New Validation and Optimization Techniques for the PerkinElmer JANUS™ platform using the ARTEL Multichannel Verification System (MVS®)

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PerkinElmer & ARTEL

ARTEL is the world wide 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™ (Liquid Handling Quality Assurance) Services.

PerkinElmer Automation and Liquid Handling business is a market leader by providing the most flexible and scaleable automated workstations capable of “on the fly” real time optimization of throughput, precision pipetting, and walk-away automation – today and in future.

The collaboration allows ARTEL to demonstrate the ability of the MVS to facilitate the performance validation and optimization for the JANUS™ Automated Workstation Product Family. PerkinElmer can also extend this measurement technology to their users thereby assisting their customers to obtain precision and accuracy information for target volumes dispensed by the JANUS platform or by other PerkinElmer liquid handlers.
Outline for LabAuto2007 Workshop

- Performance verification & goal
- MVS
- Understanding liquid handler ‘behavior’
- JANUS Automated Workstation Family
- Liquid properties & pipetting methodology
- JANUS performance verification data

Liquid Handler Performance Verification

- Goals:
  - Reproducibly deliver target volume with accuracy & precision
  - Rapidly verify transferred target volumes with standardized methodology
  - Is the instrument hitting assay-specific, or critical, target volumes?
  - How is performance of an automated liquid handler evaluated, monitored and/or maintained?
    - MVS
    - Gravimetric methods
    - Fluorescence/Absorbance methods
    - Combination
    - Other home-brew methods

Varispan VersaTip® Plus
**Liquid Handler Performance Verification**

- Volume verification method and user-specific requirements:
  - Purpose of dispensing device
  - Quantitative vs. qualitative volume transfers
  - Accuracy vs. precision
  - Reproducibility
  - Initiative
  - Responsibility of ownership
  - Time, and lack thereof
  - Resources, and lack thereof

- Pitfalls...do any of these *assumptions* sound familiar?
  - Routine use = reproducible performance
  - Quarterly or semi-annual calibration intervals are sufficient
  - Once maintenance is performed, device behavior is “like new”
  - Faith-based – “it looks like it’s working”
  - Monitoring performance is time consuming and labor intensive

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**Is Performance Optimization Necessary?**

- What if you could always be assured within minutes that a liquid handler method (script) was reliably transferring accurate volumes to the assay?

- Impact on:
  - Experiment, assay, & data integrity
  - Laboratory productivity
  - GMP/GLP compliance
  - Economics
    - reduced downstream costs
    - instrument downtime minimized
    - reduced reagent & consumable waste
    - resources (labor, time) minimized
  - Intangibles (stress, confidence, motivation, etc.)
**MVS:**
*Multichannel Verification System for Testing Liquid Handler Performance*

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**Dual-Dye Photometric Method**

- Photometric measurement of liquid volume
- Two dyes measured at two wavelengths
- Ratiometric measurements and calculation of results *per well*
- Simultaneous measurement of accuracy & precision *per channel*

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*Dispense Sample* → *Dispense Diluent* → *Photometric Measurements*
**MVS® Components**

- Characterized Microtiter Plates
- Sample Solutions
- Calibrator Plate
- Plate Shaker
- Notebook Computer with System Software & Barcode Reader
- Microtiter Plate Reader

**MVS® Mobile Workstation**

- Mobile workstation allows for 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 maximum 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
**Volumetric Sample Addition**

- Dispense Sample Solution using device under test
- Small volumes will not cover the bottom of the well.

**Non-Quantitative Diluent Addition**

- Amount of Diluent is *not* critical because the blue dye is present at the same concentration in both the Solution and the Diluent.
**Photometric Measurements**

- Bar code information passed to software for calculations
- Quantitative absorbance measurements collected for every well at 520 nm and 730 nm

**Dimensional Volume Calculation**

- Volume calculation is based on physical dimensions of truncated cone-shaped well
- Solution depth photometrically measured using Beer-Lambert Law

\[ A_\lambda = (\varepsilon_\lambda C)l = a_\lambda \cdot l \]

- where \( a_\lambda \) is the absorbance per unit pathlength

\[ l = \frac{A_\lambda}{a_\lambda} \]
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
- $a_b = \text{absorbance per unit pathlength of blue dye in both solutions}$
- $a_b$ passed to software for analysis through barcode

\[ l = \frac{A_{730}}{a_b} \]

Calculation II: Total Volume (96-well)

- Volume calculation is based on a truncated cone

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

- $\theta$ and $D$ passed to software through barcode
- Total volume calculated from liquid depth and bar-coded dimensions
**Calculation III: Sample Volume**

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

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

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**MVS: Quantifying Aqueous-based Target Volume**

- 96-well plates
  - 0.1 – 200 μL
- 384-well plates (including low-volume or low-profile)
  - 0.01 – 55 μL
### MVS Output Report

- Immediately generated after volume verification task
- Statistics are displayed by row, column, and channel
- Flagged results based on tolerance specifications
- Exportable as HTML or XML

Sample report for a 2-uL dispense for a 384-channel head into a 384-well plate (statistics not shown for the three replicate dispenses)

### MVS as an Integration Tool
Assessing Liquid Handler Performance & Facilitating Optimization
Monitoring Liquid Handler Performance After Sequential Parameter Adjustment (1 of 2)

<table>
<thead>
<tr>
<th>Sequential Experimental Reference ID</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
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<tbody>
<tr>
<td>Modified Parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New tips or Used (pre-wetted) tips</td>
<td>New</td>
<td>Used</td>
<td>Used</td>
<td>Used</td>
<td>Used</td>
<td>Used</td>
<td>Used</td>
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<tr>
<td>Aspirate Rate (µL/s)</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
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<tr>
<td>Dispense Rate (µL/s)</td>
<td>50</td>
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<tr>
<td>Leading Air Gap (µL)</td>
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<td>0</td>
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<tr>
<td>Trailing Air Gap (µL)</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Requested Volume (µL)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Sequential Parameter Adjustment

Monitoring Liquid Handler Performance After Sequential Parameter Adjustment (2 of 2)

![Graph showing MVS Output (%) for different experiments]
**Performance Data for Every Channel**

JANUS MDT P235 Head, 5-μL target volume

**Single-measurement data**: all individual channels within a device can be directly compared.

**Standardized Platform: Device-to-device Comparison**

8 μL Target volume
**Verifying “Alternative” Test Solutions**

8 replicate dispenses with an 8-μL Calibrated Syringe

<table>
<thead>
<tr>
<th>Alternative Test Solution ID</th>
<th>Relative Inaccuracy</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% Aqueous (MVS Range C)</td>
<td>-0.0%</td>
<td>-1.0%</td>
</tr>
<tr>
<td>90% DMSO in water (vol/vol)</td>
<td>-1.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>20% Glycerol in water (vol/vol)</td>
<td>1.0%</td>
<td>2.0%</td>
</tr>
<tr>
<td>50% Ethanol in water (vol/vol)</td>
<td>3.0%</td>
<td>2.0%</td>
</tr>
</tbody>
</table>

**Other Places For MVS-guided Performance Optimizations**

- Method scale-up or transfer.
- Assay or method troubleshooting.
- When preparing (reproducible) samples, mother/daughter plates, etc.
- Quick volume "spot check"; or before/after using expensive reagent.
- Employee training or during method programming.
- Monitoring all critical target volumes in an assay.
- Comparing ‘Factory’ to ‘Site’ Acceptance Testing.
- Prove regulatory compliance with NIST-traceable measurement results.
MVS – Volume Verification Method for PerkinElmer

- Performance and Traceability
  - A high level of performance and traceability is achieved through the use of the MVS dual-dye, dual-wavelength, ratiometric absorbance method.

- Data Integrity
  - Frequent verification of liquid delivery device performance is practical, quick, and easy using the ARTEL MVS®. Frequent verification provides assurance of data integrity.

- The Artel MVS method is:
  - Fast & Easy
  - Accurate
  - Precise
  - Traceable

JANUS™ Automated Liquid Handling System
Performance and Optimization

Marcus Patterson
Sr. Applications Engineer
Overview

➤ JANUS™ Platform
➤ Liquid Handling Fundamentals
➤ Performance Optimization Recommendations
➤ Performance Data using Artel MVS

JANUS™ Automated Workstation Product Family

Complete family of Liquid Handling for:
- Plate Replication
- Reformatting
- Cell-based Assays
- ELISA Assays
- Reagent Addition
- Dilutions
- DNA purification
- PCR/Sequencing setup
- Cherry picking (HITS reformatting)
- Proteomics

Powered by Packard Innovation
JANUS™ Automated Workstation

Varispan™ Pipetting Arm

- Flexible 4- or 8-tip pipetting system
- Volume Range 100nL – 5mL
- Varispan™ variable tip spacing for tubes, vials and microplates
- VersaTip® PLUS enables switching between disposable and fixed washable tips in one assay
- Perfect for serial dilutions, cherry picking and more!

JANUS™ Automated Workstation

Modular Dispense Technology™ (MDT)

- Volume range 50nL - 235μL
- Utilize any MDT pipetting head in real time and “on the fly” NO manual intervention required.
- 96- or 384-Channel MDT Dispense Heads for precision dispensing into 96-, 384- and 1536-well shallow and deepwell plates.
- Choose an MDT head to match
  - Throughput
  - Labware density
  - Optimal pipetting performance
WinPREP® Applications Software

- Easy-to-use default procedure templates
- Flexible custom procedure programming
- Predefined labware library
- Error log/database report files
- Creation and utilization of worklists
- Advanced scripting capability
- Labware integration/automation

WinPREP® Applications Software – Easy as 1, 2, 3

1. Select labware from labware library; Drop labware on deck
2. Choose pipetting template
3. Drag and drop labware on pipetting step in protocol tree
Determining Assay Criteria

- What are the minimum and maximum transfer volumes?
- What are the potential sources of contamination?
- What are the throughput requirements?
- What is the assay cost per sample?
- What are the precision and accuracy needs?
**Pipetting Methodology**

**Blowout Mode**
A volume of air is used to expel all liquid contents from the tip.

**Waste Mode**
The requested transfer volume plus an additional volume is aspirated. Excess volume is discarded after dispense.

**Contact Dispense**
Liquid contacts a solid or liquid interface during dispense.

**Non-Contact Dispense**
Liquid is ejected from tip without contacting a liquid or surface interface.

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**WinPREP® System Parameters**

- Air Gaps (TAG, SAG)
- Aspirate/Dispense Rates
- Aspirate/Dispense Delays
- Tip Type
- Default System Parameters for Aqueous Solutions
**Evaluate Liquid Properties**

**Viscosity:** The resistance to flow of a fluid due to the sum of the effects of adhesion and cohesion.

**Surface Tension:** The force that appears at the surface of a liquid and tends to pull the liquid into spherical droplets.

**Vapor Pressure:** The pressure of a vapor in equilibrium with its non-vapor phases.

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**Properties of Common Liquids**

<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th>DMSO</th>
<th>Ethylene Glycol</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Viscosity (mPa.s)</strong> *</td>
<td>0.890</td>
<td>1.987</td>
<td>16.1</td>
<td>1.074</td>
</tr>
<tr>
<td><strong>Surface Tension (mN/m)</strong> *</td>
<td>71.99</td>
<td>42.92</td>
<td>47.99</td>
<td>21.97</td>
</tr>
<tr>
<td><strong>Vapor Pressure (Pa)</strong> **</td>
<td>2338.8</td>
<td>56.0</td>
<td>8.0</td>
<td>5946.2</td>
</tr>
</tbody>
</table>

* 25°C & 1 atm pressure  
** 20°C
Recommendations per Liquid Type

➢ **Volatile Liquids (High Vapor Pressure)**
  - Waste Mode
  - Aspirate slowly
  - Reduce system air gap
  - Saturate system air gap
  - Chill reagents if possible

➢ **High Viscosity Liquids**
  - Waste Mode
  - Increase syringe pump delays
  - Aspirate & dispense slowly
  - Warm reagents if possible

Recommendations per Liquid Type

➢ **Low Viscosity/Low Surface Tension**
  - Waste Mode
  - Low dispense speed
  - Increase transport air gap

➢ **Small Volumes (<20µL)/High Reagent Cost**
  - Blowout Mode
  - Low aspirate speed
  - Decrease syringe size
Accuracy & Precision

Precision
Degree to which further measurements or calculations will show the same or similar results.

Accuracy
Degree of conformity of a measured or calculated quantity to its actual (true) value

![Diagram showing accuracy and precision]

MDT Performance Data

![Image of MDT Performance Data]

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**MDT NanoHead™**

- 50nL – 1000nL
- 384 Fixed Washable Tips
- Compound / Protein Transfers
- Tip Coatings
- 250nL CV <5%
- 250nL Inaccuracy <8%

**MDT P30 Head**

- 0.5µL - 30µL
- 384 Disposable Washable Tips
- 5µL CV <3%
- 5µL Inaccuracy <6%
MDT P235 Head

- 5µL - 235µL
- 96 Disposable Washable Tips
- 5µL CV <2%
- 5µL Inaccuracy <1%

Varispan™ Performance Data

- Disposable Tips
- Washable Fixed tips
- Or use Both
Varispan™ Accuracy Compensation

- Slope Intercept Equation
  - \( y = mx + b \)

![Linear Regression Graph](image)

\[ y = 0.9921x - 0.0379 \]
\[ R^2 = 0.9999 \]

Varispan™ Low Volume Fixed Tip Option

- 100nL – 5000nL
- Assay Miniaturization (HITS Reformatting)
- 500nL CV <10%
- 500nL Inaccuracy <3%

![Low Volume Fixed Tip Option](image)
**Varispan™ 20µL Conductive DT**

- **Varispan 20uL Conductive Tips Precision**
  - Coefficient of Variation (%)
  - Requested Volume:
    - 1µL
    - 5µL
    - 10µL

- **Varispan 20uL Conductive Tips Accuracy**
  - (+/-) % Accuracy
  - Requested Volume:
    - 1µL
    - 5µL
    - 10µL

- 1µL - 10µL
- 5µL CV <2%
- 5µL Inaccuracy <1%

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**Varispan™ 200µL Conductive DT**

- **Varispan 200uL Conductive Tips Precision**
  - Coefficient of Variation (%)
  - Requested Volume:
    - 5µL
    - 50µL
    - 175µL

- **Varispan 200uL Conductive Tips Accuracy**
  - (+/-) % Accuracy
  - Requested Volume:
    - 5µL
    - 50µL
    - 175µL

- 5µL - 200µL
- 5µL CV <2.5%
- 5µL Inaccuracy <1%
Conclusion

- Flexible laboratory automation platform
- Large dynamic range for volume transfers
- Performance optimization procedure
- Performance characterization using Artel MVS

Thank you