Validation of Both Precision-and Accuracy of Automated Liquid Handler Pipette Tips Cleaned with ‘Cold’ Atmospheric Plasma

Introduction

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 (using the newer miniaturized microtiter plates, polypropylene pipette tips and stainless pins) results in lower per-well costs and enables many laboratories to run more assays and screening campaigns.

Precision and Accuracy:

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).

Figure 1. *Visual representation of precision versus accuracy. 1a) precision 1b)accuracy 1c)represents precision and accuracy.*

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. 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.

In this study, the effects of ‘cold’ atmospheric plasma on pipetting precision and accuracy for polypropylene pipette tips and stainless steel slotted pins were demonstrated using the highly standardized, patented dual-dye, dual-wavelength MVS ratiometric absorbance method (ARTEL, Westbrook ME).

Plasma Cleaning of Pipette Tips

The TipCharger™ by IonField Systems™ uses self-contained, low-temperature, atmospheric plasma to clean pipette tips, metal cannula and pin tools associated with automated liquid handlers. Implementation of the TipCharger system provides cleaning equivalent to that of a fresh set of tips, reducing both the direct and indirect costs associated with replacing pipette tips. In this study 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) were used as well as 50nL pin tools mounted on a 96-well array (V&P Scientific, San Diego CA).

TipCharger Integration

Contaminants on the exterior of pipette tips and pin tools exposed to TipCharger-generated plasma are immediately ionized; contaminants on the inside of pipette tips are removed through a series of aspirate and dispense steps while the tips are in a cleaning station. The TipCharger cleaning stations are provided in 8, 96 and 384-well plate densities and can be taught as either a device or a consumable within the liquid handler software.

ARTEL Dual-Dye Photometric MVS Verification Method

The Dual-Dye Ratiometric Photometry method employs two colorometric dyes with distinct absorbance maxima at 520 nm (red) and 730 nm (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 were used for testing the performance of instruments over different volume ranges from 10nL to 200 μL. The concentration of blue dye was constant in all sample solutions across the volume ranges and was equal to that in the diluent. Therefore the blue dye was used as an internal standard by which to calculate solution depth in each well. An automated liquid handler was used to dispense both the sample solution and diluent into the wells of a microtiter plate. By applying the Beer-Lambert law, to the absorbance values measured for both dyes, the MVS determines both the precision and accuracy of the volume delivered by each pipetting channel of the automated liquid handler (Figure 2).

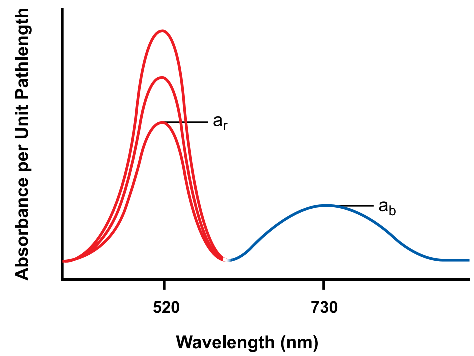


Figure 2. *ARTEL Dual-Dye Photometric MVS Detection Method. The Dual-Dye Ratiometric Photometry method employs two colorometric dyes with distinct absorbance maxima at 520nm (red dye, ar) and 730nm (blue dye, ab).*

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).

Table 1. ARTEL Dual Dye Photometric MVS Verification Method	
Plasma Exposure for 96-well Caliper/Tecan Polypropylene Pipet Tips (5 μL)	
MVS Diluent	195 μL
MVS range C (2.0 ml to 9.9 ml)	5 μL
Plasma Exposure for 96-well Caliper/Tecan Polypropylene Pipet Tips (100 μL)	
MVS Diluent	100 μL
MVS range A (50 ml to 200 ml)	100 μL
Plasma Exposure for 384-well Beckman Polypropylene Pipet Tips (5 μL)	
MVS Diluent	50 μL
MVS range B (2.5 ml to 9.9 ml)	5 μL
Plasma Exposure for 384-well Beckman Polypropylene Pipet Tips (25 μL)	
MVS Diluent	30 μL
MVS range A (10 ml to 55 ml)	25 μL
Plasma Exposure for 96-well V&P Stainless Steel Slotted pin Tools (50 nL)	
MVS Diluent	0.0 μL
MVS range D (50 nl to 199 nl)	50 μL

Results & Conclusion

Caliper/Tecan 100μL Polypropylene Pipette 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 pipette tips. The ARTEL MVS showed that wells receiving a programmed target volume of 5 μL of Sample Solution C in reality received 4.3 μL on average (CV = 3.8%). After a series of plasma exposures, tips aspirated and dispensed volumes more in line with the target volume, 4.7 μL (CV = 7.6%). Similar results were observed relative to a 100 μL target volume using the same type of pipette tip (Figure 3).

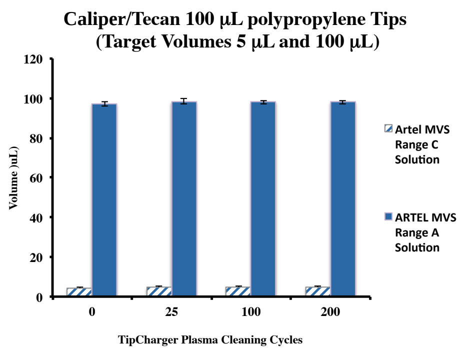


Figure 3. *Precision & Accuracy for 96-well Polypropylene Tips.*

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).

Beckman 25μL Polypropylene Pipette Tips

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

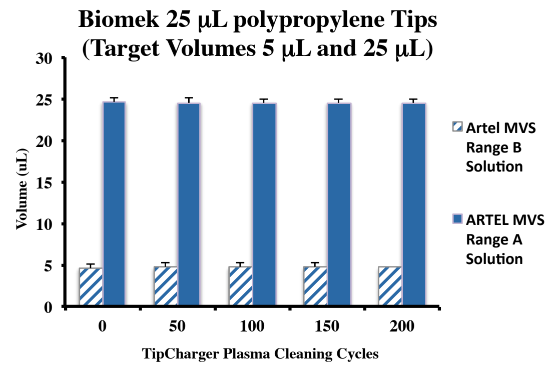


Figure 4. *Precision & Accuracy for 384-well Polypropylene Tips.*

V&P Stainless Steel 50 nL 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 50 nL of Sample Solution D in reality received 53 nL 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 53.1 nL (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).

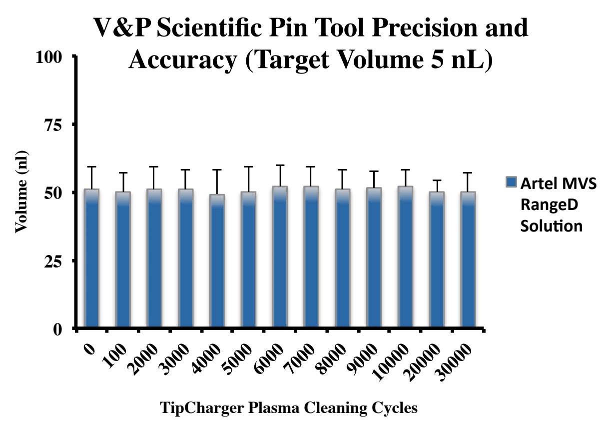


Figure 5. *Precision & Accuracy for Stainless Steel Pins.*

Summary

Regardless of the widespread adoption of disposable pipette tips, fixed cannula and pin tools, their use creates significant budgetary and logistical challenges for screening environments. 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. The data shown in this study 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 by altering surface chemistries, the length of the cleaning cycle within the TipCharger cleaning station determines the lifespan of the pipette 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. Automated environments can save on both direct and indirect costs associated with current practice with the implementation of novel technologies such as the TipCharger by IonField Systems.

Given that life science screening environments continue to make every effort to reduce time-to-market for therapeutics, the TipCharger may help play a role achieving this objective.

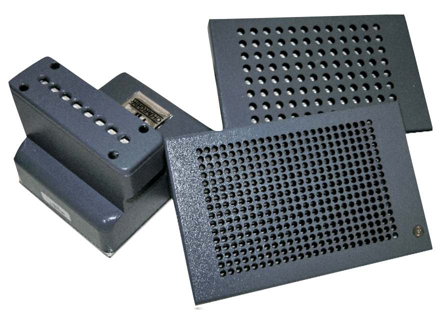
Integrating the TipCharger into Automated Assays Provides the Following Benefits:

Best in Class TipCharger cleans better than any other washing technology - in most applications the TipCharger will clean to background, so there is no difference between plasma cleaning and a new tip.

Cost Benefit TipCharger saves over 90% on the cost of the disposable tips.

Speed Incorporating the TipCharger System can result in a time savings of 10-30 seconds for every microplate processed or rack of tips cleaned.

Convenience Clear away the clutter and save time: Integrating the TipCharger System eliminates the need to store cases of new pipette tips and dispose of racks of hazardous used tips.



TipCharger Plasma Cleaning Stations
Available in 8, 96, 384 and 1536 channel versions

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About the TipCharger™

The TipCharger Cleaning System replaces existing wash stations and easily integrates with most existing and new automation platforms. The system utilizes a low temperature, atmospheric pressure plasma process that cleans metal and plastic pipette tips and pin tools. Treated surfaces are clean, dry and have uniform surface properties.

The TipCharger cleaning process reduces the incidence of micro-bubble formation and other random surface effects that degrade liquid handling precision and accuracy, even with new disposable tips.

IonField Systems' TipCharger improves the reproducibility of process results, shortens automation cycle times, reduces the number of lost runs, and eliminates environmental waste and liquid handling disposables. The overall result is increased confidence in results and a more effective and productive laboratory operation.

About IonField Systems™

IonField is an advanced technology company focused on the development of best-in-class cleaning methods for life science applications.

We provide comprehensive on-site/ phone support and ongoing services to insure a successful installation and exceptional day-to-day operation of our products.