Application Note

Precision- and Accuracy-Based Validation of Pipet Tips Used on Automated Liquid Handling Platforms Following Multiple Cleanings with 'Cold' Atmospheric Plasma

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The life science industry has been developing and utilizing automated liquid handling systems for more than two decades. Originally used in clinical chemistry environments for assays of patient samples, automated systems are now commonly used in drug discovery environments to identify and validate genomic and proteomic targets and to develop corresponding therapies. Automated liquid handling (especially with the newer miniaturized microtiter plates, polypropylene pipets and stainless pins) allows lower per-well costs and the opportunity to run more assays and more screening campaigns.

Precision and Accuracy in Automated Liquid Handling

Regardless of the type of tip (polypropylene or stainless) used, reagents must be delivered with both accuracy and precision. Often thought of as being synonymous, "accuracy" and "precision" are not interchangeable terms and do, in fact, mean very different things.

- Pipetting *accuracy* describes the deviation among a group of volumes from that of a standard volume.
- Pipetting *precision* describes the closeness of a group of volumes regardless of a standard (Figure 1).

There are two commonly used methods (gravimetric and dye-based) for determining precision and accuracy for automated liquid handlers and their various pipetting tools. In general, *gravimetric methods* require (a) weighing an empty assay plate, (b) dispensing a volume of liquid per well and (c) taking a final weight. Although relatively simple, this process allows for identifying differences among *plates*, rather than on a well-to-well basis. Variances associated with a faulty syringe or plunger within an automated liquid handler or false weights due to high evaporation rates can skew overall results but not be individually identified. Consequently, this method is often reserved for single-channel pipetting devices.

Single *dye-based methods* can provide a numeric approximation on a *well-to-well basis* providing precision data only. However, the type of dye used and accompanying detection system may not provide the sensitivity required to identify significant differences in pipetting volume. Moreover, these practices do not determine accuracy and generally provide an approximation as to the volume aspirated or dispensed. In comparison, a dual-dye photometric approach allows the user to independently calculate precision and accuracy data using one dye as an internal standard and the second as a sample.

Herein we demonstrate the effects of 'cold' atmospheric plasma on pipetting precision and accuracy for polypropylene pipet tips and stainless steel slotted pins using the highly standardized, patented dual-dye, dual-wavelength MVS ratiometric absorbance method (ARTEL, Westbrook ME).

Plasma Cleaning of Pipet Tips

The TipCharger System by Cerionx™ is used to clean and sterilize pipet tips on automated liquid handlers. The TipCharger™ System is ideal for automated screening environments because it provides pipet tip cleaning equivalent to pipet tip replacement, while drastically reducing liquid waste, the number of tip boxes, associated tip costs and logistical bottlenecks associated with tip delivery and changing.

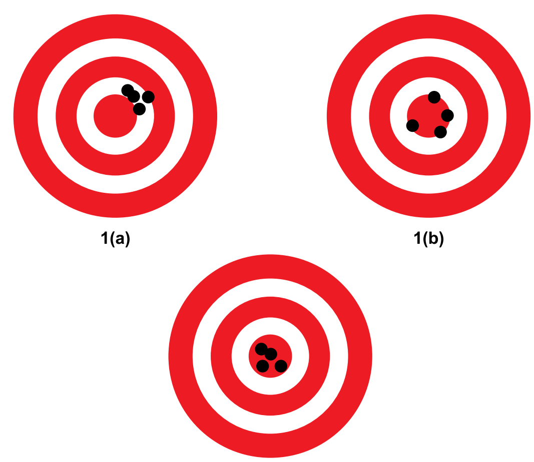

Figure 1. Visual Representation of Precision Versus Accuracy.
1 (a) represents precision; 1 (b) represents accuracy; and 1 (c) represents both precision and accuracy.

Experimental Factors for TipChager Validation

The TipCharger System is provided in 8, 96 and 384-well plate densities compliant with standard SBS footprints. TipCharger can be taught as either a device or consumable within the liquid handler software. In this study we used polypropylene tips specific to the Sciclone ALH 3000 (Caliper Life Sciences, Hopkinton MA), the TECAN EVO-MCA (The Tecan Group, Zurich SWITZERLAND), and the Biomek FX (Beckman Coulter, Fullerton CA) as well as 50nL pin tools mounted on a 96-well array (V&P Scientific, San Diego CA). The exterior of each pipetting tool is exposed to TipCharger-generated plasma, while contaminants inside polypropylene pipet tips are removed through a series of aspirate-and-dispense steps while tips are in the Cleaning Station.

ARTEL Dual-Dye Photometric MVS Verification Method

The Dual-Dye Ratiometric Photometry™ method employs two colorometric dyes with distinct absorbance maxima at 520nm (red) and 730nm (blue). MVS solutions include a series of sample solutions as well as a diluent and a baseline. Five sample solutions containing different concentrations of the red dye are used for testing the performance of instruments over different volume ranges from 10nL to 200µL. The concentration of blue dye is constant in all sample solutions across the volume ranges and is equal to that in the diluent. Therefore, the blue dye is used as an internal standard by which 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 determines both the precision and accuracy of the volume delivered by each pipetting channel of the automated liquid handler (Figure 2).

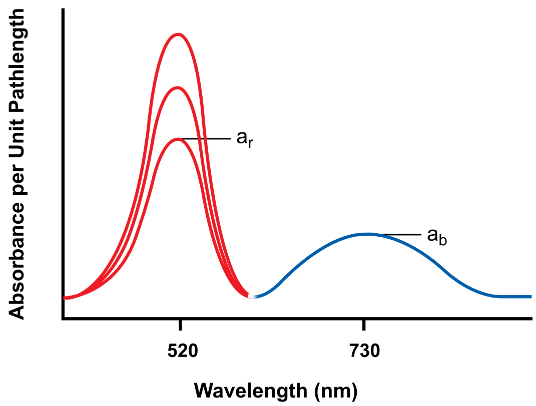

Figure 2. ARTEL Dual-Dye Photometric MVS Detection Method.
The Dual-Dye Ratiometric Photometry™ method employs two colorometric dyes with distinct absorbance maxima at 520nm (red dye, a_r) and 730nm (blue dye, a_b). Each sample solution contains two dyes. The red dye increases in concentration, while the blue dye concentration remains constant.

Table 1. ARTEL Dual-Dye Photometric MVS Verification Method	
Plasma Exposure for 96-well Caliper/Tecan Polypropylene Pipet Tips (5 µL)	
Component	**Volume Dispensed per Well**
MVS diluent	195 µL
MVS range C (2.0 mL – 9.9 mL)	5 µL
Plasma Exposure for 96-well Caliper/Tecan Polypropylene Pipet Tips (100 µL)	
Component	**Volume Dispensed per Well**
MVS diluent	100 µL
MVS range A (50 mL – 200 mL)	100 µL
Plasma Exposure for 384-well Beckman Polypropylene Pipet Tips (5 µL)	
Component	**Volume Dispensed per Well**
MVS diluent	50 µL
MVS range B (2.5 mL – 9.9 mL)	5 µL
Plasma Exposure for 384-well Beckman Polypropylene Pipet Tips (25 µL)	
Component	**Volume Dispensed per Well**
MVS diluent	30 µL
MVS range A (10 mL – 55 mL)	25 µL
Plasma Exposure for 96-well V&P Stainless Steel Slotted Pin Tools (50 nL)	
Component	**Volume Dispensed per Well**
MVS diluent	0.0 µL
MVS range D (50nL – 199 nL)	50 µL

Validating Precision and Accuracy Post-TipCharger Exposure

Polypropylene tips (both 96 and 384 formats) and stainless steel pins were exposed to TipCharger-based atmospheric plasma for a total of 30 seconds across a varying number of cycles. Post exposure, the same pipettors were then used to perform the appropriate ARTEL MVS volume verification (Table 1).

Results & Conclusion

The results demonstrate that TipCharger atmospheric plasma does not negatively impact either precision or accuracy for the various pipetting tools. In total, plasma exposure provided equivalent - *if not more accurate* - liquid handling for each of the systems described. Overall, the standard deviations and CVs for plasma treated polypropylene tips and pins were similar to that of untreated control sets across a wide range of plasma exposure cycles.

Caliper/Tecan 100µL Polypropylene Pipet Tips

Exposure to TipCharger-generated plasma generally *improved* pipetting accuracy and had little effect on CVs for Caliper and Tecan 96-well automated liquid handling pipet tips. The ARTEL MVS showed that wells receiving a programmed target volume of 5µL of Sample Solution C in reality received 4.3µL on average (CV = 3.8%). After a series of plasma exposures, tips aspirated and dispensed volumes more in line with the target volume, 4.7µL (CV = 7.6%). Similar results were observed relative to a 100µL target volume using the same type of pipet tip (Figure 3). Accuracy for this tip type was virtually unaffected throughout **200 cycles**. The slight increase in pipetting CVs was not considered to be significant when compared to the manufacturer specifications (~5% CV).

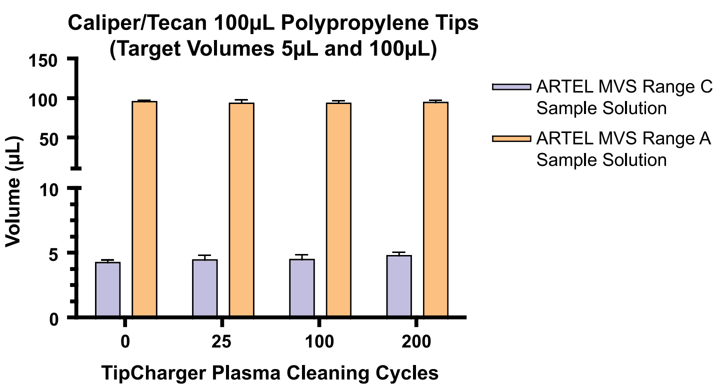

Figure 3. Precision and Accuracy for 96-well Polypropylene Tips. Results show that TipCharger plasma cleaning does not alter precision or accuracy for either high or low volumes through 200 plasma exposures, n=96. Bar graph was generated using GraphPad® Prism 4.0 software.

Beckman 25µL Polypropylene Pipet Tips

Similarly, exposure to TipCharger-generated plasma generally *improved* pipetting accuracy for low volumes versus untreated pipet tips with little effect on CVs for high volume applications using Beckman 384-well automated liquid handling pipet tips. The ARTEL MVS confirmed that control wells receiving a programmed target volume of 5µL of Sample Solution B in reality received 4.6µL on average (CV = 13.87%). After a series of plasma exposures, tips aspirated and dispensed volumes more in line with the target volume, 4.7µL, with improved precision (CV = 3.5%). Comparable results were observed with regard to a 25µL target volume using the same type of pipet tip. Untreated tips pipetted 24.6µL (CV = 2.3%) while plasma treated tips pipetted on average 24.5µL (CV = 2.4%) throughout **200 cycles** (Figure 4).

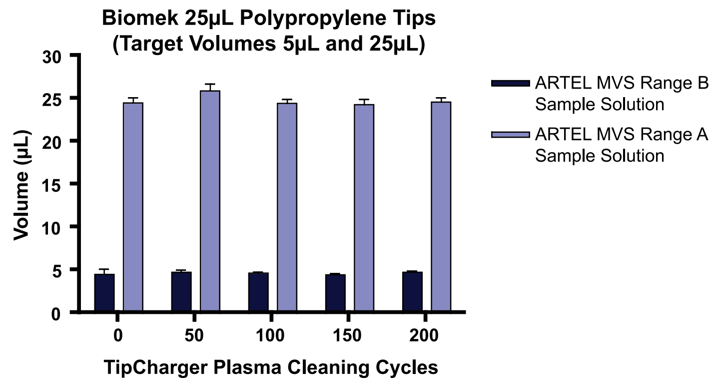

Figure 4. Precision and Accuracy for 384-well Polypropylene Tips. Results show that TipCharger plasma cleaning does not alter precision or accuracy for either high or low volumes through 200 plasma exposures, n=384. Bar graph was generated using GraphPad® Prism 4.0 software.

V&P Stainless Steel 50nL Slotted Pins

Nanoliter volumes dispensed for stainless steel slotted pins exposed to TipCharger-generated plasma were *similar* to those dispensed for untreated pin tools. The ARTEL MVS demonstrated that wells receiving a programmed target volume of 50nL of Sample Solution D in reality received 53nL on average (CV = 18%). This slight difference in volume was attributed to suboptimal liquid handling practices and acted as the overall control. After an extensive series of plasma exposures, pins dispensed volumes of 53.1nL (CV = 10%). Overall, both precision and accuracy for this tip type was unaffected throughout **30,000 cycles** (Figure 5) when compared to pin manufacturer specifications (7-8% CV).

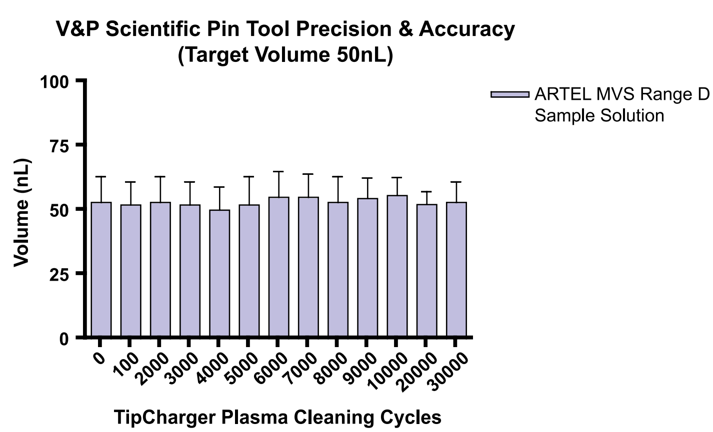

Figure 5. Precision and Accuracy for Stainless Steel Pins. Results show that TipCharger plasma cleaning does not alter precision or accuracy for 50nL volumes through 30,000 plasma exposures, n=384. Bar graph was generated using GraphPad® Prism 4.0 software.

The TipCharger™ System is protected by multiple patents and patents pending. The TipCharger mark and design, Cerionx mark and design, and TipCharger by Cerionx mark and design are trademarks owned by Cerionx, Inc. © 2006 All Rights Reserved.

Summary

Regardless of the widespread adoption of disposable pipet tips, fixed cannula and pin tools, their use creates significant budgetary and logistical challenges for screening environments. Bearing in mind that the precise transfer of reagents may be the single most crucial step in any assay, the design and application of pipettors can be crucial to best practice. The use of fixed tips and pin tools primarily allows for cost effective pipetting and assay miniaturization, but does require lengthy solvent-based wash procedures. Some avoid tip washing by using disposable polypropylene tips instead of fixed tips, but this can be cost-prohibitive and creates tremendous amounts of waste. Neither approach is perfect but each has been acknowledged as simply "a cost of doing business."

This data presents an additional option to screening environments. The data validates the premise that polypropylene tips can be repetitively cleaned with 30 second exposure to TipCharger-generated plasma and be reused at least 200 times prior to disposal with no detectable adverse effects on precision or accuracy of pipetting. It is important to note that because atmospheric plasma provides cleaning and sterilization by altering surface chemistries, the length of the cleaning cycle within the TipCharger Cleaning Station determines the lifespan of the pipet tip. Therefore, a 200 cycle mark does not necessarily represent an *upper* limit for reuse since most cleaning cycles are less than 30 seconds on average. Since plasma exposure does not affect surface chemistries on stainless steel, either metal cannula or pins can, most likely, be exposed indefinitely. Considering that tip cleaning with atmospheric plasma substitutes for either constant tip box changes after a one-time use, or a time consuming solvent-based wash protocol, automated environments can save on both direct and indirect costs associated with current practice.

Given that neither precision nor accuracy are affected throughout the plasma cleaning process, the ability to clean and reuse pipet tips, without negatively impacting transfer volumes, enables screening environments to increase throughput and further reduce the cost of screening campaigns. The implementation of novel technologies like the TipCharger System is critical to improving the bottom line of such environments. Given that life science screening environments continue to make every effort to reduce time-to-market for therapeutics, the TipCharger System may help play a role achieving this goal.