Nanoliter Volume Pin Tool Transfers as Measured by a Dual-Dye Absorbance Method

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Abstract

This poster compares the volumes of sample solutions delivered by an array of stainless steel and hydrophobic-coated pins as measured by a fluorescent-dye approach versus a dual-dye absorbance approach. The V&P Pin Tool array was used to deliver aliquots of both aqueous-based and DMSO-based FITC sample solution into test plates under controlled experimental conditions. The same testing was conducted using the dual-dye absorbance approach implemented by the ARTEL Multichannel Verification System (MVS). Each measurement method was tested side-by-side on a series of pins capable of transferring from 20 to 315 nl.

The effects of solvent composition, dye concentration, and source plate volume on actual delivered volume were compared for each system. The MVS based assays and fluorescence-based assays resulted in good general agreement. Areas of divergent results appear to be due to dye concentrations effects on the liquid surface tension or solvent/dye composition. Theories explaining these results are discussed.

Introduction

A common trend observed in life science testing over the past several years has been the ever shrinking test volume. Increasing costs of chemical compounds and commonly used solvents has pushed high throughput screening labs towards lower working volumes, specifically in the nanoliter range. Another factor driving current testing in compound screening and drug discovery labs to the nanoliter volume range is the desire to directly dispense from the source plates versus serially diluted test plates, thereby avoiding compounding dilution errors and reducing consumable costs. At test volumes in the nanoliter range, concentrations of chemical compounds are often kept very high and used essentially undiluted. Nearly all of these types of assays are dosage-dependent, thus knowing the exact volume transferred is critical for data interpretation and confidence in the final assay results. Hence, the ability to controllably dispense “known” nanoliter aliquots of samples is desired, which can readily be achieved using Pin Tools.

Pin Tools

Pin Tools pick up and transfer liquids using a combination of the liquid’s surface tension, the physical and chemical properties of the pin’s surface, and capillary action of slots or other features in the pin. This technology has been developed and used for over a decade to controllably dispense volumes in the nanoliter range, which has resulted in wide adoption of Pin Tools for low volume assays such as high-throughput screens, dose-response curves, ADME-Tox, and specificity assays. Volume delivery performance for Pin Tools has traditionally been measured using fluorescence spectroscopy based methods, mainly due to the sensitivity of common fluorescent dyes (e.g., FITC). By comparison, traditional absorbance spectroscopy has not been sensitive enough to detect the low volumes transferred by Pin Tools. However, most recently a dual-dye absorbance spectroscopy approach has been developed for measurement of volume delivery extending into the nanoliter volume range. This poster explores Pin Tool performance as measured by both fluorescence and dual-dye absorbance.

Experimental

A series of pin tools, defined in Table 1, was set into a 16 X 17 array (i.e., to deliver into a 384-well plate). The pins of each type were set into a single column of 16 individual pins. The entire array was used to transfer sample solutions using the following method: 1) the pin array was loaded with sample solution by dipping 3 times into the source plate, 2) the loaded sample solution was then delivered by dipping 6 times into the destination plate which had been pre-filled with diluent or buffer, 3) the pin array was cleaned by successive dipping into water/DMSO, then water, then isopropanol, with blotting of the tips onto an absorbent paper after each solution. Testing was conducted using three different volumes of sample solution in the source plate (25, 50, 75 µL). Sample solutions included standard aqueous MVS solution (MVS Range E), a DMSO-based MVS solution, an aqueous 0.5 mg/ml FITC solution, and a DMSO 0.5 mg/ml FITC solution. Three plates were delivered for each solution type at each source volume.

Results

Figure 1: DMSO Based Solutions

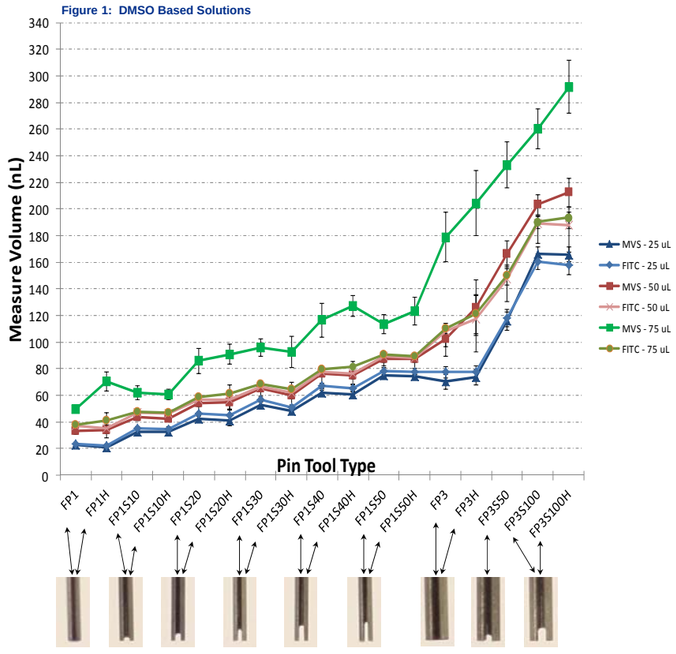


Table 1: Pin Array

Pin Type	Description
FP1	Solid Pin, 0.457mm Diameter
FP1H	Solid Pin, 0.457mm Diameter, Hydrophobic-Coated
FP1S10	10nl Slot Pin, 0.457mm Diameter
FP1S10H	10nl Slot Pin, 0.457mm Diameter, Hydrophobic-Coated
FPS20	20nl Slot Pin, 0.457mm Diameter
FP1S20H	20nl Slot Pin, 0.457mm Diameter, Hydrophobic-Coated
FP1S30	30nl Slot Pin, 0.457mm Diameter
FP1S30H	30nl Slot Pin, 0.457mm Diameter, Hydrophobic-Coated
FP1S40	40nl Slot Pin, 0.457mm Diameter
FP1S40H	40nl Slot Pin, 0.457mm Diameter, Hydrophobic-Coated
FP1S50	50nl Slot Pin, 0.457mm Diameter
FP1S50H	50nl Slot Pin, 0.457mm Diameter, Hydrophobic-Coated
FP3	Solid Pin, 0.787mm Diameter
FP3H	Solid Pin, 0.787mm Diameter, Hydrophobic-Coated
FP3S50	50nl Slot Pin, 0.787mm Diameter
FP3S100	100nl Slot Pin, 0.787mm Diameter
FP3S100H	100nl Slot Pin, 0.787mm Diameter, Hydrophobic-Coated

Figure 5: FP1 Solid pin w/ and w/o sample

Figure 6: FP1S40 Slot pin w/ and w/o sample

Figure 2: Aqueous Based Solutions – 25 µL Source Volume

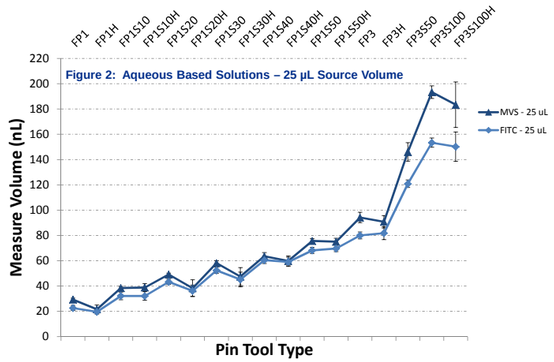


Figure 3: Aqueous Based Solutions – 50 µL Source Volume

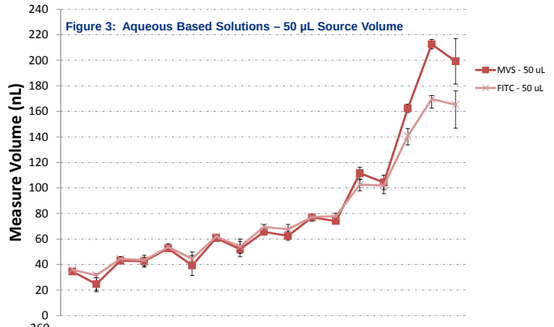
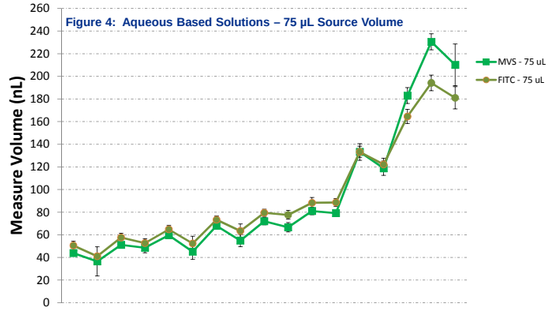


Figure 4: Aqueous Based Solutions – 75 µL Source Volume



Discussion

Figures 1-4 display the volumes delivered by each of the pin types described in Table 1. Each data point consists of data averaged over 3 recipient plates with 16 pins of each pin type, for a total of 48 replicates for each pin type. Images of each pin type can be found below Figure 1, with a description of each in Table 1. Both the DMSO and aqueous data show close agreement between Artel’s MVS method and the FITC method, except for the 75 µL DMSO source plate data (see below).

As expected, a larger source plate volume resulted in a larger transfer volume due to a higher immersion liquid level on the pins (e.g., the pins were immersed “deeper” into solution, which in effect exposed more pin surface area to the sample). This effect is more dramatic on a solid pin because the liquid on the side of the pin runs down to the hanging drop on the pin tip, and the external surface is the only mode of liquid transfer. While still present, this effect is not as significant on slot pins because the majority of transferred liquid is inside the slot itself, rendering the liquid on the pin surface less significant by comparison.

The small differences between the FITC and MVS method can be explained by the different surface tensions of the liquids. Because surface tension is critical to the exact transfer volume, liquids with different surface tension will produce different transfer volumes. This can be observed in the differences measured between the DMSO and aqueous transfer volumes in both methods. The same concept can be applied towards the differences between both the DMSO and aqueous FITC versus the MVS dyes. The aqueous data from the FITC and MVS are more agreeable than the DMSO most likely due to a more similar surface tension (between the aqueous solutions). The 75 µL DMSO MVS data is likely an anomaly due to insufficient mixing of transferred sample in the recipient plate. The standard MVS method was designed to mix 55 µL in the recipient plate. Previous mixing tests suggest the cause of the larger apparent volumes and CVs is incomplete mixing. Further study is needed using optimized mixing.

Conclusion

The preferred method for measuring volume transfer at the nanoliter scale using Pin Tools has been with a sensitive detection fluorescent method, such as FITC, in order to obtain accurate and precise results. Artel’s MVS method allows for measurement of very low volumes and, as shown herein, compares well with a fluorescent method. The MVS method can be used to determine the transfer volumes of Pin Tools just as accurately and precisely as a fluorescent methodology as long as surface tension is accounted for.

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