



Tanya Knaide
of



and

Geoffrey Schwartz
of



Present

Determining the Precision and Accuracy of Pipet Tips Exposed to the TipCharger™ System's 'Cold' Atmospheric Pressure Plasma Cleaning Using the Artel MVS® Multichannel Verification System



The Artel / Cerionx Partnership

- **Artel** is the worldwide leader in liquid delivery measurement and quality assurance. Specializing in the use of ratiometric photometry, Artel manufactures easy-to-use systems for ensuring data integrity. Products include the PCS (Pipette Calibration System), MVS (Multichannel Verification System) and LHQA Services.
- **Cerionx** develops technology-based products for automated BioPharma laboratories. The TipCharger System integrates with automated liquid handlers and generates 'cold' atmospheric pressure plasma to clean all types of pipet tips. Eliminating solvent-based wash steps and continual tip changes allows labs to operate more effectively and productively.





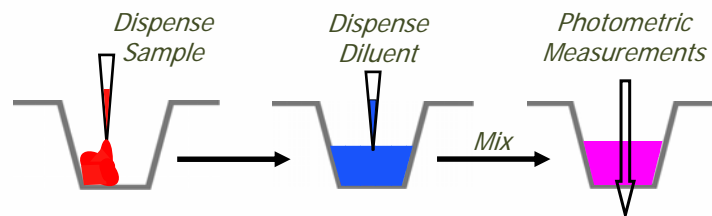
What is the Artel MVS?

- The Artel MVS is a standardized system that provides:
 - Precision & accuracy via one rapid, single-measurement for both
 - NIST-traceable results
 - Superior data to conventional methods of performance verification by employing a method conforming to the ISO 8655 standard



Dual-Dye Photometric Method

- Photometric measurement of liquid volume
- Two dyes measured at two wavelengths
- Ratiometric measurements and calculation of results





MVS Components



Characterized
Microtiter Plates



Sample Solutions



Calibrator Plate



Plate Shaker



Notebook Computer w/ System
Software & Barcode Reader



The Mobile Workstation



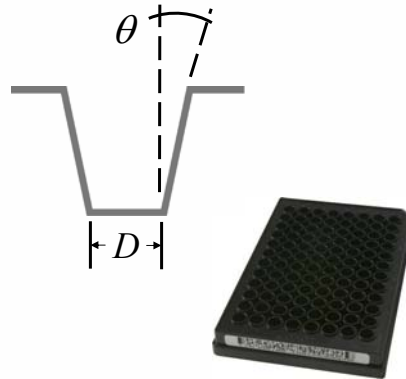
- Portability allows verification of equipment in multiple locations throughout a facility





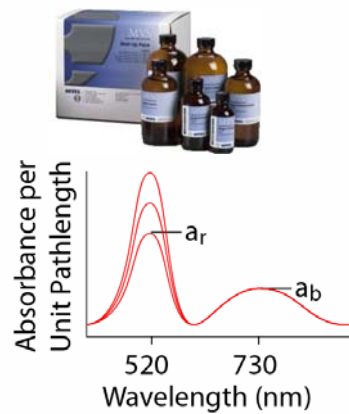
MVS Verification Plates

- Lot characterized plates
- Well dimensions traceable to national standards
- Bottom diameter and taper angle are critical to calculations
- Barcode carries necessary information about performance and dimensions



MVS Sample Solutions

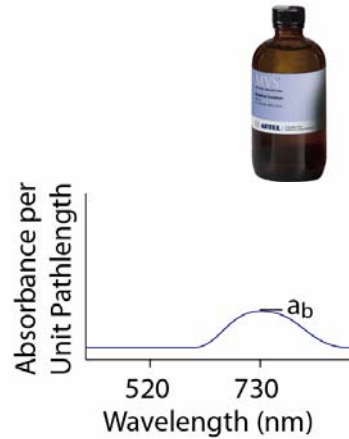
- Contain 2 dyes: red and blue
- Distinct absorbance maxima (520 & 730nm)
- Different concentrations of red dye for different volume ranges
- Blue dye at the same concentration for all ranges
- Stable and traceable to national standards





MVS Diluent

- Contains blue dye only
- Absorbance peak at 730 nm
- Concentration of blue dye same as in sample solutions
- Used to back-fill wells to working volume for low volume testing
- Stable and traceable to national standards



MVS Calibrator Plate

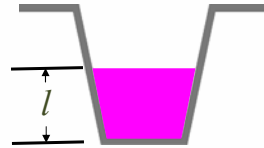
- Sealed precision cuvettes filled with same dyes as sample solutions and diluent
- Absorbance measured in factory reference spectrometer and encoded in bar code
- Bar coded absorbance traceable to national standards
- Used for daily calibration of Plate Reader output





Calculation I: Liquid Depth

- Calculate depth of liquid (pathlength) in each well
- Based on the absorbance at 730 nm
- Independent of the ratio of sample to diluent
- a_b = absorbance per unit pathlength of blue dye in both solutions
- a_b passed to software for analysis through barcode

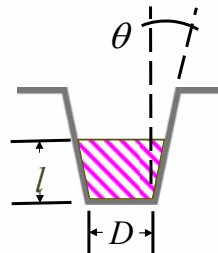


$$l = \frac{A_{730}}{a_b}$$



Calculation II: Total Volume

- Volume calculation is based on a truncated cone
- θ and D passed to software through barcode
- Total volume calculated from liquid depth and bar-coded dimensions



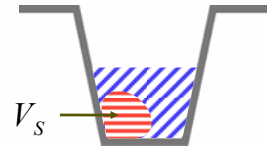
$$V_T = \pi l \frac{D^2}{4} + \pi D l^2 \frac{\tan(\theta)}{2} + \pi l^3 \frac{\tan^2(\theta)}{3}$$





Calculation III: Sample Volume

- Calculate sample volume based on total volume and measured absorbance ratios
- a_r = absorbance per unit pathlength of red dye in sample solution
- a_r passed to software for analysis through barcode



$$V_S = V_T \left(\frac{a_b}{a_r} \right) \left(\frac{A_{520}}{A_{730}} \right)$$



MVS: Quantifying Target Volume

96-well plates

0.1 – 200 μL

384-well plates

0.01 – 55 μL





MVS Multichannel Verification System

- Provides a third party, standardized system to evaluate the performance of liquid handling equipment
- Measures both precision and accuracy simultaneously
- Results are traceable to national standards
- Has been employed to evaluate in-process components such as the TipCharger by Cerionx



What is TipCharger?

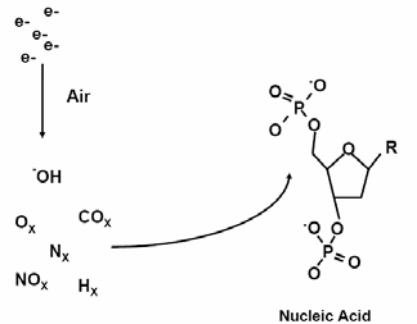
- An integrated cleaning station on automated liquid handling platforms that
 - Cleans and **sterilizes** pipet tips
 - Eliminates contamination
 - Enhances assay productivity
 - **Improves throughput**
 - **Allows for tip re-use**
 - **Eliminates hazardous waste**





Plasma-based Cleaning Solution

- Optical sensor triggers plasma production
- An electron avalanche is produced, ionizing the room air, resulting in a plasma field around each pipet tip
- Highly energetic atomic metastable species are generated and combine with contaminants on tips



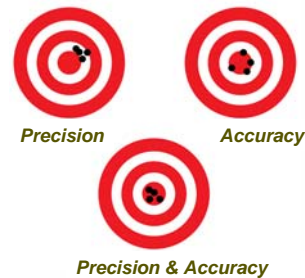
→ **Contaminants are removed at the molecular level**



Precision and Accuracy

Fluorescence-based dye tests proved unreliable in calibrating polypropylene tips used in TipCharger Systems:

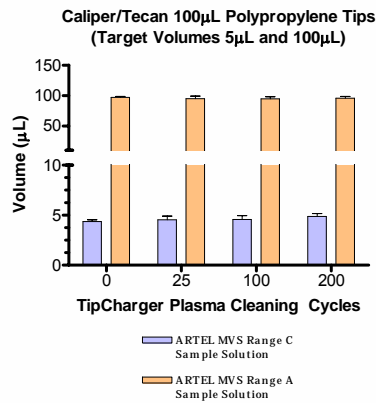
- **Non-standardized**
- **Provided reliable measurements of CVs, but not volumes**
- **Were unstable and inaccurate**





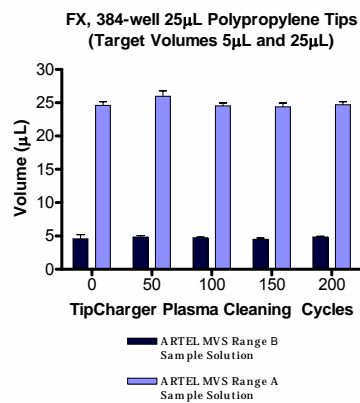
8/96-well Tips

- **Improved** pipetting accuracy
- Accuracy unaffected throughout **200 cycles**
- Little effect on CVs
 - 7.6% CV compared to manufacturer specifications of ~5%



384-well Tips

- **Improved** pipetting accuracy for low volumes (5 μ L) vs. untreated tips
- CVs unaffected throughout **200 cycles**
- Little effect on CVs for high volume (25 μ L) applications
 - TipCharger-treated = 2.4%
 - Untreated tips = 2.3%

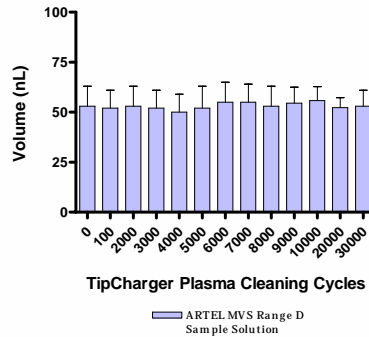




Pin Tools

- **Similar** results for TipCharger-treated and untreated pin tools
- Both precision and accuracy unaffected throughout **30,000 cycles** when compared to pin manufacturer specifications (7-8% CV)

V&P Scientific Pin Tool Precision & Accuracy
(Target Volume 50nL)



ELISA

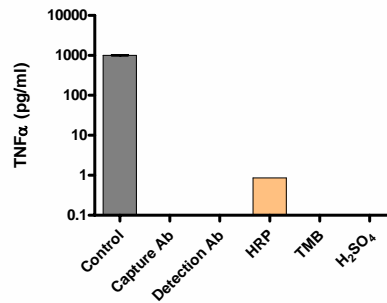
Sensitive and selective assay:

- long incubations (slow turnaround)
- lengthy plate washing protocols
- high kit cost
- requires zero sample carryover

TipCharger cleaning results:

- Cleans to almost undetectable level of 10^{-14} ... indistinguishable from new tips
- Allows reduced timelines and tip costs without any impact on assay results

Effect of TipCharger on R&D Systems
TNF α Kit (DY210)





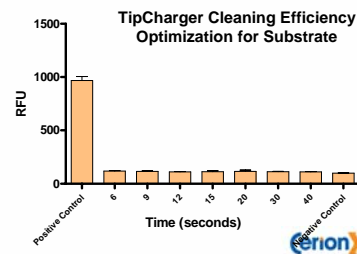
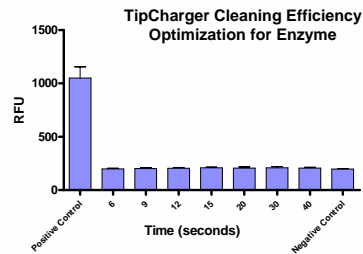
Biochemical Assays

Routine Drug Discovery screening assay:

- Ubiquitous class of targets for BioPharma
- Important for selectivity and profiling

TipCharger cleaning results:

- 6 seconds removal of either enzyme or peptide substrate
- Fully recoverable assays
- Reduced tip consumption by 75%
- Saved ~80 seconds per plate



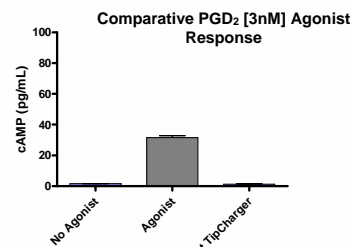
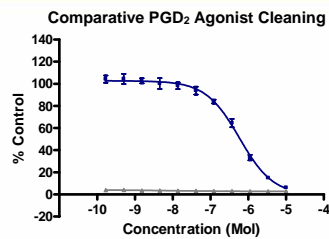
Automated cAMP

Routine Drug Discovery screening assay:

- Standard assay for GPCR screens
- Important for selectivity, profiling and MoA

TipCharger cleaning results:

- Eliminated agonist activity
- Cellular response post-TC was equivalent to absence of agonist
- Doubled assay throughput
- Eliminated frequent tip delivery and loading errors





siRNA Screening

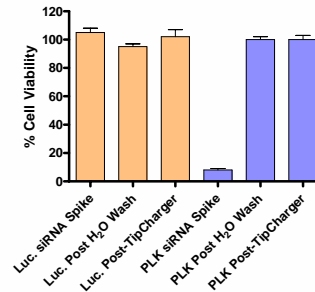
Target ID and Validation screening assay:

- Important for establishing gene function
- Provide for selectivity, profiling and MoA

TipCharger cleaning results:

- Similar to historical washing practices
- Eliminated carryover in 10 sec. vs. 60 sec.
- Reduced screening timelines by 20%
- Eliminated concern of bacterial or fungal contamination

TipCharger Reduces siRNA Carryover Equivalent to Historical Methods



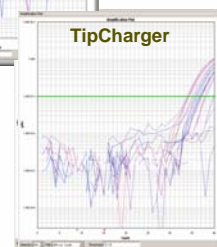
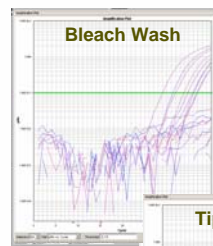
Genomic DNA via RT-PCR

Genotyping and sequencing assay:

- Important for sample ID
- Provide genetic feedback for knockout and disease model samples

TipCharger cleaning results:

- Similar to absence of gDNA
- Eliminated carryover in ~20 sec. vs. 60 sec.
- Eliminated concern of sample cross contamination
- Eliminated care & feeding of bleach wash





Genomic qPCR- Based Screens

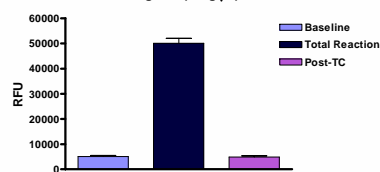
Sequencing and Target ID assay:

- Important for target validation
- Provide genetic feedback for knockout and disease model samples

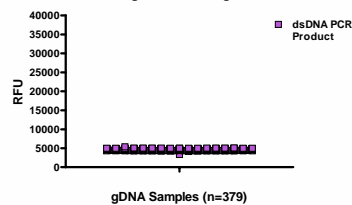
TipCharger cleaning results:

- Eliminated gDNA carryover via qPCR
- Provided better LH capabilities
- Allowed for a single box of tips/screen
- Significantly reduced tip cost and logistics

Fully Optimized TipCharger Protocols
Eliminated gDNA (30ng/μL)



Fully Optimized TipCharger Protocol
Through 55 Plates of gDNA



Automated CYP450

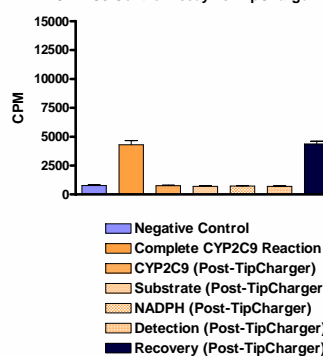
Standard ADME/Tox screening assay:

- Important for understanding drug metabolism and interaction
- Homology alone does not dictate metabolic role

TipCharger cleaning results:

- 30 sec. plasma exposure eliminated membrane-bound CYP activity (2C9, 3A4, 2D6)
- Allowed for a fully recoverable assay
- Eliminates concern of sample cross contamination

CYP2C9 Control Assay vs. TipCharger





Other TipCharger Applications:

- Cell-Based Assays
 - Cell proliferation
 - Apoptosis
 - Cytotoxicity
- Sterility
 - *E. Coli*
 - Yeast
 - Mammalian cell lines
- Proteins & Peptides
 - Kinases
 - Proteases
 - Antibodies and conjugates
 - Ligands
 - Membrane-bound proteins
- Reagents
 - General assay buffers
 - Lysis buffers
 - Most solvents
 - Transfection reagents



The TipCharger System

- Provides pipet tips cleaning and sterilization:
 - equivalent to continual tip exchanges
 - in less time than traditional wash methods
 - eliminates hazardous wash stations
- Easily integrates on liquid handling decks
 - SBS footprint
 - Does not require software integration
 - Eliminates LH care and feeding for both tips and wash solvents
- ARTEL Certified
 - Equivalent and possibly better precision and accuracy than new tips
 - Allows for reuse of pipet tips several hundred times prior to disposal





Complimentary Technologies

- The use of Cerionx **TipCharger** pipet tip cleaning with Artel **MVS** as a method of precision and accuracy assurance:
 - Provides fixed and disposable tip cleaning equivalent to new tips
 - Affords traceable accuracy and precision data in one measurement
 - Reduces/eliminates repeats due to carryover and poor liquid handling
 - Increases efficiency and reduces instrument downtime
- Both technologies are:
 - Easily integrated into automated environments
 - Work with virtually all liquid handling equipment



Demonstration

- Determine precision & accuracy representative of a Tecan Evo:
 - Aspirate 20 μ L of Range B Sample Solution
 - Dispense into 180mL of Diluent
 - Perform detection analysis
- Contaminate tips with Range B Sample Solution :
 - Clean tips via a series of mix steps in the TipCharger Cleaning Station.
 - Mix tips in 200 μ L of Diluent to elute any carryover
 - Perform detection analysis





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