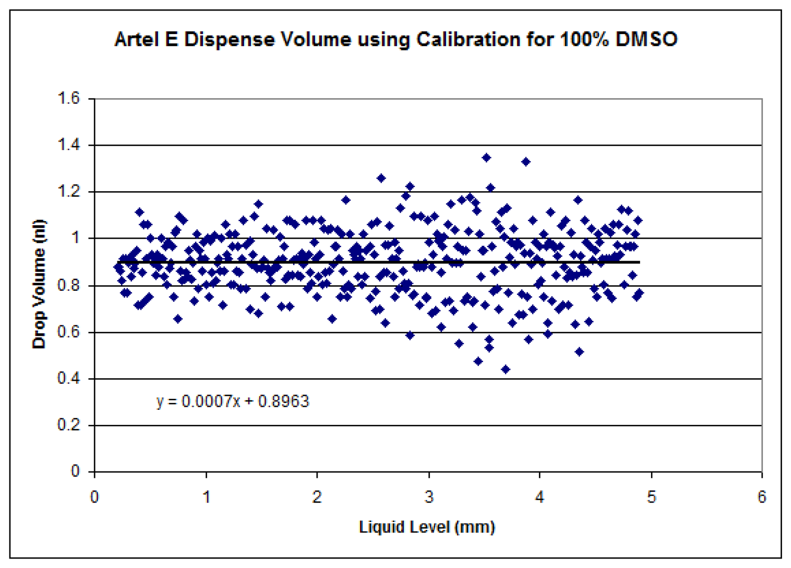

Figure 9 is data showing that the accuracy of the ATS-100 is insensitive to liquid height in the plate from which the droplet ejection occurs.

Conclusions

The ATS-100 Acoustic Transfer System is free from bias based on droplet run order. This result is clearly demonstrated by the linearity of the fluorescein data (Figure 3) which shows that the first droplet ejected is the same size as subsequent droplets within a brief series.

It was also found that when properly tuned, the ATS-100 droplet size is independent of the liquid height in the source plate (Figure 9).

The Artel MVS was capable of making accurate volume measurements down to the claimed limit of 10 nL, and perhaps as low as 5 nL (Figures 5 and 7).

At the smallest test settings of 1 nL and 2 nL the absorbance signal is small and difficult to separate from background leading to non-linear results at these smallest volumes.

Using the MVS absorbance method it was possible to show that the ATS-100 dispenses DMSO with excellent precision; typical CV results were in the range of 1 % to 3 % for volumes of 10 nL or larger (Figure 8).

Literature Cited

1. Forbush, et. al., Noninvasive Fluid Property Measurements Using Acoustic Methods, J. Laboratory Automation, 2006, 11(4) 188-194.
2. Bradshaw, et. al., Multichannel Verification System (MVS): A Dual-Dye Ratiometric Photometry System for Performance Verification of Multichannel Liquid Delivery Devices, J. Laboratory Automation, 2005, 10(1) 35-42.
3. Quintero, et. al., High-Throughput Quality Control of DMSO Acoustic Dispensing Using Photometric Dye Methods, J. Laboratory Automation, 2013, 18(4) 296-305.

Future Work

A full technical paper providing greater detail on this study is in preparation and intended to be submitted to JALA.

Further Information

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