Method Validation for Beckman Coulter Liquid Handlers Using the Artel Multichannel Verification System (MVS®)

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The Beckman Coulter/Artel Partnership

- ***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 LDQA Services.
- ***Beckman Coulter, Inc.*** combines liquid handling and laboratory automation to deliver unique research tools for today's Drug Discovery and Development and Genomics and Proteomics laboratories.
- ***The Partnership*** allows Artel to demonstrate the ability of the MVS to validate performance and optimize methods while providing Beckman the ability to extend this technology to their users thereby assisting their customers to obtain precise and accurate automated liquid handler performance.

Liquid Handler Performance

- How is performance of your automated liquid handling equipment evaluated?
- How is performance maintained?
- Do you subscribe to one of these theories?
  - Routine use = reproducible performance
  - Quarterly or semi-annual calibration intervals are sufficient
  - Once maintenance is performed, device behavior is “like new”

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Liquid Handler Performance

- When a problem with performance is discovered:
  - How much data are called into question?
  - How does this affect budget?
    - Instrument down-time
    - Consumable waste
    - Labor resources

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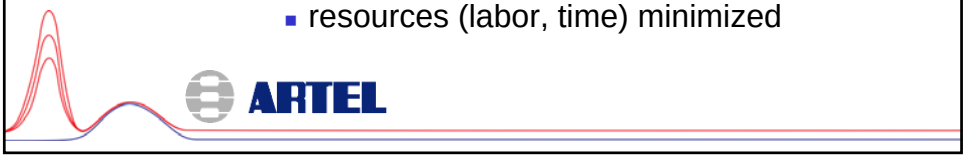
What if…

- …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

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

Method Parameters Affecting 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/cannulae
  - Max/min volume capacity
  - Fixed vs. disposable
  - Dry tip vs. wet tip
  - New tip vs. used tip
  - Carry-over
  - Tip-touches

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Multichannel Verification System (MVS®)

What is the Artel MVS®?

- Applies scientific principles to challenges faced by users of liquid handling equipment.
- Provides an easy-to-use, rapid, single-measurement platform for both precision & accuracy information well-by-well and tip-by-tip.
- Superior to conventional methods of performance verification.
- Measurement results are traceable to the National Institute of Standards and Technology (NIST).
- Method is acknowledged by ISO 8655.

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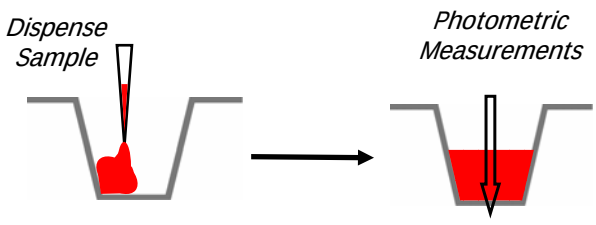
Dual-Dye Photometric Method

- 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



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MVS® Components

Characterized Microtiter Plates

Sample Solutions

Calibrator Plate

Plate Shaker

Notebook Computer w/ System Software & Barcode Reader

Microtiter Plate Reader

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MVS® Mobile Workstation

- Ability to transport the measurement device to the liquid handler.
- Mobile workstation allows for verification of equipment in multiple locations throughout a facility

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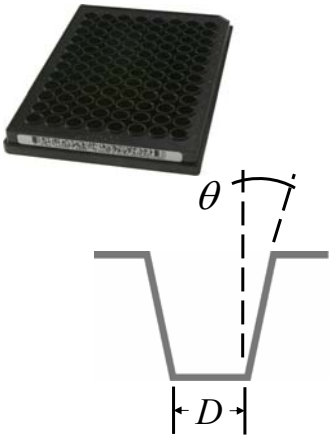
Bradshaw *et al*., *J. Assoc. Lab. Autom.*, 2005, 10, 35-42.

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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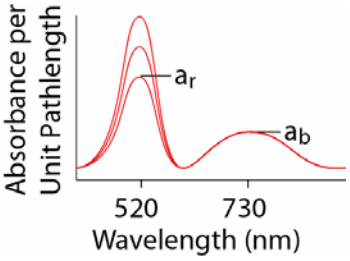
MVS® Sample Solutions

- 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



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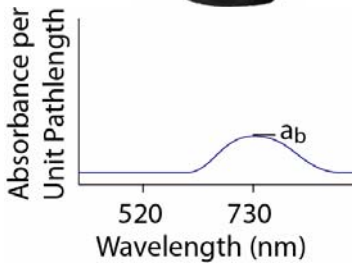
MVS® Diluent

- 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



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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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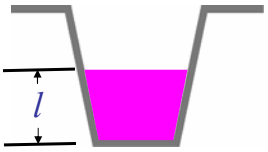
Calculation I: Liquid Depth

- 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



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

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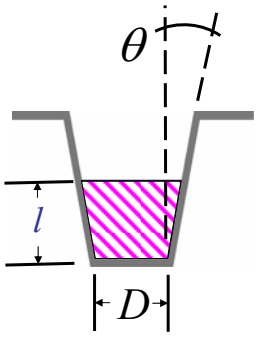
Calculation II: Total Volume

- 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



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

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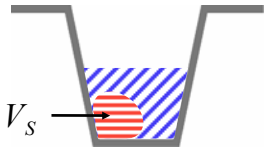
Calculation III: Sample Volume

- 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



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

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Quantifying Target Volume in Microtiter Plates

96-well, standard	0.1 – 200 µL
384-well, standard	0.03 – 55 µL
384-well, low-volume	0.019 – 28 µL
384-well, low-profile	0.01 – 20 µL

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Artel MVS NIST Traceability

Path of Traceability thru 3 measurement types:

Plate Reader Calibration	Solutions in calibrator plate cuvettes provide optical standards for daily reader correlation to NIST traceable spectrophotometer in Artel Laboratory.
Measurement of Dispensed Volumes	Physical well dimensions, absorbance per unit path length of solutions and photometric reader measurements are all NIST traceable, and necessary for calculating the dispensed sample volume.
Release testing	Release testing protocols for solutions and plates measure performance against a NIST traceable gravimetric method

Components of the Artel MVS work together to provide performance metrics about the liquid delivery capabilities of various multi channel dispensers. The accuracy and precision results generated by MVS are traceable to NIST, thus allowing standardization between laboratories.

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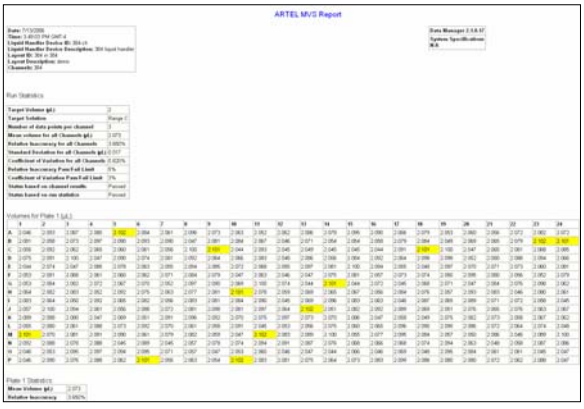
MVS Output Report

- Immediately generated after volume verification task
- Statistics are displayed by row, column, and channel
- Results that do pass tolerance specifications are flagged
- The report can be exported as HTML or XML for further analysis



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)

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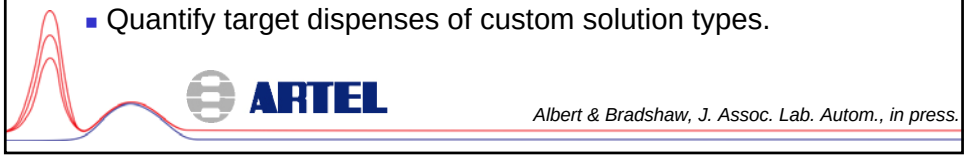
MVS as an Integration Tool

Assessing Liquid Handler Performance & Facilitating Optimization

Integrating the MVS into a process

- Calibration, OQ/PQ
- Method scale-up or transfer.
- Assay or method troubleshooting.
- Quick volume “spot check”.
- Employee training or during method programming.
- Sequential dispense trending (dispense order).
- Performance checks before and after scheduled maintenance or between calibration intervals.
- Show compliance with NIST-traceable measurement results.
- Quantify target dispenses of custom solution types.



Albert & Bradshaw, J. Assoc. Lab. Autom., in press.

Custom Test Liquids: “Alternative Solutions”

- Custom test liquids can be created to test instruments for dispensing performance for assay-specific reagents
- Concentrated dye stocks are added to the solvent-of-interest, and the software calculates the ‘Useable Volume Range (μL)” of the new solution
- Dimethly sulfoxide (DMSO) has been evaluated by Artel
- Custom solutions may require trial and error experimentation
- NIST traceability waived for any Alternative Test solution

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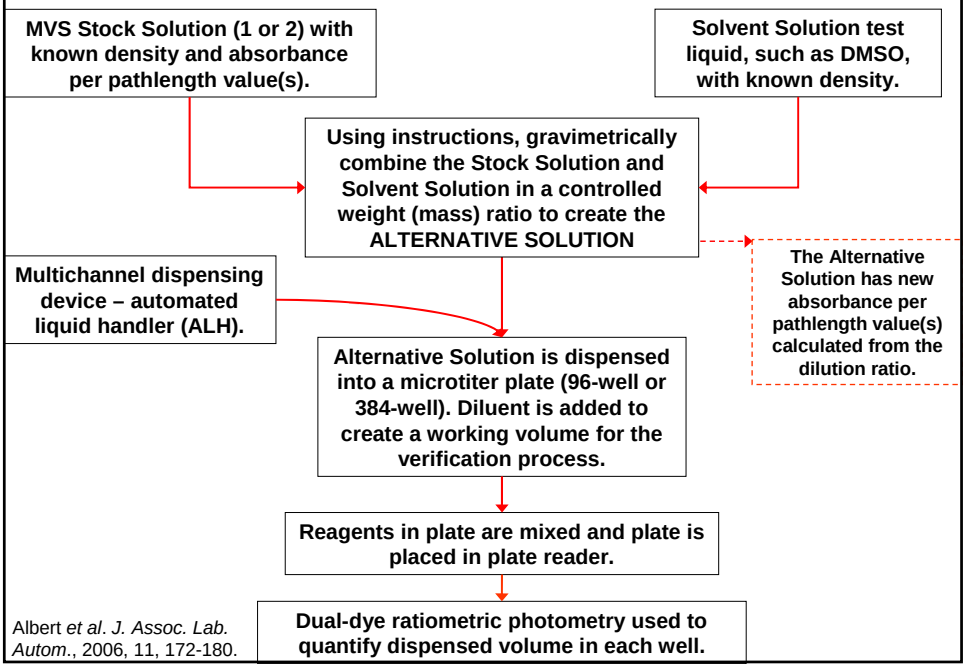
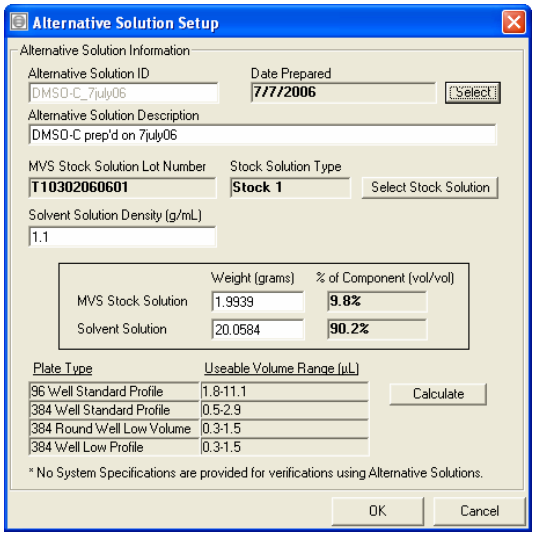
OVERVIEW of Alternative Solution Method



Albert *et al*. *J. Assoc. Lab. Autom*., 2006, 11, 172-180.

Alternative Solution Library



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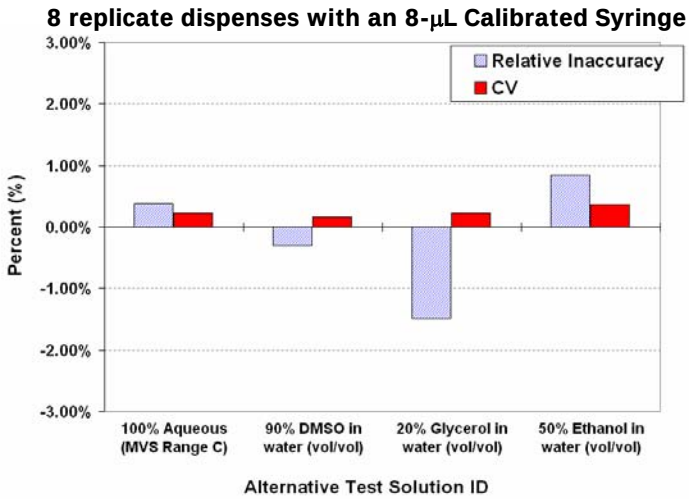
Albert *et al. J. Assoc. Lab. Autom.*, 2006, 11, 172-180.

Verifying Alternative Test Solutions



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Albert *et al. J. Assoc. Lab. Autom.*, 2006, 11, 172-180.

Other Examples of Integrating the MVS

Assessing Liquid Handler Performance & Facilitating Optimization

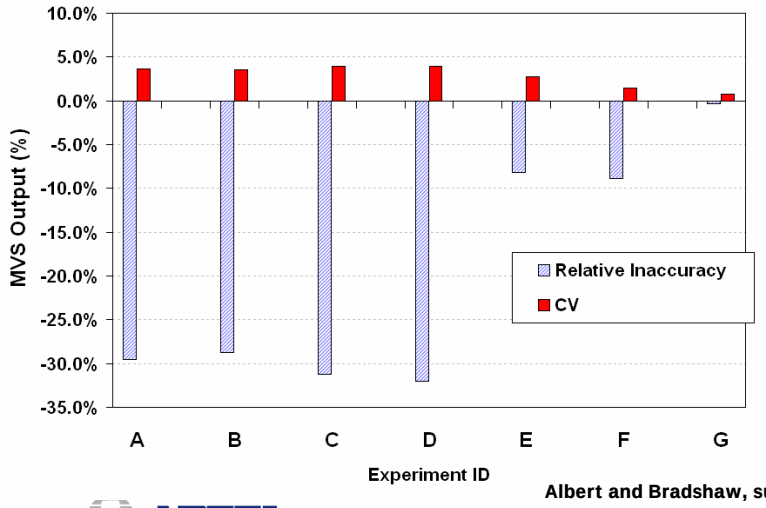
Optimizing Liquid Handler Performance via Sequential Parameter Adjustment (1 of 2)

- **Goal**
  - dispense 10 μL using a 96-channel liquid handler
- **Approach**
  - sequentially modify selected automation parameters to achieve goal

Modified Parameters	A	B	C	D	E	F	G
New tips or Used (pre-wetted) tips	New	**Used**	Used	Used	Used	Used	Used
Aspirate Rate (μL/s)	50	50	**5**	5	5	5	5
Dispense Rate (μL/s)	50	50	50	**5**	5	5	5
Leading Air Gap (μL)	0	0	0	0	**5**	5	5
Trailing Air Gap (μL)	0	0	0	0	0	**5**	5
Requested Volume (μL)	10	10	10	10	10	10	**10.88**

Albert and Bradshaw, submitted.

Optimizing Liquid Handler Performance via Sequential Parameter Adjustment (2 of 2)



Albert and Bradshaw, submitted.

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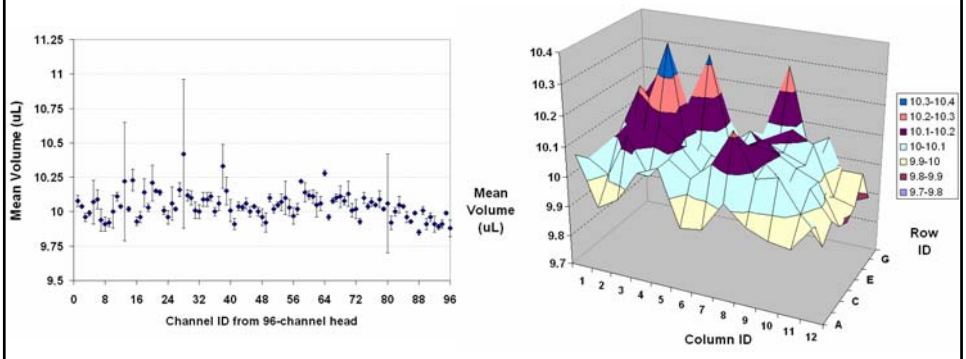
Performance Data for Every Channel

96-channel head, 10-μL target volume



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

Albert and Bradshaw, submitted.

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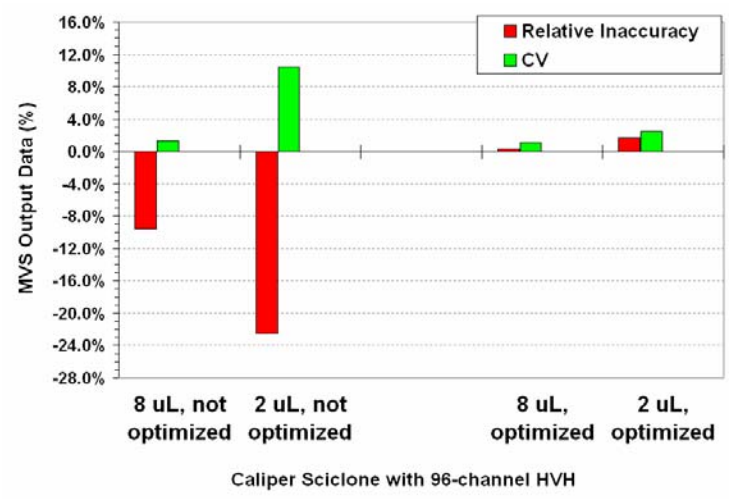
Target Volume Performance Optimization



Albert and Bradshaw, submitted.

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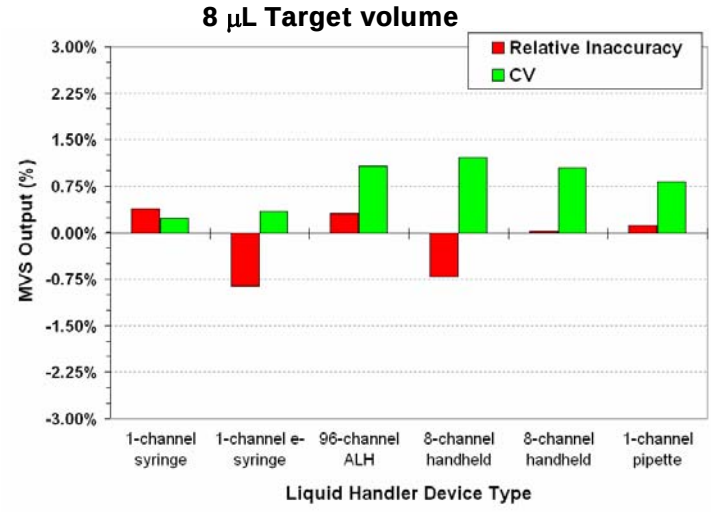
Device-to-device Comparison



Albert and Bradshaw, submitted.

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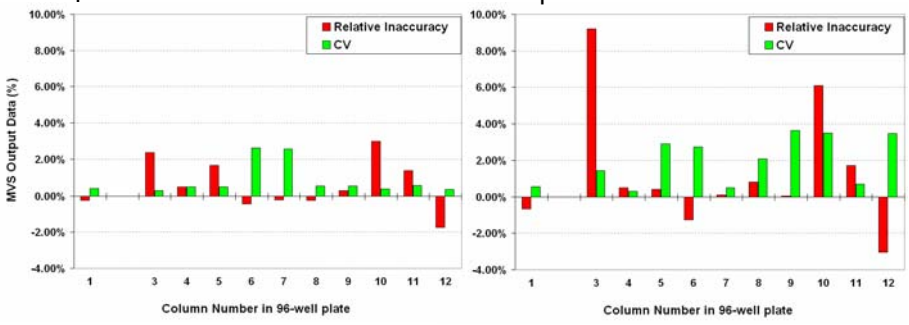
Operator-to-operator Comparison

Operator 1 | Operator 2



Column 1: "Single Dispense", 20 μL aspirated and dispensed with 8-ch electronic pipette.

Columns 3 – 12: "Multi-sequential Dispense", 200 μL aspirated and 20 μL sequentially dispensed using 8-ch electronic pipette

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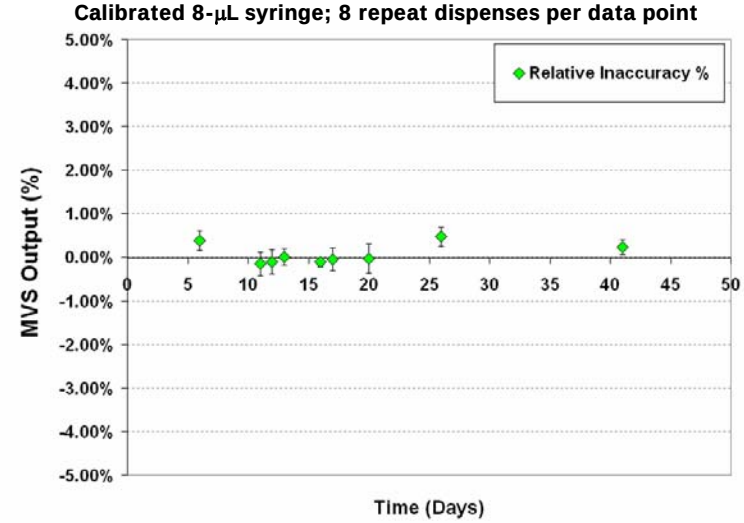
Monitoring Performance Trending Over Time

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



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Assessing Biomek FX Performance & Facilitating Method Optimization with MVS – A Case Study

Customer has Performance Concerns

- Customer is not achieving the same results with the Biomek FX as with the assay performed manually
- RT-PCR reaction setup which is primer limited.

Where is Accuracy Required?

Assay Plate

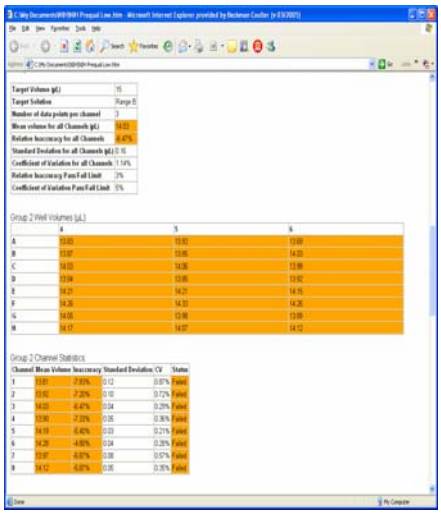
10 µL Sample	*In Excess*
15 µL Master Mix	*CRITICAL*

Place in Thermalcycler for 1½ hours

Results obtained

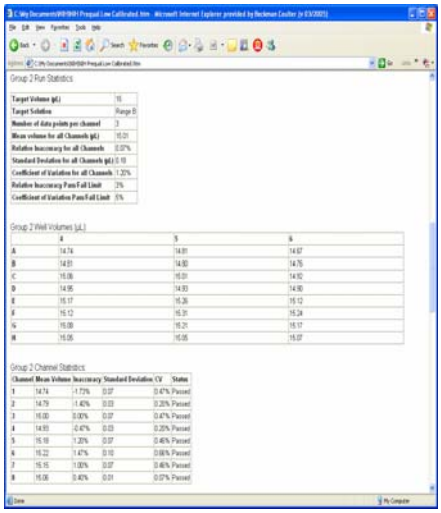
Assess Instrument Performance with MVS

- First test was “as found” with Scaling Factor and Offset set at 1 and 0, respectively



Adjust for Accuracy in Software

- Scaling Factor and Offset were adjusted to 1.045 and 0.354, respectively
- Test was repeated with new values
- Instrument meets specifications

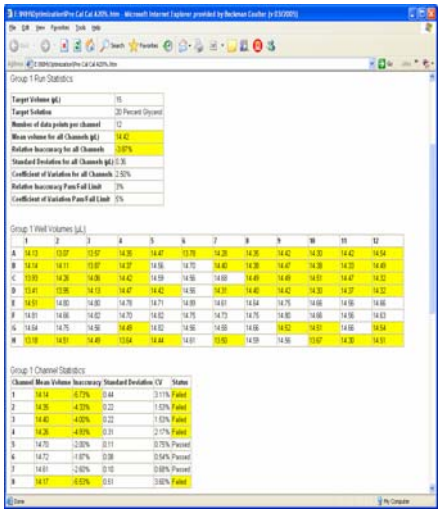


End of Story?

- Results obtained with automated assay still did not match those obtained with manual method
- What was wrong?????????

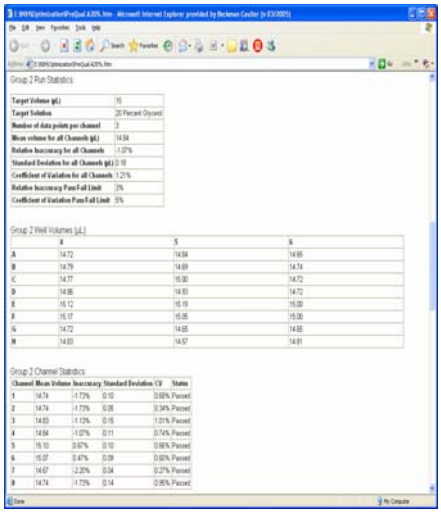
Liquid Type?

- Solution used in assay was master mix which contained approximately 20% glycerol
- Performance can be assessed with alternate solutions with MVS
- Tested instrument using same scaling factor and offset as was found previously, 1.045 and 0.354, respectively



Adjust for Accuracy in Software

- Scaling Factor and Offset were adjusted to 1.052 and 0.135, respectively
- Test was repeated with new values
- Instrument meets specifications

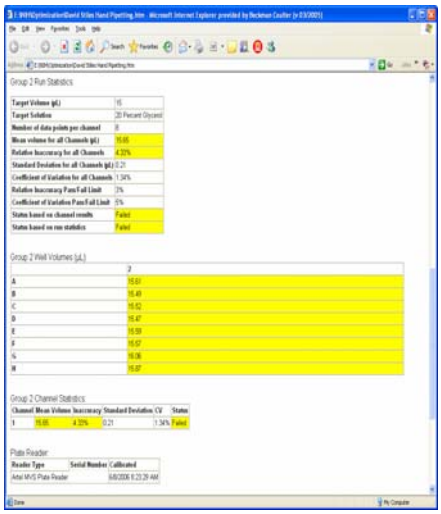


End of Story?

- Results obtained with automated assay still did not match those obtained with manual method
- What was wrong?????????

Manual Pipetting?

- Manual pipetting of technician was assessed with MVS solutions
- Why?
  - Hand pipettor could be out of specification
  - Poor pipetting technique of technician
- Technician was overpipetting the master mix
- Was this the answer?



Adjustments made

- Instrument was programmed to pipette 15.65 µL to match what technician was pipetting
- Results of manual assay and automated assay were the same in modified method

PROBLEM SOLVED!

Conclusions

- Biomek Software supports accuracy calibration for all liquid handlers.
- 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

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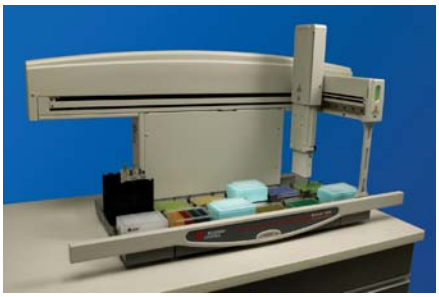
BECKMAN COULTER

Demonstration with the Biomek 3000 with the MP20 Tool



We are going to take a break

Come back for Liquid Handling 101

ARTEL precisely

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