An Automated Bioreactor Sampling Solution for Assuring On-line PAT Analytical Fidelity

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Outline

• Automated Bioreactor Sampling
• Seg-Flow Technology Overview
• Analytical Performance Case Studies
  – Methodology & Acceptance Criteria
  – YSI 2700 Biochemistry Analyzer
  – Nova BioProfile 400 Analyzer
  – Vi-CELL XR Cell Analyzer
• Summary
• Global PAT solutions provider for bio & other processing industries
  • Upstream process focus
• Enabling technologies for on-line process analytics & data management
• Headquartered in Madison, WI USA
Automated Bioreactor Sampling

• Criteria
  – not compromise the bioreactor’s sterile environment
  – establish connectivity of process systems
  – facilitate a scale-independent strategy
  – seamlessly integrate the real-time data into the process management system
  – provide rapid and precise analysis that performs as good as or better than the manual off-line analytical method
Technology Overview
Seg-Flow Technology

Sample up to 8 Bioreactors
Integrate up to 16 Accessory Pumps
Feed Control

Sample Collection & Dilution
Biochemistry Analyzers
Sample delivery to 4 Analyzers/Fraction Collectors
HPLC
Data Acquisition
Cell Counters

Sample Retention & Off-line Analytics
Nutrient & Metabolite Monitoring
Amino Acid & Product Quantity
Cell Density & Viability

Data transfer to SCADA via OPC

User Defines Analytical & Bioreactor Interface

Interconnectivity to Existing Instrumentation
Case Studies
Methodology

• Objectives:
  – Does the Seg-Flow-integrated instrument perform as good as or better than the manufacturer’s precision specifications?
    • Is the analytical fidelity preserved with the Seg-Flow system?
  – Is the Seg-Flow automated on-line analytical method comparable to the manual off-line analytical method?
Methodology

• **Evaluation:**
  – Evaluate the integrated analytical performance of three analyzers commonly used with bioreactor culture monitoring.
    • YSI® 2700 Select Biochemistry Analyzer
    • Nova® BioProfile® 400 Analyzer
    • Vi-Cell® XR Cell Analyzer
Methodology

• General Scheme:

  – Precision Evaluation
    • Within run evaluation per manufacturer’s specifications

  – Comparability Evaluation
    • Evaluate ≥ 50% of the instrument’s measurement range

  – CDM/reagent standards used in lieu of live culture
    • Assure QC of analyte concentrations
    • 0.25 – 5.0L WV: serial dilutions to attain measurement ranges
Methodology

• **General Scheme:**
  
  – Analytical instruments QC’d prior to evaluation
    • Manufacturer’s linearity, QC standards used
  
  – Seg-Flow/single instrument integration
    • Sample cycle – purge, analysis & system cleaning
      – precision & comparability studies
    • Manual sample analysis performed ≤ 5 minutes of Seg-Flow system analysis
      – comparability study
Acceptance Criteria

• Performance standards based on:
  
  • Instrument manufacturer’s precision specifications
  
  • Accepted practices and standards
Acceptance Criteria

• Precision:
  
  • 2-point linearity check
  
  • Coefficient of variation (%CV) (YSI/Nova)
    – % CV ≤ the manufacturer’s within run specification
    – compares the dispersion or variation in groups of measurements
      • $\delta/\mu \times 100\%$
  
  • Concentration average accuracy (Vi-CELL XR)
    – Average accuracy within ± 3.0% of reference standard
Acceptance Criteria

• **Accuracy:**
  
  • Qualitative evaluation of Seg-Flow System
    – No analytical errors due to Seg-Flow sample delivery
      • Ensure prescribed sample volume and timing are achieved
Acceptance Criteria

• Comparability:

  • Linear Regression Analysis
    – Determine statistical relationship of two analytical methods
    – Correlation coefficient
      • $R \geq 0.98^3$
        • Strong positive linear correlation should exist between the Seg-Flow (automated) & manual analytical methods
    – Slope
      • 95% CI should include the value of 1.0
      • slope = 1.0 (perfect)
    – Intercept
      • 95% CI should include the value of 0.0
      • intercept = 0 (perfect)

Seg-Flow Integration: YSI® 2700 Biochemistry Analyzer

**Analytes**
- D-Glucose
- L-Lactate
- L-Glutamine
- L-Glutamate
## YSI 2700 Measurement Ranges & Precision Specifications

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Measurement Range</th>
<th>Evaluated Analytical Range</th>
<th>CV (%)</th>
<th>Sample size (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Glucose</td>
<td>0 – 25.0 g/L</td>
<td>0.5 – 15.0 g/L</td>
<td>2.0</td>
<td>10</td>
</tr>
<tr>
<td>L-Lactate</td>
<td>0 - 2.7 g/L</td>
<td>0.2 – 5.0 g/L</td>
<td>2.0</td>
<td>10</td>
</tr>
</tbody>
</table>
Seg-Flow/YSI 2700 Results

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Number of Samples Analyzed</th>
<th>Theoretical concentration (g/L)</th>
<th>Measured concentration (g/L) (µ ± δ)</th>
<th>CV (%)</th>
<th>YSI CV Spec. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Glucose</td>
<td>10</td>
<td>2.00</td>
<td>2.03 ± 0.04</td>
<td>1.98</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10.00</td>
<td>10.01 ± 0.20</td>
<td>1.96</td>
<td>2.00</td>
</tr>
<tr>
<td>L-Lactate</td>
<td>10</td>
<td>0.70</td>
<td>0.64 ± 0.01</td>
<td>1.56</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.70</td>
<td>2.74 ± 0.05</td>
<td>1.94</td>
<td>2.00</td>
</tr>
</tbody>
</table>

- **YSI 2700 precision linearity demonstrated**
  - %CV acceptance criteria met
- **Seg-Flow sample delivery accuracy achieved**
  - No analytical errors due to sample delivery
Seg-Flow/YSI 2700 Results

### Glucose

- **210 mm FISP Probe**
- **Dip Tube**

### Lactate

- **210 mm FISP Probe**
- **Dip Tube**

### Glutamate

- **210 mm FISP Probe**
- **Dip Tube**

### Glutamine

- **210 mm FISP Probe**
- **Dip Tube**
## Seg-Flow/YSI 2700 Results

### Seg-Flow/YSI 2700 Statistical Comparability

<table>
<thead>
<tr>
<th>Analyte</th>
<th>FISP Sampling Probe</th>
<th>Dip Tube</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>Slope (95% CI)</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>1.00</td>
<td>1.007 (0.994 to 1.020)</td>
</tr>
<tr>
<td>L-Lactate</td>
<td>1.00</td>
<td>0.998 (0.985 to 1.010)</td>
</tr>
<tr>
<td>L-Glutamate</td>
<td>1.00</td>
<td>0.985 (0.973 to 0.997)</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>1.00</td>
<td>0.988 (0.969 to 1.007)</td>
</tr>
</tbody>
</table>

- **Comparability demonstrated for Seg-Flow & manual analytical methods**
  - Acceptance criteria met:
    - $R \geq 0.98$
    - Slope & Intercept within 95% CI
  - Irrespective of sampling mechanism used
Seg-Flow Integration: Nova BioProfile 400

**Analytes**
- Glucose
- Lactate
- Glutamine
- Glutamate
- Ammonium
- pO2
- pCO2
- pH
- Potassium
- Sodium
- Osmolality
### Nova BP 400 Measurement Ranges & Within Run Precision Specifications

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Measurement Range</th>
<th>Evaluated Analytical Range</th>
<th>CV (%)</th>
<th>Sample size (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Glucose</td>
<td>0.2 – 15.0 g/L</td>
<td>0.5 – 15.0 g/L</td>
<td>5.0</td>
<td>20</td>
</tr>
<tr>
<td>L-Lactate</td>
<td>0.2 – 5.0 g/L</td>
<td>0.2 – 5.0 g/L</td>
<td>5.0</td>
<td>20</td>
</tr>
<tr>
<td>L-Glutamate</td>
<td>0.2 – 6.0 mmol/L</td>
<td>0.2 – 5.0 mmol/L</td>
<td>5.0</td>
<td>20</td>
</tr>
<tr>
<td>pO2</td>
<td>0 – 800 mmHg</td>
<td>170 – 230 mmHg</td>
<td>5.0</td>
<td>20</td>
</tr>
<tr>
<td>pCO2</td>
<td>3 – 300 mmHg</td>
<td>18 – 50 mmHg</td>
<td>5.0</td>
<td>20</td>
</tr>
</tbody>
</table>
### Seg-Flow/Nova 400 Results

• **Nova 400 precision acceptance criteria met**  
  – Precision linearity demonstrated

• **Seg-Flow sample delivery accuracy achieved**  
  – No analytical errors due to sample delivery

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Number of Samples Analyzed</th>
<th>Theoretical concentration</th>
<th>Measured concentration ($\mu \pm \delta$)</th>
<th>CV (%)</th>
<th>Nova CV Spec. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Glucose</td>
<td>20</td>
<td>8.0 g/L</td>
<td>8.1 ± 0.1</td>
<td>1.6</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>15.0 g/L</td>
<td>15.0 ± 0.4</td>
<td>2.5</td>
<td>5.0</td>
</tr>
<tr>
<td>L-Lactate</td>
<td>20</td>
<td>2.5 g/L</td>
<td>2.4 ± 0.1</td>
<td>2.1</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>5.0 g/L</td>
<td>4.7 ± 0.1</td>
<td>2.9</td>
<td>5.0</td>
</tr>
<tr>
<td>L-Glutamate</td>
<td>20</td>
<td>2.5 mmol/L</td>
<td>2.3 ± 0.1</td>
<td>2.7</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>4.5 mmol/L</td>
<td>4.3 ± 0.1</td>
<td>2.9</td>
<td>5.0</td>
</tr>
<tr>
<td>pO2</td>
<td>10</td>
<td>185 mmHg</td>
<td>185.7 ± 2.7</td>
<td>0.9</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>220 mmHg</td>
<td>220.5 ± 4.5</td>
<td>2.0</td>
<td>5.0</td>
</tr>
<tr>
<td>pCO2</td>
<td>10</td>
<td>18 mmHg</td>
<td>18.2 ± 0.3</td>
<td>1.9</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>48 mmHg</td>
<td>47.5 ± 0.8</td>
<td>1.8</td>
<td>5.0</td>
</tr>
</tbody>
</table>
Seg-Flow/Nova 400 Results

- **Glucose**
  - Off-line Glucose (g/L) vs. SegFlow On-line Glucose (g/L)
  - Data points for 210 mm FISP Probe and Dip Tube

- **Lactate**
  - Off-line Lactate (g/L) vs. SegFlow On-line Lactate (g/L)
  - Data points for 210 mm FISP Probe and Dip Tube

- **Glutamate**
  - Off-line Glutamate (mmol/L) vs. SegFlow On-line Glutamate (mmol/L)
  - Data points for 210 mm FISP Probe and Dip Tube

- **Glutamine**
  - Off-line Glutamine (mmol/L) vs. SegFlow On-line Glutamine (mmol/L)
  - Data points for 210 mm FISP Probe and Dip Tube
Seg-Flow/Nova 400 Results

- **pO2**:
  - Off-line pO2 (mm Hg) vs. SegFlow On-line pO2 (mm Hg)
  - Linear trend observed

- **pCO2**:
  - Off-line pCO2 (mm Hg) vs. SegFlow On-line pCO2 (mm Hg)
  - Linear trend observed

- **Ammonium**:
  - Off-line Ammonium (mmol/L) vs. SegFlow On-line Ammonium (mmol/L)
  - Linear trend observed
  - 210 mm FISP Probe
  - Dip Tube
## Seg-Flow/Nova 400 Results

### Seg-Flow/Nova BP 400 Statistical Comparability

<table>
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<th>Analyte</th>
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<tbody>
<tr>
<td></td>
<td>R</td>
<td>Slope (95% CI)</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>0.99</td>
<td>0.994 (0.928 to 1.059)</td>
</tr>
<tr>
<td>L-Lactate</td>
<td>0.99</td>
<td>0.942 (0.874 to 1.009)</td>
</tr>
<tr>
<td>L-Glutamate</td>
<td>0.99</td>
<td>0.994 (0.856 to 1.131)</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>1.00</td>
<td>1.048 (0.984 to 1.113)</td>
</tr>
<tr>
<td>Ammonium</td>
<td>1.00</td>
<td>1.044 (0.994 to 1.093)</td>
</tr>
<tr>
<td>pO2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pCO2</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- **Comparability demonstrated for Seg-Flow & manual analytical methods**
  - Acceptance criteria met:
    - \( R \geq 0.98 \)
    - Slope & Intercept within 95% CI
  - Irrespective of sampling mechanism used
Seg-Flow Integration: Vi-CELL® XR Cell Analyzer

**Analytes**
- VCC
- TCC
- % Viability
- Total cell count
- Viable cell count
- μ Cell Diameter
- μ Compactness
- Aggregation Rate
- Cell Imaging

- 12 hour test duration w/ 30 minute sample frequency
- # samples represent typical 2 - 4 week cell culture sampling (1 - 2/day)
- Cell concentration calibration beads used for analysis
Seg-Flow/Vi-CELL XR Results

Mean TCC, VCC and % Viability

• **Vi-CELL XR precision acceptance criteria met**
  – Concentration Average Accuracy: ± 3.0% (n = 20)
    • ± 2.4% average concentration accuracy for VCC (n=25)
    • ± 2.0% average concentration accuracy for TCC (n = 25)
    • ± 0.5 % difference observed for % viability (n = 25)

• **Seg-Flow sample delivery accuracy achieved**
  – No analytical errors due to sample delivery
Seg-Flow/Vi-CELL XR Results

Comparability demonstrated for Seg-Flow & manual analytical methods
- Acceptance criteria met:
  - $R \geq 0.98$
  - Slope & Intercept within 95% CI

<table>
<thead>
<tr>
<th>Analyte</th>
<th>$R$</th>
<th>Slope (95% CI)</th>
<th>Intercept (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCC</td>
<td>0.98</td>
<td>1.047 (0.982 to 1.112)</td>
<td>-0.01806 (-0.05543 to 0.01931)</td>
</tr>
<tr>
<td>TCC</td>
<td>0.98</td>
<td>1.034 (0.982 to 1.085)</td>
<td>-0.01040 (-0.04090 to 0.02009)</td>
</tr>
</tbody>
</table>
Summary

• Analytical fidelity (precision) preserved for each Seg-Flow-integrated analyzer
  – YSI 2700 Biochemistry Analyzer
  – Nova BioProfile 400 Analyzer
  – Vi-CELL XR Cell Analyzer

• Seg-Flow automated and manual analytical methods are statistically comparable
  – Fully automated system for delivering precise, reliable analyses
    • cell parameters, nutrients, metabolites and product

• Enabling on-line PAT solution for real-time bioreactor culture monitoring
  – Achieve deeper process understanding & increase process efficiency
Acknowledgements

Jayson Preston
Ashley Fisher

Matthew Rhyner, PhD
Lena Lee
Ara Kulhanjian
Thank You!

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