Performance Optimization of Multichannel Liquid Handling Equipment and High Throughput Assays:

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Performance Requirements

- How is performance evaluated? Measured?
- How is performance maintained?
- Various user-specific requirements:
  - Purpose, or function, of dispensing device
  - Quantitative vs. qualitative results
  - Accuracy vs. precision
  - Reproducibility
  - Initiative
  - Responsibility of *ownership*
  - Time, and lack thereof
  - Resources, and lack thereof

Performance Assumptions

- Faith-based performance and a lack of performance metrics monitoring. *"It looks like its working"*
- Routine use = reproducible performance.
- Instruments perform *linearly* vs. target volume level.
- Quarterly or semi-annual calibration intervals are good enough.
- Once maintenance is performed, device behavior is "like new".
- Monitoring performance is time consuming and labor intensive.

Is Performance Optimization Necessary?

- Is the instrument *hitting* assay-specific, or critical, target volumes?
- What if you could always be assured – in five minutes or less - that your instrumentation was working to specification?
- Impact on:
  - Data Integrity
  - Laboratory Productivity
  - GMP/GLP compliance
  - Economics
    - reduced downstream costs
    - instrument downtime minimized
    - reduced reagent & consumable waste
    - resources (labor, time) minimized

Optimizing Performance: A *Simple* Goal

- Liquid Delivery Quality Assurance:

  Reproducibly deliver **target volume** with **accuracy & precision**.

Adjustable Assay Parameters Affect Performance

- pre- and post-air gaps
- target, or off-set, volume
- aspirate/dispense rate
- aspirate/dispense height
- on-board mixing
- wash steps
- overall speed
- wet vs. dry dispense
- dispense order
- Tips/cannulas
  - max/min volume capacity
  - fixed vs. disposable
  - dry tip vs. wet tip
  - new tip vs. used tip
  - carry-over
  - tip-touches

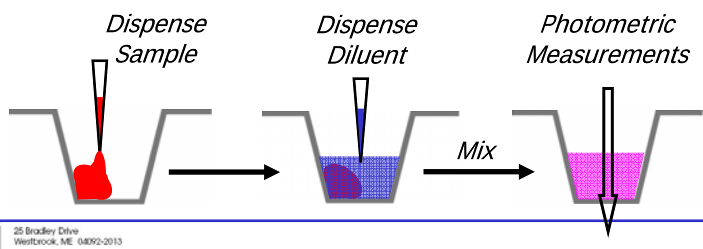
Measuring Device Performance

Artel MVS

- Applies scientific principles to challenges faced by users of liquid handling equipment.
- System concept – Complete solution to complement your process.
- Provides easy-to-use, rapid, single-measurement for both precision & accuracy.
- Superior to conventional methods of performance verification.
- NIST-traceable.
- ISO-approved.

Dual-Dye Photometric Method

- Based on the same technology as the Artel PCS® Pipette Calibration System:
  - Photometric measurement of liquid volume
  - Two dyes measured at two wavelengths
  - Ratiometric measurements and calculation of results



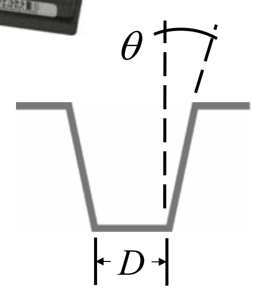
MVS™ Components

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



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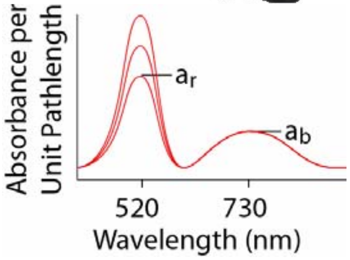
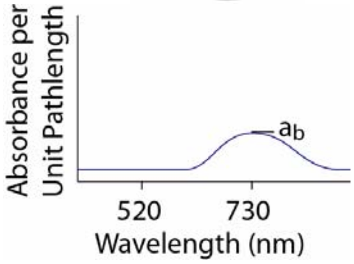
MVS™ Sample Solutions

- 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



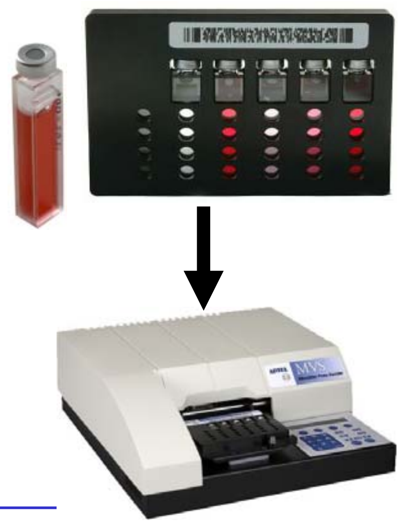
MVS™ Diluent

- 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



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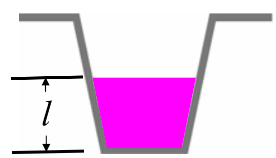
Calculation I: Liquid Depth

- 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



$$l = A_{730} / a_b$$

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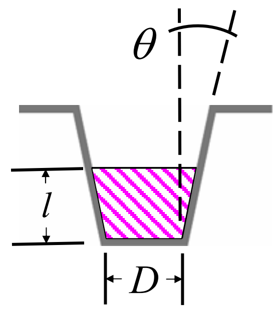
Calculation II: Total Volume

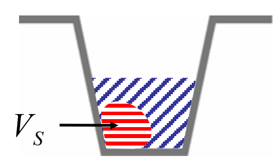
- Volume calculation is based on a truncated cone
- θ and D passed to software through barcode
- Total volume calculated from liquid depth and bar-coded dimensions



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

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



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

Demonstration with 96-channel Caliper RapidPlate

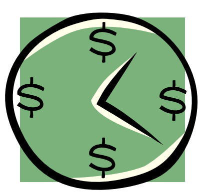
RapidPlate Optimization

- "As found" performance data for 10 μL
- Parameter adjustment
- Post-optimization performance data

So Now What Do I Do?

- Know the accuracy of Liquid Handler- What Next?
  - Close review of your application
  - Determine what accuracy you need for each step
  - Calibrate or not?
  - Verify results

It's no Big Surprise-



So Plan Wisely!

Larry's Lesson

Review Application

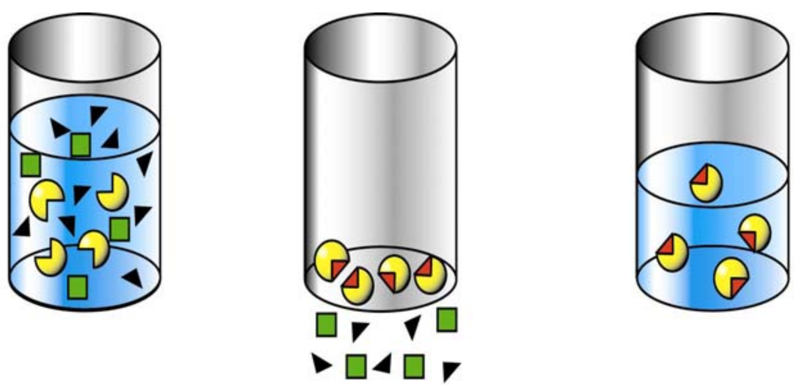
- Review each step, determine
  - What is the goal of this step?
  - Where is accuracy critical?
  - Will current performance do?
- Case Study- Radiometric Ligand Binding Assay

Radiometric Ligand Binding Assay

- Create Assay Plate
  - 25uL membranes
  - 25uL radioligand solution
  - 5uL compound
- Incubate for 1-3 hours
- Transfer Assay Mixture to Filter Plate
- Filter and Wash
- Dry Plate
- Add Scintillation Cocktail & Count

Workshop:
Lab Automation 2006

In-Plate MultiScreen Filter Plate Assay Schematics



Reaction components are added to the filter plate. Receptor binding assays are allowed to reach equilibrium. Kinase reactions are timed and stopped by the addition of acid

Bound ligands or modified substrates are retained on the filter after vacuum removal of reaction buffer (see text). Subsequent washes further removes unincorporated radioactivity.

Liquid scintillation cocktail is added. Retained radioactivity is measured directly in the filter plate

Workshop:
Lab Automation 2006

What Accuracy is Needed?

- Create Assay Plate
  - 25uL membranes — ***In Excess***
  - 25uL radioligand solution — ***In Excess***
  - 5uL compound — ***CRITICAL!!!***
- Incubate for 1-3 hours — ***N/A***
- Transfer to Filter Plate — ***All***
- Filter and Wash — ***In Excess***
- Dry Plate — ***N/A***
- Add Scintillation Cocktail & Count — ***In Excess***

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Workshop:
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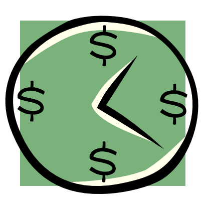
Calibrate or Not?

- Look at Known Performance
- Look at Accuracy Requirements
- Use Time to Fully Calibrate where Necessary!



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Calibration

- Create Liquid Classes
  - Liquid
  - Volume range
  - Dispensing technique
- Use Specific Liquid Class for Dispensing Step
- VERIFY RESULTS
- Compare Apples to Apples

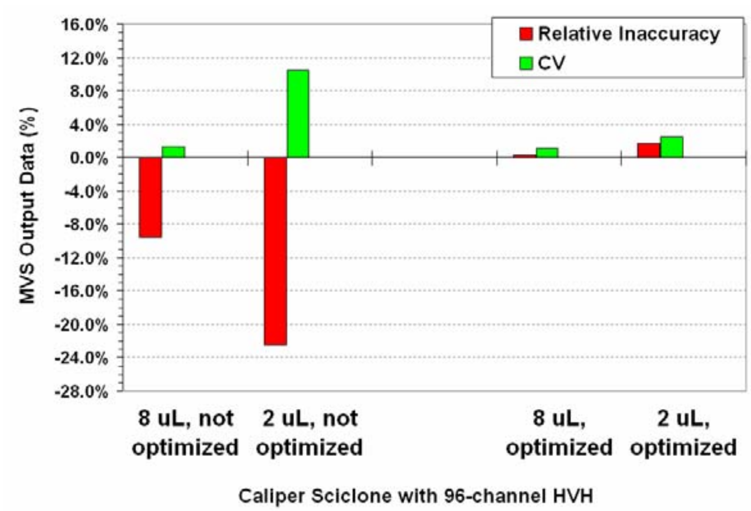
So What Can You Really Do?

- Glycerol
- Scintillation Fluid
- Technique:
  - Slow Aspirate Rate
  - PAUSE
  - Slow Dispense Step
  - PAUSE
- Calibration???
- Sciclone Calibration Examples

MVS as an Integration Tool

Assessing ALH Performance & Facilitating ALH Optimization

Sciclone Optimization



Workshop:
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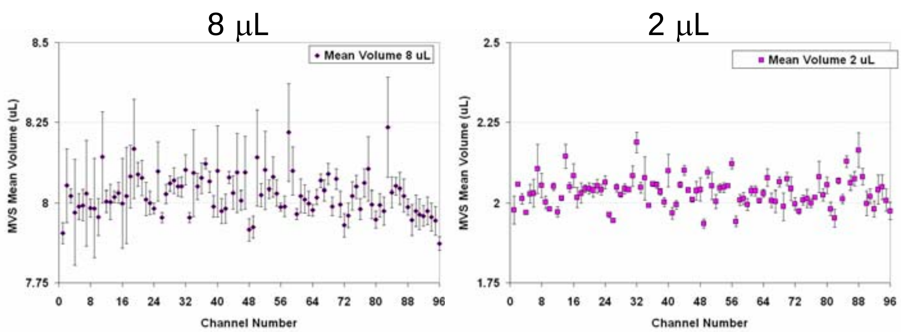
Performance Data for Every Channel



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

Shown: mean volume and standard deviation (error bars)

Workshop:
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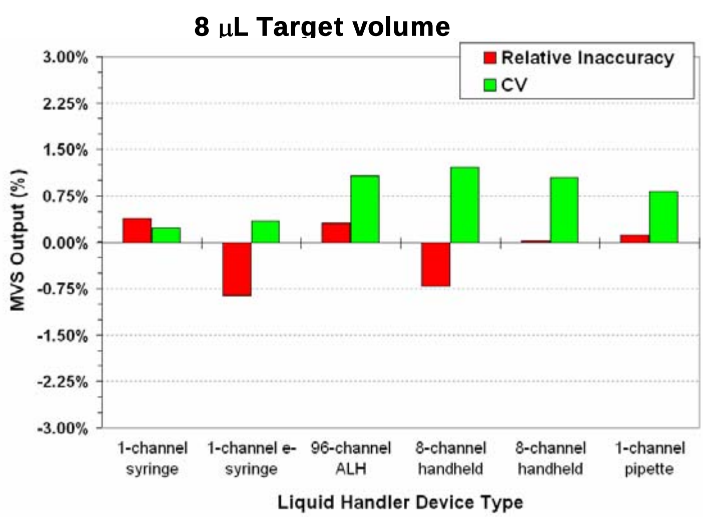
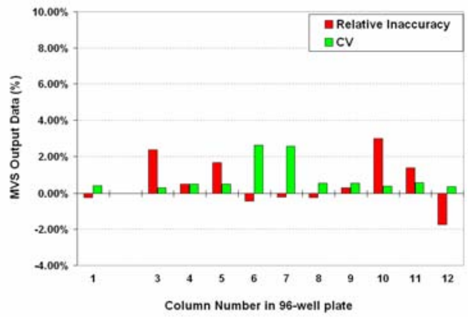
Device-to-device Comparison

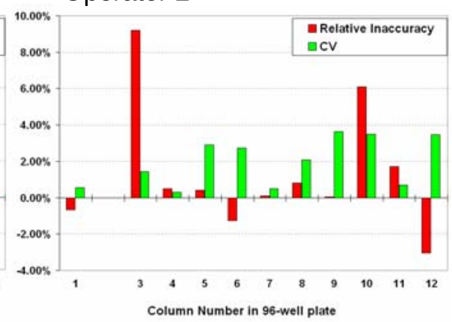


Operator-to-operator Comparison

Operator 1



Operator 2

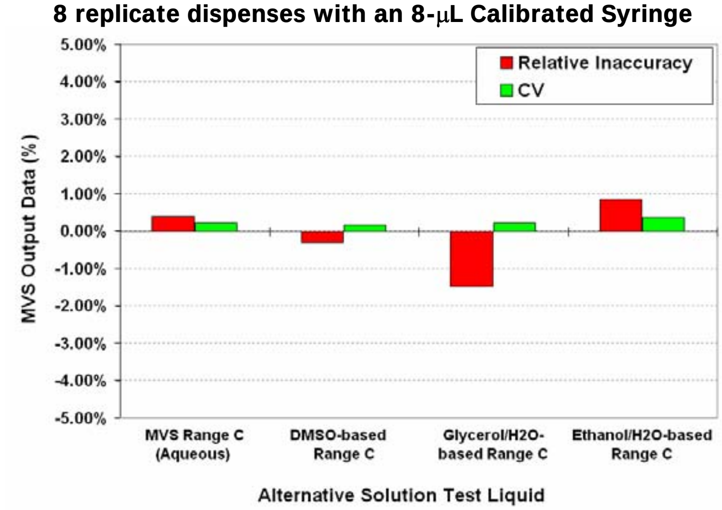


Column 1: "Single Dispense", 20 μL aspirated and dispensed.

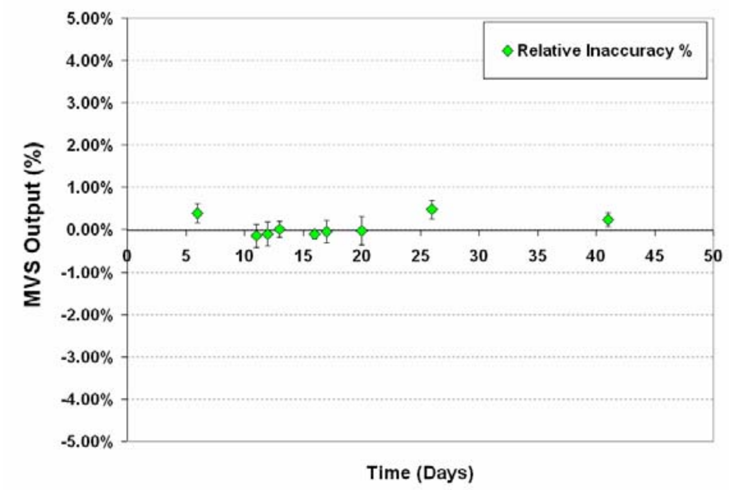
Columns 3 – 12: "Multi-sequential Dispense", 200 μL aspirated and 20 μL sequentially dispensed

Verifying Alternative Test Solutions

8 replicate dispenses with an 8-μL Calibrated Syringe

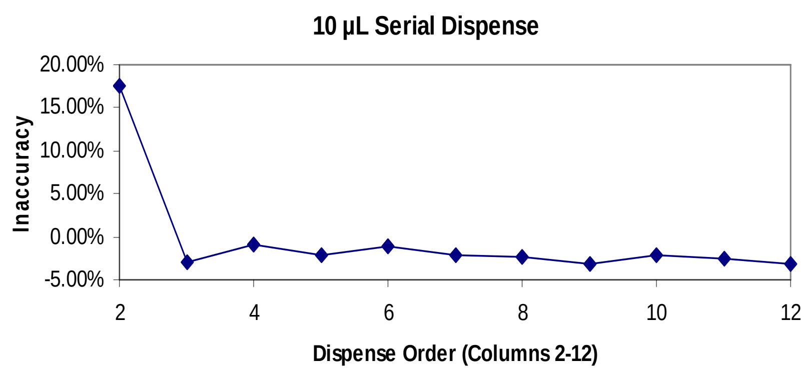


Monitoring Performance Trending Over Time



Calibrated 8-µL syringe; 8 repeat dispenses per data point

Dispense Order Trending



- The first dispense for this ALH was ~20% high, with more uniform performance for subsequent deliveries
- The effect at a test volume of 2 µL resulted in a 60% over-dispense

Other *Places* For MVS-guided Performance Optimizations

- Method scale-up or transfer.
- Assay or method troubleshooting.
- Quick volume "spot check".
- Employee training or during method programming.
- Device performance monitoring of all target volumes that are critical to every step in the assay.
- Sequential dispense trending (dispense order).
- Performance checks before and after scheduled maintenance or between calibration intervals.
- Prove regulatory compliance with NIST-traceable measurement results.

MVS: Quantifying Target Volume

- **96-well plates**
  - 0.1 – 200 μL
- **384-well plates**
  - 0.03 – 55 μL

Conclusions

- 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 to NIST
  - ISO approved