Tutorial: Improving Your Assay Performance Through Liquid Handler Optimization

Nathaniel Hentz, Ph.D.  |  Keith J. Albert, Ph.D.
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12:30-1:15 P
Outline

Part I: Effect of Liquid Handler Performance (N. Hentz)
- Review common liquid handler QC methods
  - Artel MVS technology description
- Discuss two case studies examining the effect of liquid handler variability on assay performance

Part II: Liquid Handler Optimization (K.J. Albert)
- Describe the key processes/parameters for optimizing robotic LHs
Part I: Effect of Liquid Handler Performance
# Review of Liquid Handler QC Methods and Their Relative Strengths

<table>
<thead>
<tr>
<th>Method</th>
<th>&lt;1µL</th>
<th>1-20 µL</th>
<th>&gt;20 µL</th>
<th>Precision</th>
<th>Accuracy</th>
<th>Multi-channel</th>
<th>Alternative fluids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravimetric</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Absorbance (tartrazine)</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Fluorescence (fluorescein)</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Near Infrared (977, 900 nm)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Acoustic</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dual-Dye ratiometric</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>

1Strengths are based on a variety of factors including complexity, detection limits, whether a calibration curve is needed, etc.

2Used by Molecular Devices and Biotek not necessarily for LH QC, but for pathlength correction to normalize well-to-well variability.

3Acoustic is mentioned due to prevalence of acoustic dispensing, not because it is a widely accepted method of QC.
Artel MVS Technology

Three Calculations

1. Blue dye (730 nm) is used to determine pathlength: 
   \[ l = \frac{A_{730}}{a_b} \]

2. Well liquid volume \((V_T)\) is calculated using \(l\) and well dimensions \(\theta\) and \(D\)

3. Red dye (520 nm) used to determine Sample volume \((V_S)\) is calculated using:

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

Sample Solution is dispensed into each well (left). Diluent is dispensed into each well (center) and the plate is mixed. Absorbance measurements for each dye are collected (right) and the sample volume is calculated as described above.
Common Sources of Variability for Liquid Handler Calibration Measurements

- Pipettor
- Reagents
- Tips
- Temperature
- Operator
- Plate type

These contribute directly to the liquid dispensation variability

- Mixing
- Detector

These affect the measurement process
Effect of Plate Type on Liquid Handler Performance

We see a similar trend for other volumes: ~10% difference in accuracy across different plate types.
Implications

• Plate type does make a difference when using the MVS system for liquid handler verifications
  – Artel Verification plates provide the best accuracy and precision…by design

• Liquid handlers can be miscalibrated depending on plate type used
Effect of Liquid Handling Variability on Assay Performance

- Develop model high throughput assays
  - 96-well format
  - Protein binding and enzyme classes
  - Validate and characterize
- Intentionally vary volume of each assay component by ~10% to determine effect on various inhibitor IC50s
Streptavidin:Biotin-Fluorescein Assay
Principle

Streptavidin (SA) is a tetravalent biotin-binding protein that is isolated from *Streptomyces avidinii* and has a mass of 60.0 kDa. SA has a very high affinity for biotin ($K_d = 10^{-14}$ to $10^{-15}$ M).

![Biotin-Fluorescein (B-Fl)](image)


2. Ebner, A; Marek, M; Kaiser, K; Kada, G; Hahn, CD; Lackner, B; Gruber, HJ. *Methods in Molecular Biology*, 418, 2008, 73-88. Application of biotin-4-fluorescein in homogeneous fluorescence assays for avidin, streptavidin, and biotin or biotin derivatives.
Streptavidin Assay Details

• Assay Components
  – Phosphate buffered saline, pH 7.4, containing 0.1% BSA
  – Black, non-binding 96-well plates
  – Streptavidin (SA), 3 nM final
  – Biotin-fluorescein (labeled ligand), 10 nM final
  – Inhibitors, various nM

• Experimental Conditions
  – Add 25 µL of Biotin-FL
  – Add 25 µL of inhibitor
  – Add 25 µL of SA
  – Incubate for 60 min at room temperature
  – Read fluorescence: Ex = 485 nm and Em = 515 nm

• Compare IC$_{50}$s
A Comparison of Liquid Handlers

- The Precision XS was already decided upon and characterized
- Two additional liquid handlers were explored in order to mimic real assay development and assay transfer
  - Finnpipette F2 manual 8-channel pipetter (Thermo Scientific)
  - MicroFlo Select peristaltic bulk reagent dispenser (Biotek)
Assay Variability: Manual Pipetters

Min: 790 ± 12 (1.5%)
Max: 8376 ± 428 (5.1%)
Z-factor: 0.83
n = 48
Assay Variability: Peristaltic Pipetter

Min: 1731 ± 152 (8.8%)
Max: 7002 ± 396 (5.7%)
Z-factor: 0.69
n = 96

Min: 1691 ± 156 (9.3%)
Max: 6919 ± 339 (4.9%)
Z-factor: 0.72
n = 96

Min: 1746 ± 173 (9.9%)
Max: 6887 ± 391 (5.7%)
Z-factor: 0.67
n = 96
Assay Variability: Automated Pipetter

Min: 826 ± 10 (1.2%)
Max: 7633 ± 100 (1.3%)
Z-factor: 0.95
n = 48

Min: 819 ± 6 (0.8%)
Max: 7543 ± 135 (1.8%)
Z-factor: 0.94
n = 48

Min: 804 ± 13 (1.6%)
Max: 7588 ± 88 (1.2%)
Z-factor: 0.96
n = 48
## Liquid Handler Variability Experimental Design

<table>
<thead>
<tr>
<th>Plate ID</th>
<th>Biotin-Fl, μL</th>
<th>Cmpd, μL</th>
<th>SA, μL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
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<tr>
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<td>4</td>
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<tr>
<td>5</td>
<td>23</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td>7</td>
<td>23</td>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>27</td>
<td>23</td>
</tr>
</tbody>
</table>

*n = 3 for each condition*
Inhibitors Studied

- Biotin
- Iminobiotin (I-Biotin)
- Biotin Aminohexanoic Acid (Biotin-AH)
- Desthiobiotin (DT-Biotin)
Example Potency Curve Set: Desthiobiotin
## Summary of Results for Binding Protein

<table>
<thead>
<tr>
<th>Plate ID</th>
<th>Min</th>
<th>Max</th>
<th>S/B</th>
<th>Z'</th>
<th>Biotin</th>
<th>Biotin-AH</th>
<th>DT-biotin</th>
<th>I-Biotin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1164</td>
<td>7694</td>
<td>6.6</td>
<td>0.96</td>
<td>12.1</td>
<td>10.4</td>
<td>325</td>
<td>4047</td>
</tr>
<tr>
<td>2</td>
<td>1064</td>
<td>6722</td>
<td>6.3</td>
<td>0.96</td>
<td>13.7</td>
<td>9.47</td>
<td>336</td>
<td>3922</td>
</tr>
<tr>
<td>3</td>
<td>1213</td>
<td>8354</td>
<td>6.9</td>
<td>0.96</td>
<td>12.8</td>
<td>8.75</td>
<td>313</td>
<td>3887</td>
</tr>
<tr>
<td>4</td>
<td>1106</td>
<td>7027</td>
<td>6.4</td>
<td>0.96</td>
<td>14.2</td>
<td>10.5</td>
<td>360</td>
<td>4997</td>
</tr>
<tr>
<td>5</td>
<td>1100</td>
<td>6920</td>
<td>6.3</td>
<td>0.94</td>
<td>15.9</td>
<td>10.9</td>
<td>381</td>
<td>6734</td>
</tr>
<tr>
<td>6</td>
<td>1187</td>
<td>8132</td>
<td>6.8</td>
<td>0.96</td>
<td>10.8</td>
<td>9.03</td>
<td>272</td>
<td>3740</td>
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<tr>
<td>7</td>
<td>1085</td>
<td>6814</td>
<td>6.3</td>
<td>0.96</td>
<td>15.8</td>
<td>11.3</td>
<td>505</td>
<td>7739</td>
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<tr>
<td>8</td>
<td>1142</td>
<td>7552</td>
<td>6.6</td>
<td>0.94</td>
<td>8.27</td>
<td>6.35</td>
<td>310</td>
<td>3166</td>
</tr>
</tbody>
</table>

$\Delta$ potency | 7.6   | 5.0   | 233  | 4573  |
**α-Galactosidase Assay Principle**

4-Methylumbelliferyl α-D-galactopyranoside

α-Gal

Alpha-galactosidase is a lysosomal enzyme that catalyzes the hydrolysis of terminal α-galactosyl moieties from glycolipids and glycoproteins and is a target to address Fabry’s disease.

Product formation is detected by fluorescence: Ex 365 nm/Em 448 nm

*O Motabar et al., Current Chemical Genomics, 2010, 4, 67-73*
α-Galactosidase Assay Details

• **Assay Components**
  – 50-mM citric acid, pH 4.5
  – Black, non-binding 96-well plates
  – α-Galactosidase (A-Gal), 0.001 U/mL
  – 4-MUG (fluorescent substrate), 100 µM
  – Inhibitors, various nM

• **Experimental Conditions**
  – Add 25 µL of 4-MUG
  – Add 25 µL of inhibitor
  – Add 25 µL of A-Gal
  – Incubate for 40 min at room temperature
  – Add 125 µL Stop solution
  – Read fluorescence: Ex = 365 nm and Em = 448 nm

• **Compare IC50s**
α-Galactosidase Min-Max Study

Z’{sub P1} = 0.92
Z’{sub P2} = 0.89
Z’{sub P3} = 0.94
Z’{sub P4} = 0.91
### Liquid Handler Variability Experimental Set-up

<table>
<thead>
<tr>
<th>Plate ID</th>
<th>4-MUG, μL</th>
<th>Cmpd, μL</th>
<th>A-Gal, μL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>23</td>
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<td>7</td>
<td>23</td>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>27</td>
<td>23</td>
</tr>
</tbody>
</table>

\( n = 3 \) for each condition
Example Potency Curve Set: Cmpd B8299
## α-Galactosidase Assay Results Summary

<table>
<thead>
<tr>
<th>Plate ID</th>
<th>Min</th>
<th>Max</th>
<th>S/B</th>
<th>Z'</th>
<th>#9641</th>
<th>#B8299</th>
<th>#9305</th>
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<tbody>
<tr>
<td>1</td>
<td>17.0</td>
<td>6000</td>
<td>353</td>
<td>0.96</td>
<td>0.00785</td>
<td>0.0726</td>
<td>91.6</td>
</tr>
<tr>
<td>2</td>
<td>16.0</td>
<td>5129</td>
<td>321</td>
<td>0.95</td>
<td>0.00857</td>
<td>0.0695</td>
<td>97.5</td>
</tr>
<tr>
<td>3</td>
<td>17.5</td>
<td>4781</td>
<td>273</td>
<td>0.94</td>
<td>0.00701</td>
<td>0.0669</td>
<td>113.5</td>
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<tr>
<td>4</td>
<td>17.0</td>
<td>5583</td>
<td>328</td>
<td>0.90</td>
<td>0.00789</td>
<td>0.0596</td>
<td>107.8</td>
</tr>
<tr>
<td>5</td>
<td>17.0</td>
<td>5618</td>
<td>330</td>
<td>0.97</td>
<td>0.00845</td>
<td>0.0648</td>
<td>112.9</td>
</tr>
<tr>
<td>6</td>
<td>21.0</td>
<td>5928</td>
<td>282</td>
<td>0.94</td>
<td>0.00941</td>
<td>0.0769</td>
<td>85.6</td>
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<tr>
<td>7</td>
<td>16.0</td>
<td>5638</td>
<td>352</td>
<td>0.96</td>
<td>0.00842</td>
<td>0.0678</td>
<td>104.6</td>
</tr>
<tr>
<td>8</td>
<td>17.5</td>
<td>4781</td>
<td>273</td>
<td>0.94</td>
<td>0.00701</td>
<td>0.0694</td>
<td>95.1</td>
</tr>
</tbody>
</table>

**Δ potency**

0.0024 0.0173 27.9
Part I Conclusions

• Liquid handler variability does affect assay as measured by potency
• Resolution between higher potency compounds is decreased
• Important chemical scaffolds can be missed for lower potency inhibitors
• Standardize liquid handlers during assay transfer
  – Development → automation
  – HTS → confirmation → LG/LO → SAR
  – Replacement liquid handlers

Part II: Liquid Handler Optimization
Liquid Handling Matters

Optimize Pipetting Performance to Improve Assay Quality
What Do We Know?

- Assay results are impacted by liquid handling variability
  - Assays are dependent on reagent concentration(s)
  - Reagent concentrations are volume-dependent
  - **Assay integrity is therefore dependent on accurate volume delivery**

- How do you **evaluate** your liquid handlers?
- What do you **expect** from your liquid handlers?
- How do you **select** the right liquid handler for the job?
- Are your liquid handlers **transferring** the **same volume** to all wells?
- Can you **directly compare** one robot to the next?
The MVS is a Global Standard Measurement Platform

- Compare performance regardless of user, make, model, date or location…remove robot-to-robot differences

* Measurement results are traceable to international standards thru NIST. Images from: www.tecan.com; www.beckman.com; www.agilent.com
What To Do Once You Know Performance: Two Optimization Examples

[how do you attain better accuracy and assay confidence?]
Optimizing Liquid Handler Methods Can Be Accomplished Via Manipulation to Individual Parameters

- 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
Optimizing Liquid Handler Methods Can Be Accomplished Via Manipulation to Individual Parameters

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

Simple
Optimizing Methods Using Only the Calibration Equations, not Individual Parameters

1. Measure ‘as found’ performance for specific volume, or range
2. Determine calibration factors employed in the method
3. Based on ‘as found’ performance, determine new calibration factors
4. Plug in new factors
5. Retest
6. Repeat as needed until performance is ‘as desired’ (optimized)
Optimizing Volume Transfer Performance

Beckman Biomek NX/FX/3000
Prepare the Liquid Handler Deck & Method

(Common deck for a dual bridge Biomek FX)
Increase Liquid Handling & Assay Quality – Simply By Knowing Performance

Dispense dye solutions with robot or pipette and mix.

Read Plate.

Know Performance tip-by-tip and well-by-well.
1. Pre-optimization ("As Found") Performance Assessment

<table>
<thead>
<tr>
<th>Target Volume (μL)</th>
<th>2</th>
<th>5</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVS Mean Volume (μL)</td>
<td>1.443</td>
<td>4.266</td>
<td>7.232</td>
</tr>
<tr>
<td>Relative Inaccuracy %</td>
<td>-27.85 %</td>
<td>-14.68 %</td>
<td>-9.60 %</td>
</tr>
<tr>
<td>Standard Deviation (μL)</td>
<td>0.125</td>
<td>0.053</td>
<td>0.042</td>
</tr>
<tr>
<td>CV %</td>
<td>8.66 %</td>
<td>1.24 %</td>
<td>0.58 %</td>
</tr>
</tbody>
</table>
Determine Calibration Factors Employed

1. Draw Water from P1 using the Universal technique.

2. Dispense 2 µL of Water to P3 using the Universal technique.

Determine technique ID employed

Open Technique Browser

Open Specific Technique
Scaling and Offset Factors within Calibration Tab

Based on \( y = mx + b \)
Measuring “As Found” with Calibration Factors to Determine New SLOPE

3. Plot Theoretical (y) vs. Measured (x) to determine new Scaling/Offsets

\[ y = 1.032x + 0.548 \]
\[ R^2 = 1.000 \]

4. Update Factors Before Retesting

Scaling Factor: 1.032  Offset: 0.548
# 5. Retesting with New Calibration Factors

<table>
<thead>
<tr>
<th>Target Volume (µL)</th>
<th>2</th>
<th>5</th>
<th>8</th>
<th>2</th>
<th>5</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope and Offset</td>
<td>1 and 0</td>
<td>1 and 0</td>
<td>1 and 0</td>
<td>1.032 and 0.548</td>
<td>1.032 and 0.548</td>
<td>1.032 and 0.548</td>
</tr>
<tr>
<td>MVS Mean Volume (µL)</td>
<td>1.443</td>
<td>4.266</td>
<td>7.232</td>
<td>1.999</td>
<td>4.943</td>
<td>8.084</td>
</tr>
<tr>
<td>Relative Inaccuracy %</td>
<td>-27.85 %</td>
<td>-14.68 %</td>
<td>-9.60 %</td>
<td>-0.05 %</td>
<td>-1.14 %</td>
<td>1.05 %</td>
</tr>
<tr>
<td>Standard Dev (µL)</td>
<td>0.125</td>
<td>0.053</td>
<td>0.042</td>
<td>0.104</td>
<td>0.066</td>
<td>0.037</td>
</tr>
<tr>
<td>CV %</td>
<td>8.66 %</td>
<td>1.24 %</td>
<td>0.58 %</td>
<td>5.20 %</td>
<td>1.34 %</td>
<td>0.46 %</td>
</tr>
</tbody>
</table>
Optimizing Volume Transfer Performance: Agilent Bravo Platform
### 1. Pre-optimization (“As Found”) Performance Assessment

<table>
<thead>
<tr>
<th>Target Volume (µL)</th>
<th>3</th>
<th>6</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope and Offset (respectively)</td>
<td>1 and 0</td>
<td>1 and 0</td>
<td>1 and 0</td>
</tr>
<tr>
<td>MVS Mean Volume (µL)</td>
<td>2.873</td>
<td>5.861</td>
<td>8.917</td>
</tr>
<tr>
<td>Relative Inaccuracy %</td>
<td><strong>-4.24 %</strong></td>
<td><strong>-2.32 %</strong></td>
<td><strong>-0.93 %</strong></td>
</tr>
<tr>
<td>Standard Deviation (µL)</td>
<td>0.043</td>
<td>0.077</td>
<td>0.105</td>
</tr>
<tr>
<td>CV %</td>
<td>1.49</td>
<td>1.32</td>
<td>1.18</td>
</tr>
</tbody>
</table>
2. Determine Calibration Factors Employed

Determine Liquid Class Used in Method

Based on $y = mx + b$

Open Liquid Library Editor & Specific Liquid Class

Note Calibration factors on Equation tab
3. Plot Theoretical (y) vs. Measured (x) to determine new Scaling/Offsets

4. Update Factors Before Retesting

\[ y = 0.991x + 0.165 \]

\[ R^2 = 1.000 \]
5. Retesting with New Calibration Factors

### ‘As Found’ Performance

<table>
<thead>
<tr>
<th>Target Volume (μL)</th>
<th>3</th>
<th>6</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope and Offset</td>
<td>1 and 0</td>
<td>1 and 0</td>
<td>1 and 0</td>
</tr>
<tr>
<td>MVS Mean Volume (μL)</td>
<td>2.873</td>
<td>5.861</td>
<td>8.917</td>
</tr>
<tr>
<td>Relative Inaccuracy %</td>
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<td><strong>-2.32 %</strong></td>
<td><strong>-0.93 %</strong></td>
</tr>
<tr>
<td>Standard Deviation (μL)</td>
<td>0.043</td>
<td>0.077</td>
<td>0.105</td>
</tr>
<tr>
<td>CV %</td>
<td>1.49</td>
<td>1.32</td>
<td>1.18</td>
</tr>
</tbody>
</table>

### Optimized Performance

<table>
<thead>
<tr>
<th>Target Volume (μL)</th>
<th>3</th>
<th>6</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVS Mean Volume (μL)</td>
<td>0.991 and 0.165</td>
<td>0.991 and 0.165</td>
<td>0.991 and 0.165</td>
</tr>
<tr>
<td>Relative Inaccuracy %</td>
<td><strong>1.07 %</strong></td>
<td><strong>0.10 %</strong></td>
<td><strong>0.12 %</strong></td>
</tr>
<tr>
<td>Standard Deviation (μL)</td>
<td>0.043</td>
<td>0.064</td>
<td>0.0.825</td>
</tr>
<tr>
<td>CV %</td>
<td>1.42</td>
<td>1.06</td>
<td>0.91</td>
</tr>
</tbody>
</table>
Application Notes Describing Each Optimization Process

Optimizing Accuracy Performance on a Beckman Coulter Biomek Using the Artel MVS

ABSTRACT:
This application note discusses one approach to optimizing pipetting accuracy on a Beckman Coulter liquid handler using MVS mimetic information. Pipetting accuracy can be improved in a number of ways, such as by adjusting individual pipette variables (aspirate rates, dispense rates, gape, mix steps, etc.) and measuring and analyzing the volume resulting after each successive adjustment. The accuracy of the final volume delivered is dependent on the adjustment of the pipette variables. The focus of this application note is on optimizing pipetting accuracy on a Beckman Coulter Biomek liquid handler. The MVS system supports verifying both tip-to-tip precision and accuracy values. The accuracy of the pipetting performance is dependent on the adjustment of the pipette variables. The focus of this application note is on optimizing pipetting accuracy on a Beckman Coulter Biomek liquid handler.

INTRODUCTION:
This application note is based on the same process as described in Reference 1. This approach is intended to show that pipetting accuracy can be improved by simply adjusting the calibration values within the method's liquid class, which is a process that also works for other liquid handlers. The approach discussed here, the individual pipette variables, is relatively unimportant to the optimization process. The focus of this approach is on optimizing pipetting accuracy on a Beckman Coulter Biomek liquid handler. The MVS system supports verifying both tip-to-tip precision and accuracy values. The accuracy of the pipetting performance is dependent on the adjustment of the pipette variables. The focus of this application note is on optimizing pipetting accuracy on a Beckman Coulter Biomek liquid handler.

Optimizing Accuracy Performance on an Agilent Bravo Platform Using the Artel MVS

ABSTRACT:
This application note discusses one approach to optimizing pipetting accuracy on an Agilent Bravo liquid handler using MVS mimetic information. Pipetting accuracy can be improved in a number of ways, such as by adjusting individual pipette variables (aspirate rates, dispense rates, gape, mix steps, etc.) and measuring and analyzing the volume resulting after each successive adjustment. The accuracy of the final volume delivered is dependent on the adjustment of the pipette variables. The focus of this application note is on optimizing pipetting accuracy on an Agilent Bravo liquid handler. The MVS system supports verifying both tip-to-tip precision and accuracy values. The accuracy of the pipetting performance is dependent on the adjustment of the pipette variables. The focus of this application note is on optimizing pipetting accuracy on an Agilent Bravo liquid handler.

INTRODUCTION:
This application note is based on the same process as described in Reference 1. This approach is intended to show that pipetting accuracy can be improved by simply adjusting the calibration values within the method's liquid class, which is a process that also works for other liquid handlers. The focus of this application note is on optimizing pipetting accuracy on an Agilent Bravo liquid handler. The MVS system supports verifying both tip-to-tip precision and accuracy values. The accuracy of the pipetting performance is dependent on the adjustment of the pipette variables. The focus of this application note is on optimizing pipetting accuracy on an Agilent Bravo liquid handler.
Did You Know…. Two successive, inaccurate liquid transfers can cause reagent concentration errors to be >50% off?

Calibrating Liquid Handlers: Applying the Artel MVS to Correct the Accuracy of Pipetting Performance

Abstract

Laboratories utilizing liquid handling devices appreciate the efficiency benefits that these important instruments provide; however, modest yet-volatile uncertainties inherent in this technology can contribute to inaccurate results. Assays are dependent on accurate concentrations, and inaccurate concentrations can lead to incorrect results, which can be disastrous. Therefore, maintaining accurate concentrations can be very different within the standard deviation, particularly when using multichannel dispensers. Fortunately, Artel can help users monitor and control these variations.

Materials & Methods

All transfer solutions were aqueous and the target volumes were measured with the Artel MVS. Verification of accuracy was performed using a single-channel handheld pipet. According to the Artel MVS protocol, the volume was pipetted 10 times in calibration and was measured 30 times. The volume was then measured using a single-channel handheld pipet. This protocol was repeated for both the handheld pipet and the MVS.

Results

The results demonstrated that the MVS was more accurate than the handheld pipet. The MVS was able to deliver the target volume with a standard deviation of 0.05%, while the handheld pipet had a standard deviation of 0.15%. This demonstrates the importance of using accurate and reliable dispensers in laboratory settings.

Conclusion

The Artel MVS can help laboratories maintain accurate concentrations and deliver precise liquid volumes, leading to more reliable and reproducible results. 

References


Jul 1, 2013 (Vol. 33, No. 13)
Understand Your Assay Process and Determine Where Performance Needs Improvement (1 of 2)

**Standardization:**
Using a Universal Method to Assess Liquid Handler Performance & Behavior

- **Measure Residual Volume** in a well after Sample Aspiration
- Test Performance with **DMSO, non-Aqueous or custom reagents** (some limitations)
- **Measure Mixing Efficiency** in Microplates
Understand Your Assay Process and Determine Where Performance Needs Improvement (2 of 2)

Facilitate Method Transfer & troubleshooting

Compare **Acoustic Dispenser Performance**

Compare **disposable tip performance** vs. ‘economics’
Users of Automated Liquid Handlers Need To…

- Gain control of liquid handling QA & QC processes
- Increase confidence in assay results
- Become more proficient in liquid handler use and problem solving
- Learn how to diagnose and identify common factors that affect method transfer
- Understand liquid handler behavior for assay-specific tasks
- Recognize how liquid handler settings affect volume transfer accuracy
Thank You.