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**

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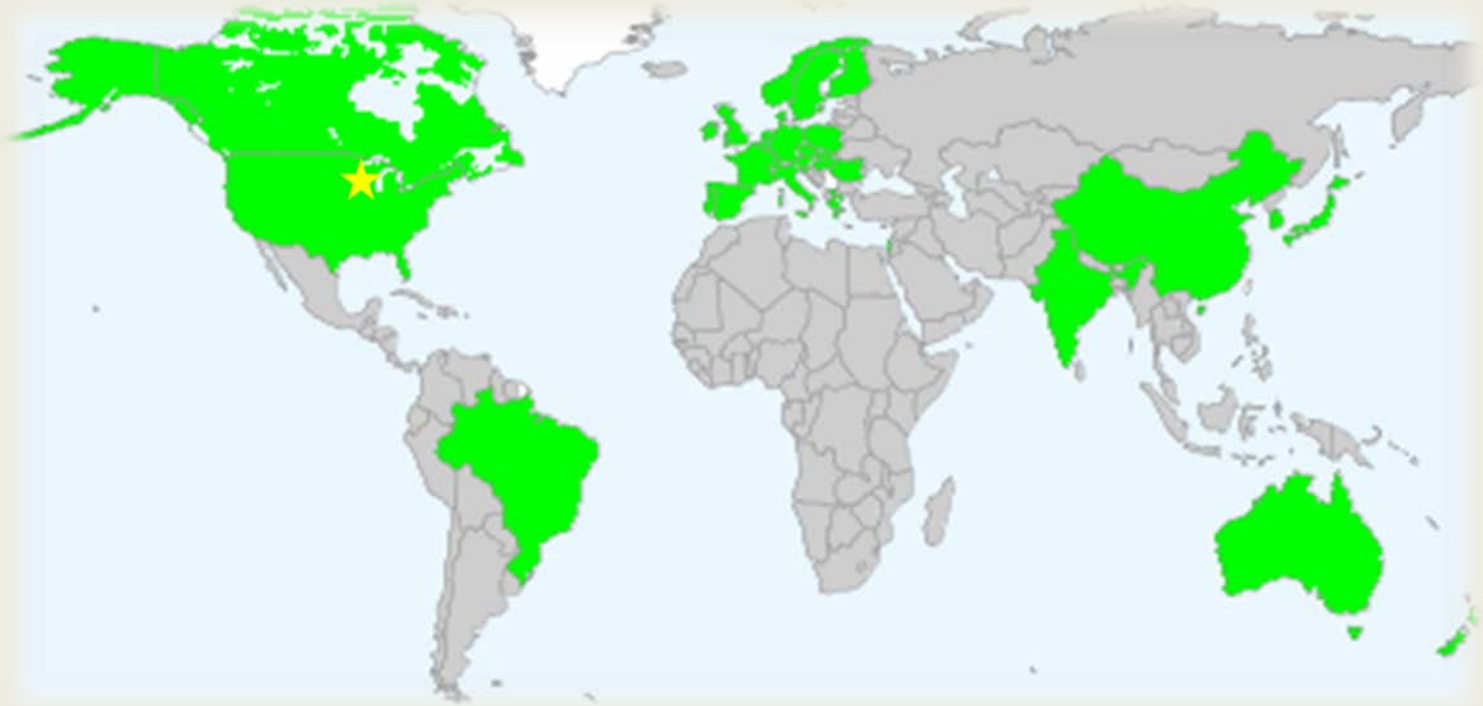

- **Global PAT solutions provider for bio & other processing industries**
  - Upstream process focus
- **Enabling technologies for on-line process analytics & data management**
- **Headquartered in Madison, WI USA**

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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

SEGFLOW®

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Seg-Flow Technology

Sample up to 8 Bioreactors

Integrate up to 16 Accessory Pumps

Feed Control

Sample Collection & Dilution

Sample Retention & Off-line Analytics

Biochemistry Analyzers

Nutrient & Metabolite Monitoring

Sample delivery to 4 Analyzers/Fraction Collectors

HPLC

Amino Acid & Product Quantity

Data Acquisition

Data transfer to SCADA via OPC

Cell Counters

Cell Density & Viability

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[2]
      - δ/μ x100%
  - 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 ≥ 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)**

3 *Krause, S. Good Analytical Method Validation Practice, Deriving Acceptance Criteria for the AMV Protocol: Part II. J. Val. Tech. 162-178. (Feb 2003)*

Seg-Flow Integration: YSI® 2700 Biochemistry Analyzer

Analytes

- D-Glucose
- L-Lactate
- L-Glutamine
- L-Glutamate

Seg-Flow Integration: YSI® 2700 Biochemistry Analyzer

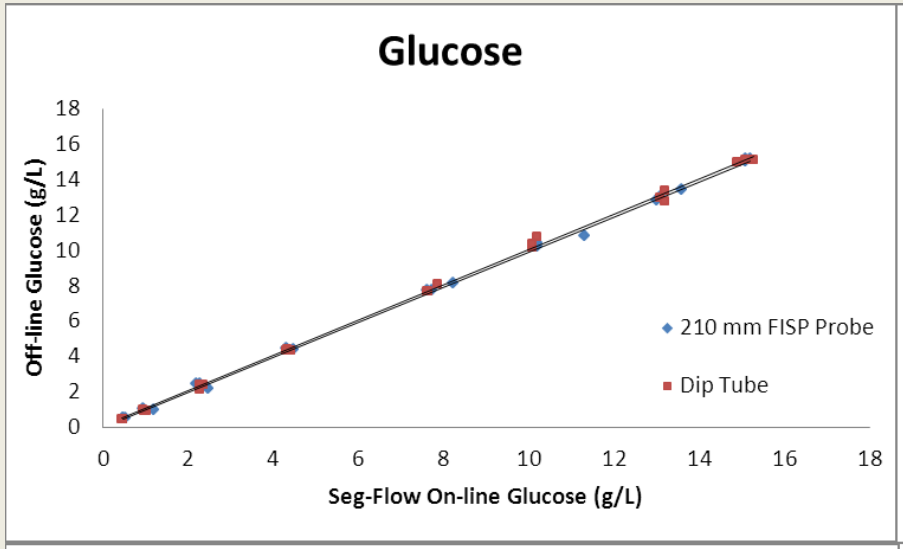
YSI 2700 Measurement Ranges & Precision Specifications				
Analyte	**Measurement Range**	**Evaluated Analytical Range**	**CV (%)**	**Sample size (n)**
D-Glucose	0 – 25.0 g/L	0.5 – 15.0 g/L	**2.0**	**10**
L-Lactate	0 - 2.7 g/L	0.2 – 5.0 g/L	**2.0**	**10**

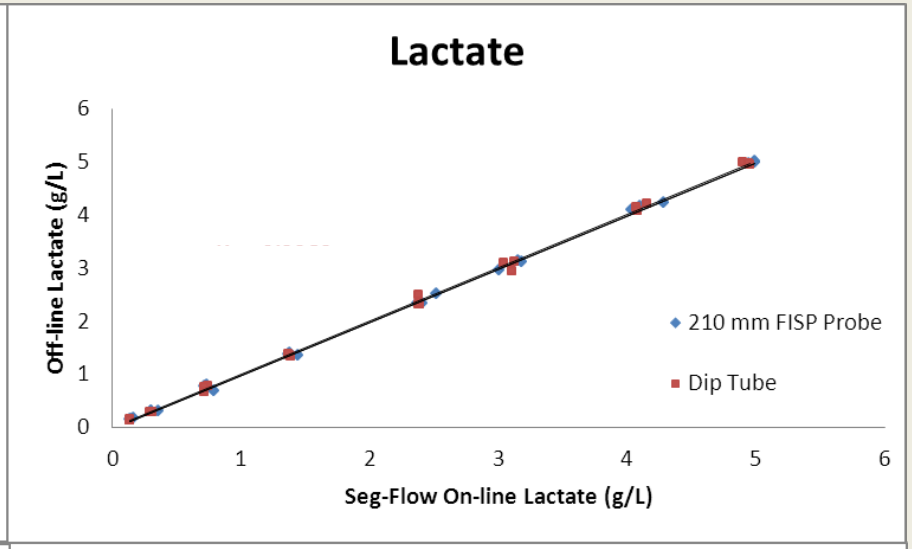
Seg-Flow/YSI 2700 Results

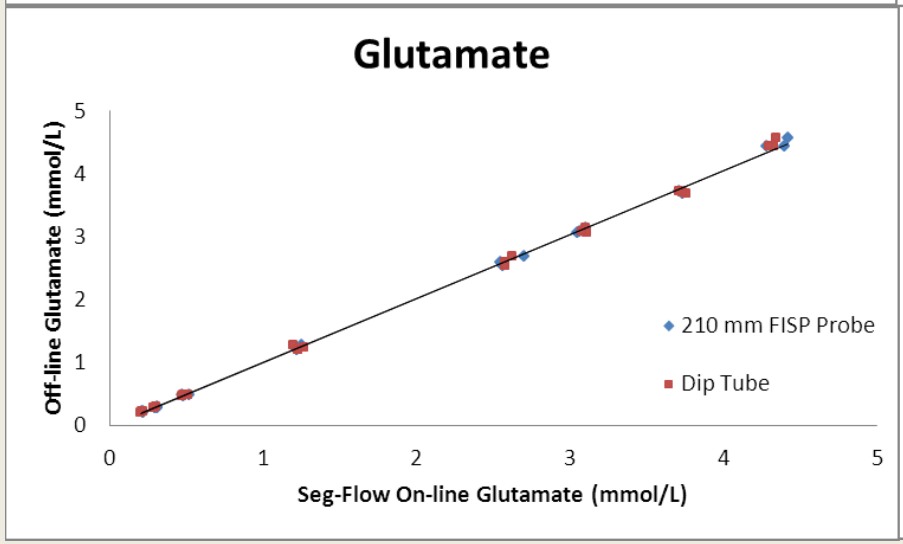
Seg-Flow/YSI 2700 within run precision					
Analyte	**Number of Samples Analyzed**	**Theoretical concentration (g/L)**	**Measured concentration (g/L) (μ ± δ)**	**CV (%)**	**YSI CV Spec. (%)**
D-Glucose	10	2.00	2.03 ± 0.04	**1.98**	2.00
	10	10.00	10.01 ± 0.20	**1.96**	2.00
L-Lactate	10	0.70	0.64 ± 0.01	**1.56**	2.00
	10	2.70	2.74 ± 0.05	**1.94**	2.00

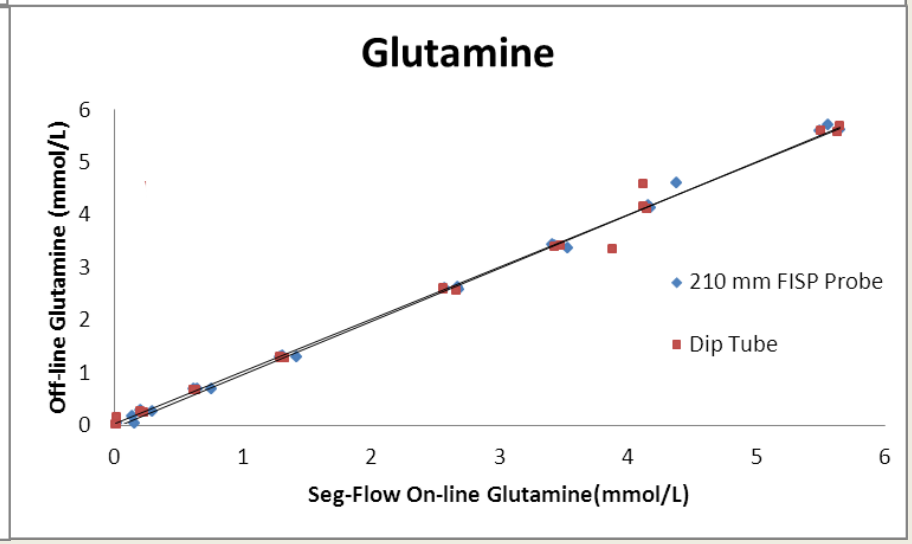
- **YSI 2700 precision linearity demonstrated**
  - %CV acceptance criteria met
- **Seg-Flow sample delivery accuracy achieved**
  - No analytical errors due to sample delivery

Seg-Flow/YSI 2700 Results









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Seg-Flow/YSI 2700 Results

Seg-Flow/YSI 2700 Statistical Comparability						
Analyte	FISP Sampling Probe			Dip Tube		
	R	Slope (95% CI)	Intercept (95% CI)	R	Slope (95% CI)	Intercept (95% CI)
D-Glucose	1.00	1.007 (0.994 to 1.020)	0.0048 (-0.1087 to 0.1183)	1.00	0.999 (0.983 to 1.014)	-0.0385 (-0.1710 to 0.0940)
L-Lactate	1.00	0.998 (0.985 to 1.010)	0.028 (-0.007 to 0.062)	1.00	0.992 (0.977 to 1.007)	0.013 (-0.028 to 0.053)
L-Glutamate	1.00	0.985 (0.973 to 0.997)	0.0148 (-0.0150 to 0.0445)	1.00	0.983 (0.967 to 1.000)	-0.0385 (-0.1710 to 0.0940)
L-Glutamine	1.00	0.988 (0.969 to 1.007)	0.0411 (-0.0161 to 0.0984)	1.00	0.988 (0.969 to 1.007)	0.0411 (-0.0161 to 0.0984)

- **Comparability demonstrated for Seg-Flow & manual analytical methods**
  - Acceptance criteria met:
    - R ≥ 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

Seg-Flow Integration: Nova BioProfile 400

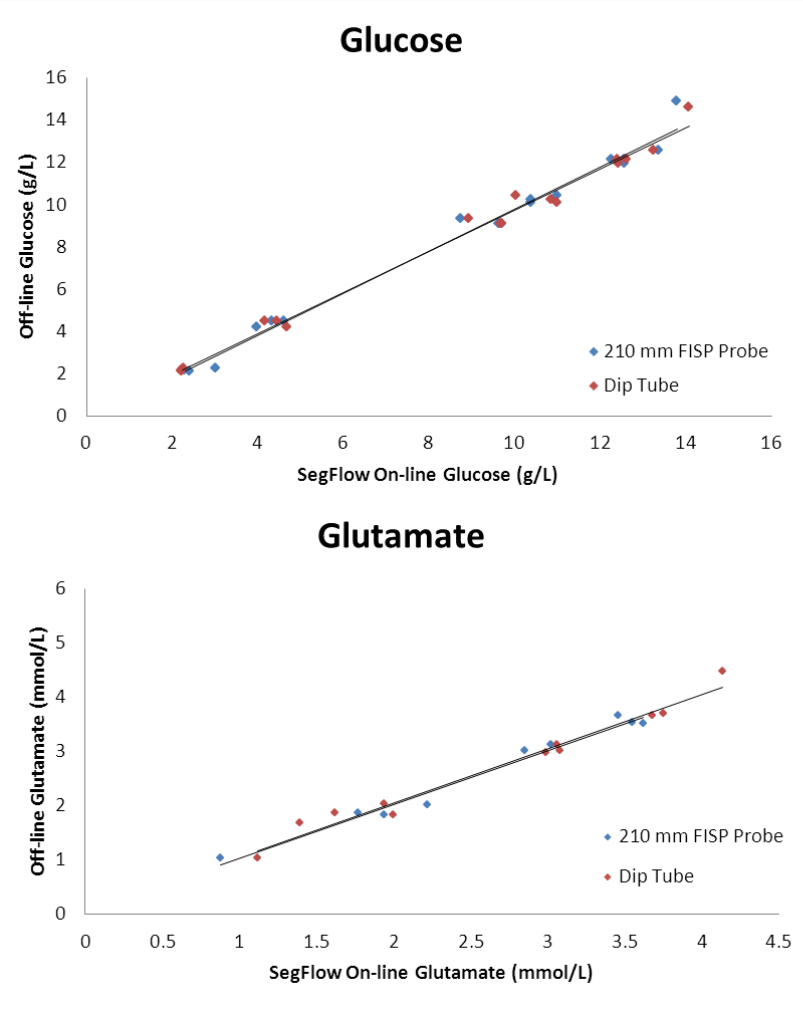
Nova BP 400 Measurement Ranges & Within Run Precision Specifications				
Analyte	**Measurement Range**	**Evaluated Analytical Range**	**CV (%)**	**Sample size (n)**
D-Glucose	0.2 – 15.0 g/L	0.5 – 15.0 g/L	**5.0**	**20**
L-Lactate	0.2 – 5.0 g/L	0.2 – 5.0 g/L	**5.0**	**20**
L-Glutamate	0.2 – 6.0 mmol/L	0.2 – 5.0 mmol/L	**5.0**	**20**
pO2	0 – 800 mmHg	170 – 230 mmHg	**5.0**	**20**
pCO2	3 – 300 mmHg	18 – 50 mmHg	**5.0**	**20**

Seg-Flow/Nova 400 Results

Seg-Flow/Nova 400 within run precision					
Analyte	**Number of Samples Analyzed**	**Theoretical concentration**	**Measured concentration (μ ± δ)**	**CV (%)**	**Nova CV Spec. (%)**
D-Glucose	20	8.0 g/L	8.1 ± 0.1	**1.6**	5.0
	20	15.0 g/L	15.0 ± 0.4	**2.5**	5.0
L-Lactate	20	2.5 g/L	2.4 ± 0.1	**2.1**	5.0
	20	5.0 g/L	4.7 ± 0.1	**2.9**	5.0
L-Glutamate	20	2.5 mmol/L	2.3 ± 0.1	**2.7**	5.0
	20	4.5 mmol/L	4.3 ± 0.1	**2.9**	5.0
pO2	10	185 mmHg	185.7 ± 2.7	**0.9**	5.0
	10	220 mmHg	220.5 ± 4.5	**2.0**	5.0
pCO2	10	18 mmHg	18.2 ± 0.3	**1.9**	5.0
	10	48 mmHg	47.5 ± 0.8	**1.8**	5.0

- **Nova 400 precision acceptance criteria met**
  - Precision linearity demonstrated
- **Seg-Flow sample delivery accuracy achieved**
  - No analytical errors due to sample delivery

Seg-Flow/Nova 400 Results



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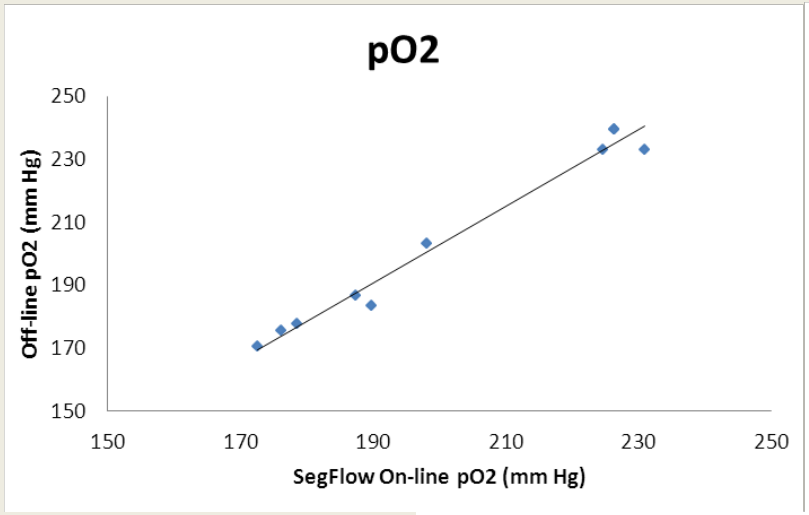
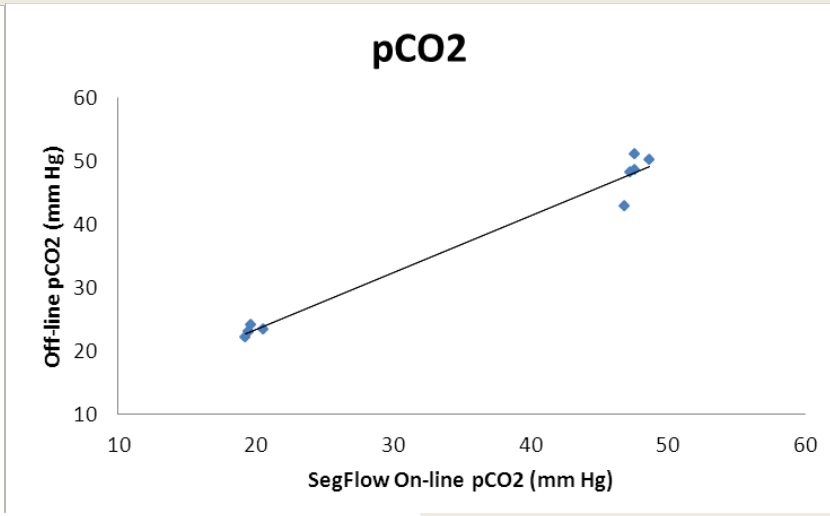
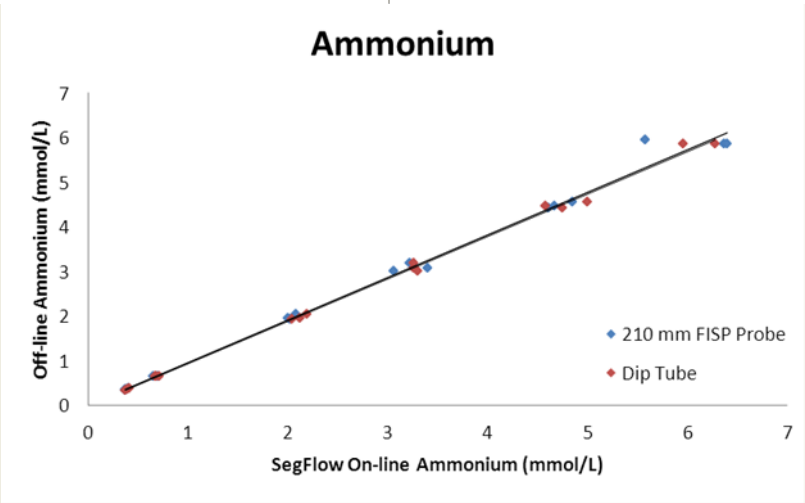
Seg-Flow/Nova 400 Results







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Seg-Flow/Nova 400 Results

Seg-Flow/Nova BP 400 Statistical Comparability						
Analyte	FISP Sampling Probe			Dip Tube		
	R	Slope (95% CI)	Intercept (95% CI)	R	Slope (95% CI)	Intercept (95% CI)
D-Glucose	**0.99**	0.994 **(0.928 to 1.059)**	0.260 **(-0.346 to 0.867)**	**0.99**	1.024 **(0.966 to 1.082)**	0.027 **(-0.508 to 0.563)**
L-Lactate	**0.99**	0.942 **(0.874 to 1.009)**	-0.126 **(-0.337 to 0.086)**	**0.99**	0.998 **(0.897 to 1.099)**	-0.332 **(-0.680 to 0.016)**
L-Glutamate	**0.99**	0.994 **(0.856 to 1.131)**	-0.003 **(-0.383 to 0.376)**	**0.99**	0.986 **(0.868 to 1.103)**	-0.007 **(-0.341 to 0.327)**
L-Glutamine	**1.00**	1.048 **(0.984 to 1.113)**	-0.106 **(-0.338 to 0.126)**	**0.99**	1.053 **(0.951 to 1.156)**	-0.218 **(-0.536 to 0.099)**
Ammonium	**1.00**	1.044 **(0.994 to 1.093)**	-0.003 **(-0.171 to 0.165)**	**1.00**	1.028 **(0.993 to 1.064)**	0.058 **(-0.052 to 0.168)**
pO2	-	-	-	**1.00**	0.924 **(0.816 to 1.032)**	13.98 **(-6.84 to 34.80)**
pCO2	-	-	-	**0.98**	1.066 **(0.842 to 1.290)**	-3.97 **(-13.50 to 5.57)**

- **Comparability demonstrated for Seg-Flow & manual analytical methods**
  - Acceptance criteria met:
    - R ≥ 0.98
    - Slope & Intercept within 95% CI
  - Irrespective of sampling mechanism used

Seg-Flow Integration: Vi-CELL® XR Cell Analyzer

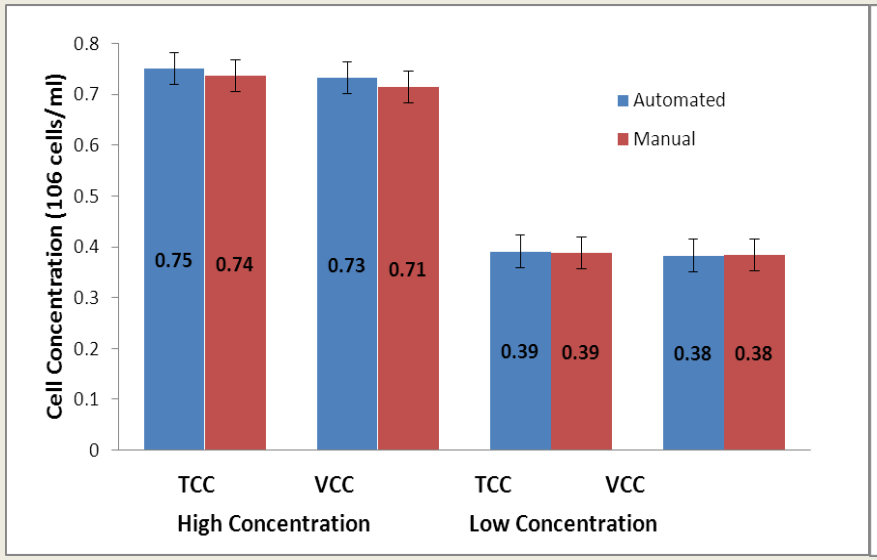
Analytes

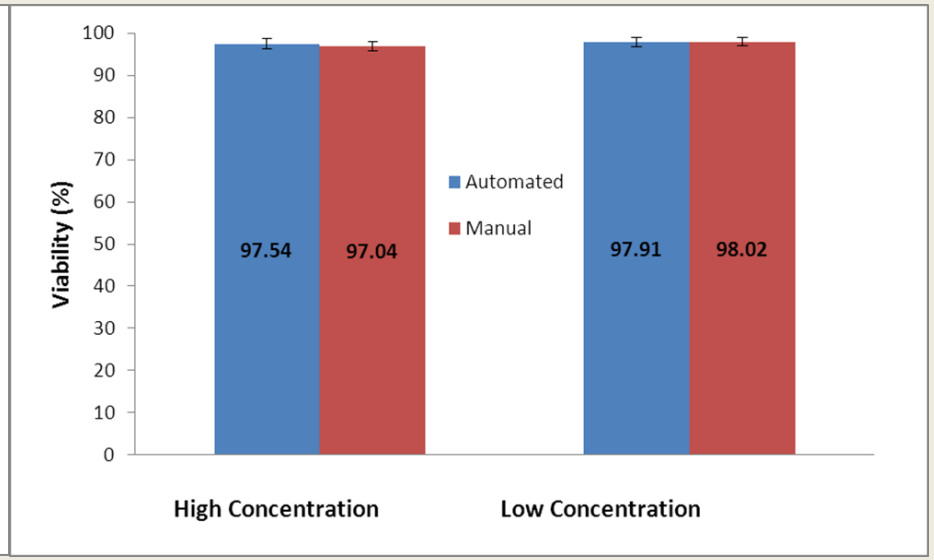
- **VCC**
- **TCC**
- **% Viability**
- Total cell count
- Viable cell count
- µ Cell Diameter
- µ Compactness
- Aggregation Rate
- Cell Imaging

- 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

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Seg-Flow/Vi-CELL XR Results

Seg-Flow/Vi-CELL XR Statistical Comparability			
Analyte	**R**	**Slope (95% CI)**	**Intercept (95% CI)**
VCC	**0.98**	1.047 **(0.982 to 1.112)**	-0.01806 **(-0.05543 to 0.01931)**
TCC	**0.98**	1.034 **(0.982 to 1.085)**	-0.01040 **(-0.04090 to 0.02009)**

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

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

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Thank You!

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