Nova BioProfile FLEX® Automated Analyzer

ANALYTICAL STUDIES

Cell Culture Monitoring Via an Auto-Sampler and an Integrated Multi-Functional Off-Line Analyzer
Genentech Inc. Oceanside, CA, USA
Genentech Inc. South San Francisco, CA, USA

Facilitating Multisite Bioprocess Transfer: Multi-Instrument and Multi-Platform Comparability and Long Term Stability of Nova Biomedical’s BioProfile® Chemistry and Gas Analyzers
Nova Biomedical, Waltham, MA, USA

PAT Tools for Accelerated Process Development and Improvement: Application to Cells on Microcarriers with Continuous Control of Metabolites Concentrations Using Nova Biomedical Flex Sampling and Analysis System
USP, Sanofi Pasteur, Lyon, France

Performance Evaluation of an Automated Bioreactor Sampling System for Mammalian Cell Cultivation
BioTherapeutics Pharm Sci, Pfizer Inc, St. Louis, MO, USA

Consolidated Single Analyzer Measurement of Key Cell Culture Constituents in the Development of Mammalian Cell Processes
Massachusetts Institute of Technology, Cambridge, MA, USA
Table of Contents

Cell Culture Monitoring Via an Auto-Sampler and an Integrated Multi-Functional Off-Line Analyzer.......................................................... 3
Gayle E. Derfus and Daniel Abramzon, Meg Tung, David Chang, Robert Kiss, Ashraf Amanullah Genentech Inc.

Facilitating Multisite Bioprocess Transfer: Multi-Instrument and Multi-Platform Comparability and Long Term Stability of Nova Biomedical’s BioProfile® Chemistry and Gas Analyzers............................................... 15
Matthew McRae, John McHale, Scott Granger, Brian Goulart, Elizabeth Kilcoyne, Roystein Bulman, Nova Biomedical, Waltham, MA, USA

PAT Tools for Accelerated Process Development and Improvement: Application to Cells on Microcarriers with Continuous Control of Metabolites Concentrations Using Nova Biomedical Flex Sampling and Analysis System........................................................................... 27
Eric Calvosa, Jean-Marc Guillaume, Bioprocessing R&D, USP, Sanofi Pasteur, Lyon. Cosette Deyirmendjian, Roystein Bulman, Andrei Malic, Nova Biomedical Europe

Performance Evaluation of an Automated Bioreactor Sampling System for Mammalian Cell Cultivation........................................................................... 31
Kavi Mehta, Erwin Y. Yu, Susan Casnocha BioTherapeutics Pharm Sci, Pfizer Inc, St. Louis, MO 63017

Consolidated Single Analyzer Measurement of Key Cell Culture Constituents in the Development of Mammalian Cell Processes................................................. 37
Carissa M. Moore and Jean-François P. Hamel Department of Chemical Engineering Massachusetts Institute of Technology, Cambridge, MA 02139
Cell Culture Monitoring Via an Auto-Sampler and an Integrated Multi-Functional Off-Line Analyzer

Gayle E. Derfus and Daniel Abramzon, Meg Tung, David Chang, Robert Kiss, Ashraf Amanullah
Genentech Inc.

This report describes the successful use of a novel integrated multi-functional analyzer and automated sampling system for mammalian cell culture bioprocess monitoring. The results suggest that the system has the potential to dramatically reduce the manual labor and associated errors involved in monitoring mammalian cell bioprocesses, without altering the quality of the data collected. The extensive testing across multiple instruments, multiple laboratory sites, and multiple operators gives a sound indication of the performance to be expected in a typical bioprocess development setting where many bioreactors are maintained. The automation of cell culture bioprocess monitoring will allow for more robust, operator-independent processes, with the potential for feedback control, resulting in increased efficiency, process understanding and process reproducibility while at the same time reducing the resources required to operate the cultures.
Cell Culture Monitoring Via an Auto-Sampler and an Integrated Multi-Functional Off-Line Analyzer

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Mammalian cell-based bioprocesses are used extensively for production of therapeutic proteins. Off-line monitoring of such cultivations via manual sampling is often labor-intensive and can introduce operator-dependent error into the process. An integrated multi-functional off-line analyzer, the BioProfile FLEX (NOVA Biomedical, Waltham MA) has been developed, which combines the functionality of three off-line analyzers (a cell counter, an osmometer, and a gas/electrolyte & nutrient/metabolite bio-profile analyzer) into one device. In addition, a novel automated sampling system has also been developed that allows the BioProfile FLEX to automatically analyze the culture conditions in as many as ten bioreactors. This is the first report on the development and function of this integrated analyzer and an auto-sampler prototype for monitoring of mammalian cell cultures. Evaluation of the BioProfile FLEX was conducted in two separate laboratories and involved two BioProfile FLEX analyzers and two sets of reference analyzers (Nova BioProfile 400, Beckman-Coulter Vi-Cell AS, and Advanced Instruments Osmometer 3900), 13 CHO cell lines and over 20 operators. In general, BioProfile FLEX measurements were equivalent to those obtained using reference analyzers, and the auto-sampler did not alter the samples it provided to the BioProfile FLEX. These results suggest that the system has the potential to dramatically reduce the manual labor involved in monitoring mammalian cell bioprocesses without altering the quality of the data obtained, and integration with a bioreactor control system will allow feedback control of parameters previously available only for off-line monitoring.

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Keywords: mammalian cell culture, process monitoring, BioProfile flex, auto-sampler, chinese hamster ovary

Introduction

Bioprocesses based on mammalian cell culture are used extensively for industrial production of therapeutic proteins. Cell culture monitoring is essential for the development, optimization, and control of highly productive and robust processes. Some culture parameters such as pH, dissolved oxygen, and temperature, can be monitored using in situ sensors, which has allowed the implementation of automated feedback control of these parameters. With the ability to control these aspects of cell culture microenvironment, reproducible, scalable, and robust bioprocesses can be implemented.

Other important culture parameters, however, are still typically monitored via manual sampling and several off-line analyzers. Some of these parameters are used to trigger in-process actions, such as the addition of a feed in response to an off-line nutrient concentration reading. Other parameters, such as cell viability, dissolved gases, nutrients, osmolality, and cellular by-products, are tracked as metrics of culture reproducibility. Thus this information is important both for bioprocess implementation and for understanding the impact of culture conditions on performance, productivity, and product quality.

Off-line monitoring of these important parameters is labor-intensive due to the manual nature of collecting...
samples, presenting them to several analyzers, and entering data. This large number of manual steps introduces operator-dependent variability and error into the process. In addition, each sample taken manually requires an at-risk breach of the sterility barrier, which can increase the probability of culture contamination.

With the goals of reducing the manual labor involved in bioprocess monitoring and improving data reliability, various new online and at-line monitoring tools have been developed over the last decade. Many of these advances have been successfully applied to cell culture systems, including optical density probes for monitoring cell density; near infrared spectroscopy (NIR) for monitoring various cell culture constituents; flow injection analysis (FIA) for sample delivery to offline analyzers; online high performance liquid chromatography (HPLC) for monitoring amino acid and other metabolite concentrations; and automated samplers connected to off-line analyzers such as HPLC for at-line monitoring. Although NIR technology could, in theory, preclude the need for off-line and at-line culture analyses, significant challenges remain for the successful industrial application of the technology for cell culture monitoring. These include establishment of robust calibration and data analysis methods that can be implemented routinely by nonexpert operators as well as development of biomass quantification methods for mammalian cells as previously shown for microbial cultures.

Thus the need remains for conventional off-line cell culture monitoring. A recent advancement in off-line cell culture monitoring was the introduction of integrated analyzer technology. The BioProfile FLEX (Nova Biomedical, Inc., Waltham, Massachusetts) combines the functionality of three off-line analyzers (a bio-profile analyzer, a cell counter, an osmometer) into one device. The analyzer design is modular in nature, including gas/electrolyte, nutrient/metabolite, cell density/viability, and osmometer modules (shown in Figure 1B). An IgG module is also available, but was not tested here. The gas/electrolyte and nutrient/metabolite modules use ion-selective electrode potentiometry, amperometry, and enzymatic reaction-dependent biosensors. The cell density/viability module was designed by Nova Biomedical to count cells and assess viability based on the traditional trypan blue exclusion method. Briefly, the module first mixes the cell-containing sample with trypan blue, and the mixture is incubated so that dead cells absorb the trypan blue. The sample then settles on the translucent bottom of a flow cell. The flow cell is moved past a fixed 10x microscope objective using a precision motion control system, with high resolution images obtained at 40 locations. Cell counts and viability are quantified by image processing based on user-defined constraints such as live/dead cell brightness and average cell sizes. The osmometer module is a freezing point osmometer model manufactured by Advanced Instruments specifically for this application. Table 1 shows the analytes measured by each module, and BioProfile FLEX analytical ranges for each analyte are published by Nova Biomedical. Each module can be selected individually, and if all modules are selected the BioProfile FLEX requires 1 mL sample volume and 7.5 min to run the analyzes. Results and user input are exported electronically in spreadsheet format.

Although the integrated analyzer and its electronic data output streamline some of the manual labor involved in cell culture monitoring, a new automated sampling system has been designed specifically for sample output to the BioProfile FLEX (Nova Biomedical) that could eliminate manual sampling altogether. The system consists of a switching pumping module (SPM) designed to fit directly underneath the BioProfile FLEX, up to 10 reactor valve modules (RVM) placed adjacent to the bioreactors, as well as containers for cleaning solution, saline flush, and waste collection (Figure 1). The bioreactor, RVM, and SPM are connected via 1 mm inner diameter (0.8 mL/m hold-up volume) stainless steel lines of lengths configured to fit a given bioreactor arrangement. The stainless steel lines are connected to the SPM and RVM using compression fittings. The line connecting the RVM and
the bioreactor extends through the head-plate to the bottom of the bioreactor, with a very small (~1 mL) dead volume in the line.

The SPM drives all fluid movement within the auto-sampler system via a single 5 mL syringe pump, and each RVM serves to protect the sterility of one bioreactor. The direction and location of fluid flow is determined by the opening and closing of various valves throughout the system. The SPM contains 10 sample inlet ports (one for each RVM). To pull a sample from a given bioreactor, the SPM inlet port valve opens and the pump is activated to draw a vacuum against a two-way valve within the RVM. The RVM valve opens only when the pressure gradient, determined by a built-in pressure sensor, is such that flow will be out of the bioreactor. After a predetermined sample volume flows from the bioreactor to the SPM, both valves close, preventing any further fluid exchange with the bioreactor. The sample is then pumped to a holding coil inside the SPM, and in-line air detectors (one located at the outlet of the RVM, and another at the inlet of the SPM) are used to ensure that the desired sample volume has been obtained and pressure has stabilized before the SPM delivers a portion of the sample to the BioProfile FLEX. Following sample aspiration by the BioProfile FLEX, all remaining sample is pumped to a single waste container placed next to the SPM. The SPM then pumps an alcohol-based cleaning solution through the entire sample flow path, followed by saline flush solution, in preparation for the next sample.

The culture volume required for automated sampling depends on the length of the stainless steel lines connecting the RVM and SPM, and is determined such that the first portion of the sample plug acts as a prime of the line, and the BioProfile FLEX takes 1 mL for analysis from the end of the sample plug. The auto-sampler adds roughly 3 min to the total sample time per reactor. For 10 bioreactors, the entire system occupies roughly 0.7 m² of lab bench space (Figure 1A). Once the system is installed (SPM, RVMs in place, performed by the vendor), the only set-up required for each culture run is connecting each bioreactor to its RVM, priming the bioreactor-RVM line, and setting the automated sampling schedule. Maintenance of the system includes replacing flush and cleaning fluid supplies and emptying waste containers during culture runs, and cleaning the bioreactor-RVM connection lines between cultures.

The objectives of this work were twofold. The first was to evaluate the performance of the BioProfile FLEX in terms of (i) equivalence of its measurements to those of currently utilized instruments, and (ii) inter-instrument reproducibility among three separate BioProfile FLEX analyzers. The second was to assess the function of a prototype auto-sampler in terms of (i) reliability of communication between the auto-sampler and the BioProfile FLEX, (ii) maintenance of culture sterility, and (iii) equivalence of BioProfile FLEX measurements from automated and manual samples.

### Materials and Methods

For the BioProfile FLEX evaluation, industrial suspension-adapted Chinese Hamster Ovary (CHO) cell lines from Gentech Inc. were cultivated. The cell lines were derived from a dihydrofolate reductase minus (dhfr-) CHO host, and genetically engineered to secrete IgG1 recombinant proteins of interest using a dhfr/methotrexate selection method similar to that described previously. Cells were maintained in proprietary medium adapted from DMEM and Ham’s F 12 media. Cultivations were carried out in 2-L bioreactors controlled by a DeltaV based BioNet system (Broadley-James, Irvine, CA) or by digital control units (Sartorius, Edgewood, NY). The bioreactors (Applikon Inc., Foster City, CA) were equipped with calibrated dissolved oxygen, pH, and temperature probes. Temperature control was achieved via a heating blanket. Dissolved oxygen was controlled on-line through sparging with air and/or oxygen, and pH was controlled through additions of CO₂ or 1 M Na₂CO₃. Cultures were monitored over the course of 12–14 days via daily samples (9 mL) extracted manually from the reactor via a sample port containing a one-way check valve and a syringe attachment valve. A portion of each sample was presented to one or more off-line analyzers, as described later in detail for each specific study.

### BioProfile FLEX versus reference analyzers

The performance of the BioProfile FLEX was assessed by comparing BioProfile FLEX measurements to those of the instruments currently utilized for cell culture monitoring, using cell culture samples obtained as described earlier. The currently utilized instruments, herein referred to as reference analyzers, include a bio-profile analyzer that employs ion-selective electrode potentiometry, amperometry, and enzymatic reaction-dependent biosensors (BioProfile 400, Nova Biomedical, Inc., Waltham, Massachusetts), a trypan blue exclusion and digital imaging based cell counter (Vi-Cell AS, Beckman Coulter, Inc., Fullerton, California), and freezing point osmometer (Model 3900, Advanced Instruments, Inc., Norwood, Massachusetts). The analytes measured by each instrument are shown in Table 1.

### Table 1. Analytes Measured by Each BioProfile Flex module, and the Reference Analyzers Used for Comparison for Each Analyte

<table>
<thead>
<tr>
<th>FLEX module</th>
<th>Analyte</th>
<th>Symbol</th>
<th>Units</th>
<th>Reference analyzer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas, Electrolyte</td>
<td>pH</td>
<td>pH</td>
<td>pH units</td>
<td>Nova BioProfile 400</td>
</tr>
<tr>
<td></td>
<td>Partial pressure oxygen</td>
<td>pO₂</td>
<td>mmHg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Partial pressure carbon dioxide</td>
<td>pCO₂</td>
<td>mmHg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ammonium ion</td>
<td>NH₄⁺</td>
<td>mM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Potassium ion</td>
<td>K⁺</td>
<td>mM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium ion</td>
<td>Na⁺</td>
<td>mM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calcium ion</td>
<td>Ca²⁺</td>
<td>mM</td>
<td>NA</td>
</tr>
<tr>
<td>Nutrient, Metabolite</td>
<td>Glucose</td>
<td>glu</td>
<td>g/L</td>
<td>Nova BioProfile 400</td>
</tr>
<tr>
<td></td>
<td>Lactate</td>
<td>lac</td>
<td>g/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glutamine</td>
<td>glu</td>
<td>mM</td>
<td>Beckman-Coulter Vi-Cell AS</td>
</tr>
<tr>
<td></td>
<td>Glutamate</td>
<td>glu</td>
<td>mM</td>
<td></td>
</tr>
<tr>
<td>Cell Density</td>
<td>Viable cell density</td>
<td>vcd</td>
<td>le5 cells/mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Viability</td>
<td>viab</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Live cell diameter</td>
<td>diam</td>
<td>μm</td>
<td></td>
</tr>
<tr>
<td>Osmometer</td>
<td>Osmolality</td>
<td>osmo</td>
<td>mOsm/kg</td>
<td>Advanced Instruments 3900</td>
</tr>
</tbody>
</table>

The objectives of this work were twofold. The first was to evaluate the performance of the BioProfile FLEX in terms of (i) equivalence of its measurements to those of currently utilized instruments, and (ii) inter-instrument reproducibility among three separate BioProfile FLEX analyzers. The second was to assess the function of a prototype auto-sampler in terms of (i) reliability of communication between the auto-sampler and the BioProfile FLEX, (ii) maintenance of culture sterility, and (iii) equivalence of BioProfile FLEX measurements from automated and manual samples.
For this study, a cell culture sample consisted of a single 9 mL draw from a bioreactor via a syringe, presented to the BioProfile 400 (~0.8 mL) and BioProfile FLEX (~1 mL) directly from the syringe, and to the Vi-Cell AS (~1 mL) and Osmometer 3900 (~200 μL) in tray mode. Care was taken to minimize the elapsed time between presentation of sample to the BioProfile 400 and BioProfile FLEX to minimize effects of off-gassing and cellular metabolism between measurements. This study was conducted in two separate laboratories, and involved two BioProfile FLEX analyzers with the original software version (upgrades have since been released), two sets of reference analyzers, 13 CHO cell lines and over 20 operators. Each operator obtained samples of a single cell type (from his/her cultures ongoing for other studies), and presented them to a single set of analyzers.

During the evaluation period, the BioProfile FLEX and reference analyzers were tested daily using quality control (QC) standards of known analyte concentrations. The QC standards and the expected mean values and standard deviations for each analyte were provided by Nova Biomedical. Each analyzer was designated as “passing QC” for a given analyte if its output was within three standard deviations of the expected mean value for each QC standard. For each analyte, a cell culture sample result was removed from the evaluation dataset if it was collected during a day when any analyzer did not pass QC for that analyte. An example is shown in Figure 2.

For each analyte, data were analyzed by (a) linear regression of the BioProfile FLEX measurements versus the reference analyzer measurements for cell culture samples and QC standards, (b) calculation of the difference between the BioProfile FLEX values and reference analyzer values for cell culture samples, and (c) qualitative comparison of analyte time-course profiles produced via automated sampling to those generated by the reference analyzers for cell culture samples. Linear regression fits were calculated and the linear regression slope, intercept, and correlation coefficient were tabulated. For each cell culture sample, the difference between the BioProfile FLEX value and the reference analyzer value was computed, and these difference values were averaged to get an overall average difference between the two analyzers. The percentage of BioProfile FLEX measurements that differed from the reference analyzer measurement by less than 10% and 25% (or by less than 0.1 and 0.15 pH units, for pH) was also computed for each analyte.

**Inter-BioProfile FLEX variability assessment**

Variability among three different BioProfile FLEX analyzers was assessed essentially as described for the comparison of the BioProfile FLEX and reference analyzers, with two exceptions: (1) the cell culture samples were presented to all three BioProfile FLEX analyzers and (2) QC standard results were not used to eliminate cell culture data. Thus, all cell culture sample data were included in the analysis, regardless of whether or not the analyzers passed QC, in an attempt to capture the potential “worst case” operating performance of the analyzers as it is possible that in certain settings the instruments will not be qualified daily prior to use. Data were analyzed by linear regression and difference calculation as described earlier.

**Auto-sampler prototype evaluation**

The utility of the auto-sampler was evaluated in terms of (i) reliability of communication between the auto-sampler and the BioProfile FLEX, (ii) culture sterility maintenance, and (iii) equivalence of BioProfile FLEX measurements from automated and manual samples. The impact of automated sampling on BioProfile FLEX measurements for cell culture samples was determined for two separate 12-day cultures, each consisting of two 2-L bioreactors run as described earlier.

Bioreactors were autoclaved after assembly with the stainless steel line for connection to an auto-sampler prototype RVM. Before connection of the bioreactor to the RVM, the interior fluidics of the RVM were cleaned with isopropyl alcohol (IPA). The stainless steel line was connected to the RVM using a compression fitting, following copious irrigation of both ends of the fitting with IPA. Each RVM was connected to the prototype SPM with a 3 m length, 1 mm inner diameter, stainless steel line. Sample volume was set at 7.5 mL. Up to four automated samples were collected from each reactor per day. For each automated sample, a manual sample was subsequently collected as described earlier, and presented to the BioProfile FLEX when analysis of the automated sample was completed. For each analyte, the data were analyzed by (a) linear regression of the automated sample measurements versus the manual sample measurements, (b) calculation of the difference between the automated and manual measurements, and (c) qualitative comparison of analyte time-course profiles produced via automated sampling to those produced via manual sampling.

**Results**

**BioProfile FLEX versus reference analyzers**

Batch and fed-batch cell culture samples using a total of 13 different cell lines were analyzed, with 230–664 measurements per analyte included in the final dataset after some measurements were eliminated from the analysis due to the analyte level being outside the specified ranges of the

![Figure 2. Example of measurements produced by the BioProfile FLEX (solid symbols) and reference analyzer (open symbols) for three different quality control (QC) standard solutions (triangles, diamonds, squares). Acceptance ranges for each solution represent ±3 standard deviations from the expected mean value (provided by Nova Biomedical), and are shown as dotted lines. Each arrow indicates an example of a QC standard not falling within the acceptance range for pH, in which cases all cell culture sample pH measurements from that day were eliminated from the data set.](image-url)
showed linear regression slopes and 1.28. Similarly, for cell culture samples many analytes, glutamate, for which the slope was higher than expected, at the reference analyzers for QC samples of all analytes except low intercept values relative to the data range) to those of the analyzers for QC samples run during the same time period that the slope for cell culture samples can be predicted from (data not shown). In turn, depends on maintenance of the sensors on both analyzers. However, the BioProfile FLEX software was subsequently modified, resulting in elimination of this offset, but at the same time introducing a variable linear regression slope, with values between 1.15 and 1.30 (data not shown). For dissolved oxygen and (pO₂) carbon dioxide (pCO₂), the linear regression slopes were slightly different than expected, at 0.6 and 1.2, respectively. Subsequent experiments have demonstrated that this offset is variable for both analytes, ranging from 0.4 to 0.6 g/L for glucose, and 0 to 0.3 g/L for lactate. In addition, in this range a small absolute difference between the two analyzers’ measurements was less than 0.1 and 0.15 pH units of each other, rather than 10% and 25%.

For viable cell density measurements, correlation was good for the overall data set (R² = 0.99, slope = 0.86) including all 13 CHO cell lines, and the overall offset was 2.2±5 cells/mL over the range of 0–100e5 cells/mL. However, it should be noted that cell count parameters on the BioProfile FLEX must be optimized to achieve results similar to those of the reference analyzer. In addition, for certain cell lines the two instruments did not agree well under the optimized parameters used in the study. Further optimization of the cell count parameters could result in better BioProfile FLEX-reference analyzer agreement for a larger number of cell lines.

Complete 12 day time course profiles were obtained using the BioProfile FLEX and all reference analyzers for five cultivations (two cell lines). By way of example, representative pH, glucose, and viable cell density profiles are shown for two cultivations in Figure 3, for qualitative visualization of the agreement between the BioProfile FLEX and the reference analyzers.

**Table 2. Summary of Results from BioProfile FLEX (FLEX) versus Reference Analyzers (Ref) Comparison**

<table>
<thead>
<tr>
<th>QC standards</th>
<th>Linear regression</th>
<th>Cell culture samples</th>
<th>% of samples for which abs (FLEX-Ref)/Ref ≤ 10%</th>
<th>25%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R²</td>
<td>slope</td>
<td>int</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>0.994</td>
<td>1.05</td>
<td>-0.3</td>
<td></td>
</tr>
<tr>
<td>pO₂</td>
<td>0.94</td>
<td>1.07</td>
<td>-9.8</td>
<td></td>
</tr>
<tr>
<td>pCO₂</td>
<td>0.97</td>
<td>1.12</td>
<td>-3.4</td>
<td></td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>0.99</td>
<td>1.03</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>K⁺</td>
<td>0.96</td>
<td>0.06</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Na⁺</td>
<td>0.95</td>
<td>0.94</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>Gluc</td>
<td>0.99</td>
<td>1.08</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Lac</td>
<td>0.95</td>
<td>1.13</td>
<td>-0.4</td>
<td></td>
</tr>
<tr>
<td>Glut</td>
<td>0.97</td>
<td>1.28</td>
<td>-0.4</td>
<td></td>
</tr>
<tr>
<td>Vcd</td>
<td>0.99</td>
<td>1.06</td>
<td>-0.2</td>
<td></td>
</tr>
<tr>
<td>Viab</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Diam</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Osmo</td>
<td>0.99</td>
<td>0.99</td>
<td>0.8</td>
<td></td>
</tr>
</tbody>
</table>

Total number of samples (N) and linear regression correlation coefficient (R²), slope, and intercept (int) are shown for both QC standards and cell culture samples. For cell culture samples, the average difference (ave diff) between measurements from the two methods, and the percent of samples for which the difference between the two analyzers’ measurements was less than 10% and 25% are also shown. Units for intercepts and average differences are listed in Table 1.

*For pH, the last two columns represent the percent of samples where the difference between the two analyzers’ measurements was less than 0.1 and 0.15 pH units of each other, rather than 10% and 25%.

For viable cell density measurements, correlation was good for the overall data set (R² = 0.99, slope = 0.86) including all 13 CHO cell lines, and the overall offset was 2.2±5 cells/mL over the range of 0–100e5 cells/mL. However, it should be noted that cell count parameters on the BioProfile FLEX must be optimized to achieve results similar to those of the reference analyzer. In addition, for certain cell lines the two instruments did not agree well under the optimized parameters used in the study. Further optimization of the cell count parameters could result in better BioProfile FLEX-reference analyzer agreement for a larger number of cell lines.

**Inter-BioProfile FLEX variability analysis**

In general, the data produced by the three different BioProfile FLEX analyzers was comparable (Table 3). Linear regression slopes were within 15% of 1.0, and correlation coefficients were above 0.88 for all analytes except for live cell diameter, indicating that the variability among instruments is minimal. Comparing any BioProfile FLEX against another, the two analyzers produced measurements that differed from each other by less than 25% for at least 90% of samples for most analytes. Exceptions include glutamine, ammonium, pO₂, and glucose. For this study, many samples had glucose and lactate concentrations below 0.5 g/L, which is near the lower limit of the specified range for those analytes (0.2 g/L). In addition, in this range a small absolute difference results in a large percent difference.

The three analyzers were very comparable for pH, with 96–100% of measurements differing by less than 0.1 pH
units for any two analyzers. Because of a laboratory supply stock limitation, a glutamine membrane was not available for FLEX 1 during this experiment, and as such the section of Table 3 comparing FLEX 1 and FLEX 3 do not show glutamine data. Representative time course culture profiles obtained using all three BioProfile FLEX analyzers are shown for two cultivations in Figure 4.

Auto-sampler prototype evaluation

All automated samples were successfully delivered to the BioProfile FLEX as scheduled, with a total of 57–87 measurements per analyte included in the final dataset. Culture sterility was maintained for over 65 days of high frequency automated sampling (1–4 samples per day), and the system functioned properly during two 12-day cultures without breaking down. For most analytes the auto-sampler did not alter the BioProfile FLEX measurements compared with those of manual samples (Table 4), with linear regression coefficients and slopes within 15% of 1.0, negligible offsets, and over 80% of automated sample measurements differing from manual sample measurement by less than 10%. The main exception to this finding was for pO₂, where the correlation coefficient and slope were near zero and over half of automated sample values differed from manual sample values by more than 25%. This result, perhaps, is not unexpected as elaborated on in the discussion section. Representative time course culture profiles obtained via automated and manual sampling are shown for two cultivations in Figure 5.

Discussion

These results demonstrated that the BioProfile FLEX and auto-sampling system have the potential to rapidly (~10 min per sample) generate online data comparable to that generated using three separate analyzers and manual sampling, using low sample volumes (10 mL per sample). BioProfile FLEX data was, in general, comparable to that of the reference analyzers. The variability between any two BioProfile FLEX analyzers was minimal for most analytes. Finally, data collected via automated sampling correlated very well with data from manual sampling. Taken together the results suggest that the cumbersome task of manually sampling bioreactors could become fully automated with such a system without compromising the quality of the data obtained.

The BioProfile FLEX performed well in syringe mode in terms of equivalence to reference analyzers, though for some analytes this was not evident in the summary data in Table 2. In certain cases, the performance of the BioProfile FLEX was improved over that of the reference analyzer. For example, glutamine and glutamate measurements depend nonlinearly on sample glutamine and glutamate concentrations for the reference analyzer, whereas this relation is linear over the range of the BioProfile FLEX (data not shown), resulting in higher but more accurate measurements in the range of 3–5 mM compared with those of the reference analyzer. This improvement resulted in lower correlation coefficients, higher average offsets, and more than half of BioProfile FLEX measurements differing from the reference analyzer measurement by more than 25% though it appeared that the BioProfile FLEX functioned properly.

In the case of live cell diameter, BioProfile FLEX measurements were consistently ~3.5–4.0 μm larger than those reported by the reference analyzer, probably due to the difference in the way the two analyzers process the cells for counting. Although both analyzers use the trypan blue exclusion method to identify live and dead cells, they differ in their cell imaging methodologies. The BioProfile FLEX allows the cells to settle in a glass well for up to 60 s before imaging. The reference analyzer, on the other hand, pumps the suspended cells over a stationary camera that takes an image each time the sample moves. In the latter case, several different cellular cross sections are likely obtained, whereas with the former, the images include the largest cross section of all cells.

While the slope (1.08) and offset (10 mmHg) were acceptable for dissolved oxygen (pO₂), the correlation coefficient of 0.6, along with the fact that only 73% of measurements
differed from the reference analyzer measurement by less than 25%, indicate a relatively large degree of variability for this analyte. This variability is likely due to gas entrainment, off-gassing, or cellular metabolism in the sample during operator manipulation or during the time between presentation to the BioProfile FLEX and the reference analyzer. This hypothesis is supported by the good correlation between the BioProfile FLEX and the BioProfile 400 for QC standard samples ($R^2$, slope of 0.94, 1.07 respectively).

For other analytes, the original data set showed unexpectedly high linear regression slopes (NH$_4^+$ and pCO$_2$) or non-negligible inter-analyzer offsets (glucose and lactate) in the BioProfile FLEX versus reference analyzer study. Subsequent studies, however, suggested that these slopes and offsets are not inherent to every BioProfile FLEX-reference analyzer pair, but instead represent variability in the relative performance between two analyzers. Subsequent experiments also suggested that two analyzers’ relative performance for cell culture samples could be predicted by the relative performance of QC standards on those two analyzers. Thus the variability described here could be eliminated by maintaining the analyzers such that QC measurements are at the mean expected value for these analytes. It is important to understand and consider these potential performance relationships when transferring a process between sites using the BioProfile FLEX and sites utilizing other analyzers.

In general the inter-BioProfile FLEX variability was minimal for most parameters. One exception was for analytes in

### Table 3. Summary of Results for Culture Samples Presented to Three Different BioProfile FLEX analyzers (FLEX 1, FLEX 2, FLEX 3)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>N</th>
<th>slope</th>
<th>int</th>
<th>$R^2$</th>
<th>% of samples for which (FLEX1-FLEX3)/FLEX1 ≤ 10%</th>
<th>% of samples for which (FLEX2-FLEX3)/FLEX2 ≤ 10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>57</td>
<td>0.99</td>
<td>0.1</td>
<td>0.96</td>
<td>96*</td>
<td>100*</td>
</tr>
<tr>
<td>pO$_2$</td>
<td>51</td>
<td>0.96</td>
<td>-3.2</td>
<td>0.88</td>
<td>39</td>
<td>42</td>
</tr>
<tr>
<td>pCO$_2$</td>
<td>60</td>
<td>0.92</td>
<td>0.4</td>
<td>0.96</td>
<td>60</td>
<td>88</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>5</td>
<td>1.11</td>
<td>0.6</td>
<td>0.99</td>
<td>86</td>
<td>88</td>
</tr>
<tr>
<td>K$^+$</td>
<td>51</td>
<td>0.96</td>
<td>0.8</td>
<td>0.97</td>
<td>94</td>
<td>99</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>57</td>
<td>1.01</td>
<td>0.4</td>
<td>0.998</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>gluc</td>
<td>63</td>
<td>1.1</td>
<td>-0.3</td>
<td>0.96</td>
<td>21</td>
<td>41</td>
</tr>
<tr>
<td>lac</td>
<td>64</td>
<td>1.1</td>
<td>0.1</td>
<td>0.99</td>
<td>64</td>
<td>74</td>
</tr>
<tr>
<td>gln</td>
<td>76</td>
<td>1.1</td>
<td>-0.3</td>
<td>0.96</td>
<td>56</td>
<td>103</td>
</tr>
<tr>
<td>glu</td>
<td>65</td>
<td>0.93</td>
<td>0.4</td>
<td>0.93</td>
<td>65</td>
<td>111</td>
</tr>
<tr>
<td>vcd</td>
<td>65</td>
<td>0.98</td>
<td>1.6</td>
<td>0.97</td>
<td>94</td>
<td>91</td>
</tr>
<tr>
<td>viab</td>
<td>65</td>
<td>0.88</td>
<td>1.5</td>
<td>0.86</td>
<td>91</td>
<td>111</td>
</tr>
<tr>
<td>diam</td>
<td>33</td>
<td>0.98</td>
<td>11.4</td>
<td>0.95</td>
<td>97</td>
<td>48</td>
</tr>
<tr>
<td>osmo</td>
<td>33</td>
<td>0.98</td>
<td>11.4</td>
<td>0.95</td>
<td>97</td>
<td>48</td>
</tr>
</tbody>
</table>

For each pair of analyzers, the total number of samples (N); linear regression correlation coefficient ($R^2$), slope, and intercept (int); and the percent of samples for which the difference between the two analyzers’ measurements was less than 10% and 25% are shown. Units for intercepts and differences are listed in Table 1.

*For pH, the last two columns represent the percent of samples where the difference between the two analyzers’ measurements was less than 0.1 and 0.15 pH units, rather than 10% and 25%. Flex 1 versus FLEX 2 comparison not shown for brevity.

Figure 4. Cell culture profiles for glucose (A), lactate (B), ammonium (C), pH (D), pCO$_2$ (E), osmolality (F), viable cell density (vcd) (G), viability (H), and glutamate (I) tracked via three separate BioProfile FLEX analyzers (triangles vs. diamonds vs. squares) for two different cell culture processes (open vs. closed symbols).
the nutrient/metabolite module, where the percentage of samples where the two analyzers’ results were within 10% of each other was lower than expected (ranging from 21 to 81% for the various analytes and analyzer pairs, Table 3), due to the absolute value of most measurements being low relative to the analytical range. For example, for 92% of samples, the absolute difference between two analyzers’ glucose results were less than 1.5 g/L when comparing any two BioProfile FLEX analyzers. Similarly, for 99.6% of samples the absolute difference between two BioProfile FLEX analyzers’ lactate results was less than 0.5 g/L. In both cases, the absolute differences observed would not affect the outcome of a typical cell culture process. It is noted that the data from the BioProfile FLEX versus reference analyzer study represent the proper function of all analyzers due to careful tracking of QC standards and, at the same time, high operator-related variability due to a large number of operators contributing to the study. In contrast, the results from the inter-BioProfile FLEX variability study represent potentially higher analyzer-related variability due to inclusion of all cell culture samples regardless of QC results, but less operator-related variability as this portion of the study was conducted by only three operators.

The integration of three analyzers into one eliminates some manual labor associated with cell culture monitoring, and also allows for integrated electronic data transfer, increasing efficiency and accuracy of data tracking and the potential for feedback control based on BioProfile FLEX output. An even greater increase in efficiency is realized when the BioProfile FLEX is connected to the cell cultures via the automated sampling system. For example, current sampling practices in a process development setting require roughly 0.2 h of manual labor per reactor per day including data entry time. Assuming 500 two-week cultivations per year sampled twice daily, automating the sampling process could save 2,800 h of manual labor per year.

The auto-sampler prototype performed as expected, with excellent correlation between measurements from automated and manual samples for all analytes except pO2, most likely due to culture metabolism or air leakage into the RVM and/or manual sample syringe. Although further efforts directed at improving these methods could result in better agreement in pO2 measurements, the information is not typically considered crucial off-line information. Total volume extracted from the bioreactor per sample, ~5–10 mL, was equivalent to the volume required for manual sampling with the existing configuration (9 mL including prime). Currently the auto-sampler system does not include a fraction collecting module, meaning that manual samples would still need to be collected in situations where, for example, packed cell

Table 4. Summary of Results from Automated versus Manual Sampling Method Comparison

<table>
<thead>
<tr>
<th>N</th>
<th>Linear regression</th>
<th>% of samples for which (auto-man)/man ≤</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R²</td>
<td>slope</td>
</tr>
<tr>
<td>pH</td>
<td>85</td>
<td>0.93</td>
</tr>
<tr>
<td>pO2</td>
<td>84</td>
<td>0.00</td>
</tr>
<tr>
<td>pCO₂</td>
<td>82</td>
<td>0.99</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>86</td>
<td>1.00</td>
</tr>
<tr>
<td>K⁺</td>
<td>87</td>
<td>0.98</td>
</tr>
<tr>
<td>Na⁺</td>
<td>86</td>
<td>1.00</td>
</tr>
<tr>
<td>gluc</td>
<td>77</td>
<td>0.99</td>
</tr>
<tr>
<td>lac</td>
<td>57</td>
<td>1.00</td>
</tr>
<tr>
<td>gln</td>
<td>57</td>
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<tr>
<td>glu</td>
<td>78</td>
<td>0.94</td>
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<tr>
<td>vcd</td>
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<tr>
<td>viab</td>
<td>87</td>
<td>0.98</td>
</tr>
<tr>
<td>osmo</td>
<td>81</td>
<td>0.86</td>
</tr>
</tbody>
</table>

The total number of samples (N); linear regression correlation coefficient (R²), slope, and intercept (int); the average difference between automated and manual measurements (ave diff), and the percent of samples for which the difference between the two sampling methods’ measurements was less than 10% and 25% are shown. Units for intercepts and differences are listed in Table 1.

* For pH, the last two columns represent the percent of samples where the difference between automated and manual measurements was less than 0.1 and 0.15 pH units, rather than 10% and 25%.

Figure 5. Cell culture profiles for glucose (A), lactate (B), ammonium (C), pH (D), pCO₂ (E), osmolality (F), viable cell density (vcd) (G), viability (H), and cell size (I) tracked via automated sampling (open symbols) or manual sampling (closed symbols), for two different cell culture processes (triangles vs. diamonds).
volume measurements or sample retains are required. Nevertheless, the excellent performance of the prototype in terms of sterility maintenance, scheduling reliability and sample fidelity indicate that the system has the potential to be a very robust commercial product.

The integrated sampling and analysis system represents a breakthrough for cell culture monitoring, in that many important culture parameters can be measured from a single automated sampling event without repeated risk of sterility breach. This is in contrast with other automated monitoring systems, which often monitor only one or two parameters, requiring complex computational analysis or calibration/validation for each new culture system, or are incompatible with aseptic conditions. This suggests that the system tested here has the potential for widespread implementation in an industrial setting although, due to the complexity of the system, the long term robustness of the system should first be evaluated.

Conclusions

This report describes the successful use of a novel integrated multi-functional analyzer and automated sampling system for mammalian cell culture bioprocess monitoring. The results suggest that the system has the potential to dramatically reduce the manual labor and associated errors involved in monitoring mammalian cell bioprocesses, without altering the quality of the data collected. The extensive testing across multiple instruments, multiple laboratory sites, and multiple operators gives a sound indication of the performance to be expected in a typical bioprocess development setting where many bioreactors are maintained. The automation of cell culture bioprocess monitoring will allow for more robust, operator-independent processes, with the potential for feedback control, resulting in increased efficiency, process understanding and process reproducibility while at the same time reducing the resources required to operate the cultures.

Acknowledgments

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Literature Cited


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Facilitating Multisite Bioprocess Transfer: Multi-Instrument and Multi-Platform Comparability and Long Term Stability of Nova Biomedical’s BioProfile® Chemistry and Gas Analyzers

Matthew McRae, John McHale, Scott Granger, Brian Goulart, Elizabeth Kilcoyne, Roystein Bulman, Nova Biomedical, Waltham, MA 02454

In this study, the BioProfile analyzers have proven to be extremely robust and reliable, while providing high quality data that cell culture scientists and engineers demand.

The results of this study show a high level of comparability between the BioProfile analyzers, not only among the same analyzers but across platforms. In addition, comparability was also demonstrated between both the new and aged analyzers, providing evidence of the long-term robustness and the quality of data that can be generated from the BioProfile analyzers. Nova Biomedical’s BioProfile analyzers provide the tools to facilitate multisite bioprocess transfer in the Biopharmaceutical industry.

As a result of data generated in this study and proof of comparability between BioProfile® systems, this Biopharmaceutical company has fully integrated Nova’s analyzers for use in their cell culture development and manufacturing. The data presented proved complete comparability of results across multiple sites.
Facilitating Multisite Bioprocess Transfer: Multi-Instrument and Multi-Platform Comparability and Long Term Stability of Nova Biomedical’s BioProfile® Chemistry and Gas Analyzers

Matthew McRae, John McHale, Scott Granger, Brian Goulart, Elizabeth Kilcoyne, Roystein Bulman

Abstract

The Biopharmaceutical industry has grown exponentially and more companies are now operating in a global market with sites thousands of miles apart. The need is now even greater for robust bioprocess monitoring solutions that can provide consistent instrument-to-instrument results.

The seamless transfer of information across multiple sites relies heavily on the comparability of process data from various technologies, ensuring effective monitoring and control of critical process parameters. This study provides data supporting comparability of the BioProfile® (Nova Biomedical, Waltham, MA) chemistry and gas analyzers across several development and manufacturing sites in the United States. In addition, the long-term performance stability of the BioProfile systems was also tested. Five BioProfile FLEX and four BioProfile 100 Plus analyzers were used to determine linearity, precision, accuracy, and instrument-to-instrument comparability. The age of the instruments used for this study ranged from new to over 8 years old, with several hundred samples to over 20,000 samples run on a given analyzer.

The results of this study show a high level of comparability between the BioProfile® analyzers. In addition, comparability was also demonstrated between both the new and aged analyzers, providing evidence of the long-term robustness and the quality of data that can be generated from the BioProfile analyzers. Nova Biomedical’s BioProfile analyzers provide the tools to facilitate multisite bioprocess transfer in the Biopharmaceutical industry.

Introduction

Nova Biomedical’s BioProfile line of chemistry, gas, and cell density analyzers are used extensively in the Biopharmaceutical industry. Uses include cell line optimization, media development, process development, bioreactor characterization, and commercial product process monitoring. With the ability to measure pH and gases, nutrients and metabolites, electrolytes, osmolality, IgG, phosphate, and cell density and viability, the array of cell culture analytical systems available by Nova Biomedical provide single-system solutions for almost any cell culture application.

Development and commercial manufacturing facilities have grown from just a few to over several hundred small-scale bioreactors with manufacturing capacities in the hundreds of thousands of liters. It is nearly impossible to utilize the same analytical devices in manufacturing suites that were used in the development of a process. It is imperative that scientists and engineers feel confident that comparable results can be achieved between multiple analytical systems across parallel development labs and up through commercial manufacturing suites. Furthermore, manufacturing campaigns are run months to years after a process has been developed, requiring robust analytical technologies that scientists and engineers can rely on.

This study was performed in partnership with one of the largest Biopharmaceutical companies in the United States, with global sites in over thirty countries. In order to guarantee success with the manufacture of their product lines, absolute comparability of their analytical devices is necessary. Having tested a number of other analyzers, the customer acknowledged Nova Biomedical’s analytical technologies as the only devices available on the market today that can provide robust solutions for their laboratories and manufacturing suites. In addition, Nova provides the quality control of reagents and consumables as well as the technical resources capable of providing support and services facilitating technology transfer.

After the final site expansion, the strategic initiative was given to globally standardize their cell culture analytical devices. As part of this initiative, Nova’s Applications Support Organization drafted and executed this cross-site correlation study with the goal of providing robustness data, linearity and precision data, and ultimately the interchangeability of equipment.
Material & Methods

System Configuration and Initial Setup

Prior to running any samples, the BioProfile 100 Plus and BioProfile FLEX analyzers were inspected for proper system function, maintenance needs, and up-to-date software versions. All consumables were inspected to ensure that no sensor or tubing was out of date or past expiration. Replacement of any consumables past expiration was performed. A review of the error logs on the BioProfile FLEX and the BioProfile 100 Plus also showed that there had been no significant hardware related errors on the systems. Reagent pack status was also inspected. To eliminate reagent packs as a source of variability, all reagent packs with less than 50% remaining fluid were replaced. This would ensure that the same reagents were used throughout this multi-day study.

Quality Control Verification

For this study, a single lot of QC’s for each level was used. Table 1 summarizes the lot and expiration data information for the QC and Linearity standards used in this study. The Expected Range (the range within which verifies proper system function) for each parameter and each level was programmed into the BioProfile FLEX and the BioProfile 100 Plus analyzers. All levels of Quality Control were equilibrated to room temperature prior to analysis. Per the SOP outlined on the Quality Control Insert sheet, ampules were shaken for 10 seconds and analyzed within 30 seconds of opening. A separate ampule was used for each instrument when replicates were analyzed to minimize equilibration with room air and to ensure each sample was analyzed at approximately the same time from opening.

Table 1: QC and Linearity lot information

<table>
<thead>
<tr>
<th>Standard</th>
<th>Lot #</th>
<th>Expiration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality Control Level 1</td>
<td>105621</td>
<td>Nov-12</td>
</tr>
<tr>
<td>Quality Control Level 2</td>
<td>105834</td>
<td>Nov-12</td>
</tr>
<tr>
<td>Quality Control Level 3</td>
<td>105718</td>
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<tr>
<td>Quality Control Level 4</td>
<td>104437</td>
<td>Oct-12</td>
</tr>
<tr>
<td>Quality Control Level 5</td>
<td>103055</td>
<td>Sep-12</td>
</tr>
<tr>
<td>Linearity Low</td>
<td>011470</td>
<td>May-12</td>
</tr>
<tr>
<td>Linearity High</td>
<td>011471</td>
<td>May-12</td>
</tr>
</tbody>
</table>

Levels 8 and 9 provide for the measurement of particles for the BioProfile FLEX CDV module. These levels contain polystyrene beads and are used to determine proper fluid flow, dilution, and calibration of the CDV module. Level 8 and 9 were measured on all FLEX CDV modules to confirm proper function of the CDV fluidics and imaging system.

Sample Setup and Preparation

For this study, it was important to use samples of known concentration, when available. For pH, gas, electrolyte, nutrient/metabolite, and osmolality measurements, Nova’s Quality Control material were used. These controls are manufactured to provide standards of known concentration across the analytical range. Control Levels 1, 2, and 3 provide for the measurement of pH, pCO2, pO2, Glucose, Lactate, NH4+, Na+, K+, Ca++, and Osmolality. Levels 4 and 5 provide for the measurement of Glucose, Lactate, Glutamine, and Glutamate. To provide a greater sample set and additional points along the linear range of the analyzers, various standards were diluted with Water for Injection (WFI).
Cell culture samples obtained from a bioreactor were used for viable cell density, total cell density, and viability measurements. Unless otherwise noted, all sample sets were analyzed in triplicate on each analyzer.

Calculations

The following calculations were used to determine the mean, standard deviation, and coefficient of variation for the data generated in this study.

Arithmetic Mean:

$$\bar{x} = \frac{1}{n} \sum_{i=1}^{n} x_i$$

Standard Deviation:

$$\sigma = \sqrt{\frac{1}{n-1} \left( \sum_{i=1}^{n} x_i^2 \right) - \bar{x}^2}$$

Coefficient of Variation:

$$C_v = \frac{\sigma}{\bar{x}}$$

Data Analysis

All data from this study was either transposed from the paper printouts or exported via USB Flash Drive (BioProfile FLEX) to Microsoft Excel Spreadsheet for graphing and data analysis. The graphs represented in this study show results compared to a reference FLEX analyzer. This analyzer was arbitrarily chosen among the group of instruments used in this study. It is identified as FLEX 1.
Results

Figure 1 shows the pH linearity profile for the BioProfile FLEX analyzers. Linearity was very strong with correlation coefficients of greater than 0.99. In addition, the instrument exhibited a high level of comparability with a standard deviation of 0.03 pH units and a coefficient of variability of 0.4%.

Figure 1: pH Linearity Profile

![pH Linearity Profile](image)

Figure 2 and Figure 3 shows the pO2 and pCO2 linearity profiles for the BioProfile FLEX analyzers, respectively. Linearity was very strong with correlation coefficients of greater than 0.98 for both pO2 and pCO2. In addition, the instrument exhibited a high level of comparability with a standard deviation of 2.29 and 7.94 mmHg and a coefficient of variability of 5.2% and 7.7% for pO2 and pCO2, respectively.

Figure 2: pO2 Linearity Profile

![pO2 Linearity Profile](image)

Figure 3: pCO2 Linearity Profile

![pCO2 Linearity Profile](image)
Results

Figure 4 and Figure 5 show the glucose and lactate linearity profiles for the BioProfile analyzers, respectively. Linearity was very strong with correlation coefficients of greater than 0.99 for both glucose and lactate. In addition, the instrument exhibited a high level of comparability with a standard deviation of 0.11 and 0.06 g/L and a coefficient of variability of 4.9% and 2.9% for glucose and lactate, respectively.

**Figure 4: Glucose Linearity Profile**

![Glucose Linearity Profile](image)

**Figure 5: Lactate Linearity Profile**

![Lactate Linearity Profile](image)

Figure 6 and Figure 7 show the glutamine and glutamate linearity profiles for the BioProfile analyzers, respectively. Linearity was strong with correlation coefficients of greater than 0.97 for both glutamine and glutamate. In addition, the instrument exhibited a high level of comparability with a standard deviation of 0.13 and 0.07 mmol/L and a coefficient of variability of 6.0% and 5.2% for glutamine and glutamate, respectively.

It should be noted that the linearity and variability for glutamine are not as strong for as the other parameters tested. Though the samples were controlled in the best possible manner (samples stored on ice prior to analysis), the instability of glutamine often results in unusually high variability in large experiments such as this.
Results

Figure 6: Glucose Linearity Profile

Figure 7: Lactate Linearity Profile

Figure 8 shows the osmolality linearity profile for the BioProfile FLEX analyzers. Linearity was very strong with correlation coefficients of greater than 0.999. In addition, the instrument exhibited a high level of comparability with a standard deviation of 2.99 mOsm/kg pH units and a coefficient of variability of 1.0%.

Figure 8: Osmolality Linearity Profile
Results

Figure 9 shows the Total Cell Density linearity profile for the BioProfile FLEX analyzers. In the figure, results obtained on the BioProfile CDV module are compared to results obtained on the Cedex cell counter, the reference instrument used in this laboratory. Data was generated from bioreactor cultures seeded at varying densities, providing a range of total cell density and viability samples. Due to intellectual property concerns, the viability data is not shown. However, a range of cultures were analyzed with viabilities ranging from approximately 45% to 99% viability. BioProfile FLEX viabilities were within 3% compared to the Cedex analyzers.

Figure 9: Total Cell Density Linearity Profile

![Figure 9: Total Cell Density Linearity Profile](image)

Figure 10 and Figure 11 show the NH4+ and Na+ linearity profiles for the BioProfile analyzers, respectively. Linearity was very strong with correlation coefficients of greater than 0.99 for both glucose and lactate. In addition, the instrument exhibited a high level of comparability with a standard deviation of 0.09 and 2.23 mmol/L and a coefficient of variability of 5.3% and 2.5% for NH4+ and Na+, respectively.

Figure 10: NH4+ Linearity Profile

![Figure 10: NH4+ Linearity Profile](image)

Figure 11: Na+ Linearity Profile

![Figure 11: Na+ Linearity Profile](image)
Results

Figure 12 and Figure 13 show the K+ and Ca++ linearity profiles for the BioProfile analyzers, respectively. Linearity was very strong with correlation coefficients of greater than 0.99 for both glucose and lactate. In addition, the instrument exhibited a high level of comparability with a standard deviation of 0.09 and 0.02 mmol/L and a coefficient of variability of 2.8% and 2.2% for K+ and Ca++, respectively.

Figure 12: K+ Linearity Profile

![K+ Linearity Profile](image)

Figure 13: Ca+ Linearity Profile

![Ca+ Linearity Profile](image)

Table 3 shows the precision analysis for the combined measurements on the BioProfile analyzers. Nova Biomedical provides published specifications for all parameters (excluding CDV) as measured on a single analyzer for both within run and day-to-day precision. This study incorporated nine BioProfile Instruments, with samples analyzed over the course of several weeks. The precision specifications listed below show that, not only can one expect a single BioProfile instrument to meet published specifications for precision, but multiple instruments can also fall well within the published specifications of a single instrument. This data further supports the BioProfile analyzers as extremely robust analytical tools for cell culture development and manufacturing process monitoring.

Table 3: Precision Analysis Summary

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean Standard Deviation</th>
<th>Mean %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>0.03</td>
<td>0.4%</td>
</tr>
<tr>
<td>pCO₂ (mm Hg)</td>
<td>7.94</td>
<td>7.7%</td>
</tr>
<tr>
<td>pO₂ (mm Hg)</td>
<td>2.29</td>
<td>5.2%</td>
</tr>
<tr>
<td>Glucose (g/L)</td>
<td>0.11</td>
<td>4.9%</td>
</tr>
<tr>
<td>Lactate (g/L)</td>
<td>0.06</td>
<td>2.9%</td>
</tr>
<tr>
<td>Glutamine (mmol/L)</td>
<td>0.13</td>
<td>6.0%</td>
</tr>
<tr>
<td>Glutamate (mmol/L)</td>
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<td>5.2%</td>
</tr>
<tr>
<td>Ammonium (mmol/L)</td>
<td>0.09</td>
<td>5.3%</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>2.23</td>
<td>2.5%</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
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<td>2.2%</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>0.09</td>
<td>2.8%</td>
</tr>
<tr>
<td>Osmolality (mOsm/kg)</td>
<td>2.99</td>
<td>1.0%</td>
</tr>
</tbody>
</table>
Summary

In this study, the BioProfile analyzers have proven to be extremely robust and reliable, while providing high quality data that cell culture scientists and engineers demand.

The results of this study show a high level of comparability between the BioProfile analyzers, not only among the same analyzers but across platforms. In addition, comparability was also demonstrated between both the new and aged analyzers, providing evidence of the long-term robustness and the quality of data can be generated from the BioProfile analyzers. Nova Biomedical’s BioProfile analyzers provide the tools to facilitate multisite bioprocess transfer in the Biopharmaceutical industry.

As a result of data generated in this study and proof of comparability between BioProfile® systems, this Biopharmaceutical company has fully integrated Nova’s analyzers for use in their cell culture development and manufacturing. The data presented proved complete comparability of results across multiple sites.
PAT Tools for Accelerated Process Development and Improvement: Application to Cells on Microcarriers with Continuous Control of Metabolites Concentrations Using Nova Biomedical Flex Sampling and Analysis System

Eric Calvosa, Jean-Marc Guillaume, Bioprocessing R&D, USP, Sanofi Pasteur, Lyon. Cosette Deyirmendjian, Roystein Bulman, Andrei Malic, Nova Biomedical Europe.

The Nova Biomedical FLEX and autosampler system can be implemented to provide an online feedback process control loop on metabolite concentrations (Glc, Gln, Glu, Lac, NH4+) continuously in microcarrier manufacturing processes. Process monitoring using the BioProfile FLEX sampling and analysis system with OPC improves process control.
**Summary**

The trend within the biopharmaceutical industry to include PAT (Process Analytical Technology) approaches in manufacturing processes, applies early in development activities. With an anchorage dependent cell line (Vero), in-line sampling for real-time process monitoring and process adjustment is challenging due to non-homogeneous cultures and potential clogging caused by microcarriers in the culture.

In this study, we have tested the Nova Biomedical FLEX automated sampling and analysis system with OPC (Open Productivity & Connectivity) for continuous feedback control of metabolite concentrations in the culture.

At first we demonstrated that glutamine, glucose and glutamate consumption was dependent on cell growth phase. In a second step, metabolite concentrations were controlled in the bioreactor using the FLEX sampling and analysis system with OPC. During this step, metabolite concentrations were continuously retro-controlled in the culture via sterile in-line bioreactor sampling and Nova analysis. Once established on non-infected cells, the system was further applied to a model viral (rabies) productivity process for 14 days. Cell culture experiments were carried out with sampling and analysis automatically performed every hour to continuously control and monitor the virus production process.

Thus it is possible to control and adjust in real-time, the required concentration of metabolites to sustain controlled cell metabolism, especially as viral infection and production progresses. The Nova Biomedical FLEX autosampler and analyzer gives reliable results and provides improved process control in development activities. In conclusion, we say that process monitoring using the FLEX sampling and analysis system with OPC provides improved process control.

**Materials and Methods**

**I Vero Cell Kinetics**

![Vero Cell Kinetics Diagram]

At the beginning of this study, a kinetic of Vero cells in serum-free medium culture is tracked by metabolite consumption. The qGlutamine and qGlucose were at the maximum level at the beginning of cell growth when metabolites concentrations were at the highest level. The cell growth was at the maximum on day 2 and 3 (PDL cumul). On day 4, the cell growth decreased, linked to a lower concentration of key metabolites.

To maintain glucose and glutamine concentration at low level, an OPC Nova analysis system was used and a medium injection with specific glucose and glutamine concentrations. Metabolite concentration was tested every hour to retro-control the medium injection. In this study, a FISP sampling probe with 0.2 of porosity was used to avoid blocking the autosampler. The data obtained with manual sampling control was similar to automated analysis. At the end of the process, the glucose concentration decreased due to fresh medium addition in the bioreactor.

**II Virus Infected Cells**

![Virus Infected Cells Diagram]

Kinetics of glutamine and glucose were tracked in viral infected cells for 14 days. The sampling system was adjusted to limit flow restrictions. A new FISP probe was used with a higher porosity of 20 μm. During the study, cell lysis caused by the virus restricted the sampling flow of the system at lower probe porosity. A very high correlation between manual and automated sampling was obtained.

The image on the left shows Vero cells on microcarriers before the infection by rabies virus. The image on the right shows Vero cells after two weeks of infection.

**Conclusion:**

The Nova Biomedical FLEX and autosampler system can be implemented to provide an online feedback process control loop on metabolite concentrations (Glc, Gln, Glu, Lac, NH4+) continuously in microcarrier manufacturing processes.
In early 2009, the BioProfile FLEX® Autosampler from NOVA Biomedical was introduced to the biotech market. Pfizer purchased a commercial unit and put it through extensive trials to ensure it met three main requirements to be successfully implemented in our vision of the “lab of the future.”

These three main requirements are:

1) to maintain sterility of the bioreactor for an extended period of time; up to 3 weeks for a fed-batch process,
2) to interface with various in-process analytical instruments, and
3) to maintain the fidelity of cell cultivation samples (e.g. no sample dilution, no loss of gas components during sample processing).

In this study, we have demonstrated that the BioProfile FLEX® Autosampler has met all three requirements. This Automated Sampling System Could Be Used to Provide Greater Process Understanding with Real-time Sample Analysis while minimizing FTE Burden. In addition a robust automated sampling system is a cornerstone of future applications of Process Analytical Technology.
Performance Evaluation of an Automated Bioreactor Sampling System for Mammalian Cell Cultivation

Kavi Mehta, Erwin Y. Yu, Susan Casnocha

BioTherapeutics Pharm Sci, Pfizer Inc, St. Louis, MO 63017

Introduction

Two common goals of biotherapeutics development are reduction in timelines and increased process understanding. Both of these require increased throughput in process development labs. One way to increase throughput of bioreactor process development is to increase the number of bioreactors and/or increase the frequency of bioreactor sampling; however these approaches have an associated increased level of resource commitment. In order to address the need for more reactors and a higher frequency of reactor sampling without dramatically increasing resources automation of sampling and in-process analytics becomes a necessity. In early 2009, the FLEX™ autosampler from NOVA Biomedical was introduced to the biotech market. Pfizer purchased a commercial unit and put it through extensive trials to ensure it met three main requirements to be successfully implemented in our vision of the “lab of the future.”

These three main requirements are: 1) to maintain sterility of the bioreactor for an extended period of time; up to 3 weeks for a fed-batch process, 2) to interface with various in-process analytical instruments, and 3) to maintain the fidelity of cell cultivation samples (e.g. no sample dilution, no loss of gas components during sample processing). In this study, we have demonstrated that the FLEX™ Autosampler has met all three requirements.

The Nova Biomedical FLEX™ Autosampler system can sample up to 9 bioreactors based on a user-defined, pre-set schedule. The sample is transferred to the FLEX™ Bioanalyzer that analyzes culture nutrients, metabolites, gases, electrolytes, and viable cell density.

Materials and Methods

• Recombinant CHO cell cultures grown in chemically defined media with the addition of a chemically-defined nutrient feed

• 1L Applikon Bioreactor

• NOVA Autosampler samples from 2 Bioreactors and delivers to FLEX™ for all analyses

• Data Obtained from Online Analysis was Compared to Data Obtained from Manual Samples taken from the reactor by hand and run on the same FLEX™
Conclusions

This Automated Sampling System Met All Three Requirements Outlined in the Introduction:

• Successfully Met Sterility Requirement

• Metabolite and Gas analysis: \( \Delta \text{pH} \leq 0.035 \), \( \Delta \text{pCO}_2 \leq 10\% \), \( \Delta \text{Glucose} \leq 10\% \), \( \Delta \text{Lactate} \leq 10\% \)

• Cell Count Analysis: \( \Delta \text{Viable Cell Count} \leq 10\% \), \( \Delta \text{Viability} \leq 2\% \)

• This Automated Sampling System Could Be Used to Provide Greater Process Understanding with Real-time Sample Analysis while minimizing FTE Burden. In addition a robust automated sampling system is a cornerstone of future applications of Process Analytical Technology.
The goal of this study was to evaluate the results produced by one fully integrated instrument (BioProfile FLEX) in comparison to other established and separate analyzers.

The BioProfile FLEX instrument successfully processed cell culture samples and measured cell concentration, cell viability, and osmolality in addition to nutrient and metabolite concentrations. BioProfile FLEX uses an improved sample dilution system which is different from BioProfile 400. This difference can account for some degree of variance between the measurements of the two instruments. The integrity of the nutrient and metabolite measurements was maintained in BioProfile FLEX as compared to BioProfile 400, with R2 values for pH, ammonium, glutamine, and glucose ranging from 0.99 – 0.94.
Consolidated Single Analyzer Measurement of Key Cell Culture Constituents in the Development of Mammalian Cell Processes

Carissa M. Moore and Jean-François P. Hamel

Department of Chemical Engineering
Massachusetts Institute of Technology, Cambridge, MA 02139

Background

In a typical cell culture process for making therapeutics, cultures need to be closely monitored for their viability, genetic stability, production potential, and for potential contamination. It is essential that the levels of key nutrients and metabolites as well as cell concentration be known. This allows the operator to maintain the culture appropriately, by using the correct feeding strategy and being aware of the changes in the culture and cell metabolism during the process. Typically, when cell culture samples are taken, they must be analyzed for cell concentration, nutrient and metabolite concentrations, and osmolality on separate instruments. This process is time consuming, requires multiple sample transfers, and can make data collection cumbersome. Nova BioProfile® FLEX simplifies this complex monitoring system by allowing all of these analyses to occur and data to be collected in one place.

The BioProfile FLEX instrument under study incorporates a Cell Density and Viability analyzer along with a Freezing Point Osmometer and a Chemistry analyzer. For Cell Density and Viability measurements, BioProfile FLEX uses an automated Trypan Blue dye exclusion assay similar to that used by current technologies, such as the CEDEX cell counter. One major difference between the CEDEX and BioProfile FLEX is the method of sample positioning. In the BioProfile FLEX analyzer, the sample is allowed to settle on a slide and the flowcell is then moved from one position to the next as the camera in the Image Processing System takes one image at each position and processes the result. Conversely, in the CEDEX, the flowcell is stationary while the sample is passed by the camera as images are taken. This difference allows BioProfile FLEX to have more precise focus and display clearer images. BioProfile FLEX contains an Advanced Instruments Freezing Point Osmometer, which is essentially equivalent to their model 210 Osmometer. However, BioProfile FLEX incorporates a 20-sample tray that results in less frequent operator maintenance because the sample tubes need only to be changed every 20 samples rather than being prepared for each sample. Also, the Osmometer sample is pipetted directly into the sample tube avoiding the manual step in the 210.

When incorporating multiple modules into a single analyzer, it is important that the time sequence for sample analysis take into account both time sensitive tests and efficiency. BioProfile FLEX accomplishes this by measuring gases and electrolytes first, followed by cell density, osmolality, and finally, nutrients and metabolites.

Objectives

The goal of this research is to evaluate the results produced by one fully integrated instrument (BioProfile FLEX) in comparison to other established and separate analyzers. The study uses samples from CHO cells, hybridoma cells, and cancer cells to compare the performance of BioProfile FLEX to a chemistry analyzer (Nova BioProfile 400), an automated cell counter (CEDEX), and an Osmometer (Advanced Instruments 210). Use of these instruments and cell lines allows Bio-Profile FLEX to be evaluated for a number of important parameters including such as pH, ammonium, glutamine, glucose, cell concentration and viability, and osmolality. This study will test the validity of BioProfile FLEX’s cell concentration and osmolality analysis and determine if these typically separate analyzers can successfully be incorporated with nutrient and metabolite analysis while maintaining the integrity of all measurements. This comparison will be done using a linear regression of the data from BioProfile FLEX compared to the corresponding data from individual reference analyzers. Only samples taken when the instruments meet quality control specifications and that are within the instruments’ range will be included.
Materials and Methods

Outline of the BioProfile FLEX Analysis Cycle:

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Less than 1.0 mL of sample is aspirated into the system.</td>
</tr>
<tr>
<td>2.</td>
<td>The Gas/Electrolyte Module sample (~ 350 μL) is positioned, and the Module's analysis cycle is started.</td>
</tr>
<tr>
<td>3.</td>
<td>The Cell Density and Viability sample (200 μL) is dispensed into its Transfer Well, and the Module's analysis cycle is started.</td>
</tr>
<tr>
<td>4.</td>
<td>The Osmometer sample (20 μL) is dispensed into its Sample Tube, and the Module's analysis cycle is started.</td>
</tr>
<tr>
<td>5.</td>
<td>The Nutrient/Metabolite Module sample (40 μL) is dispensed into its Transfer Well, and its analysis cycle started.</td>
</tr>
<tr>
<td>6.</td>
<td>Results for each Module are reported as soon as they are available.</td>
</tr>
</tbody>
</table>

BioProfile FLEX incorporates the following measurement technologies:

**Cell Density and Cell Viability Module: The Trypan Blue Exclusion Assay for Cell Viability**

The assay is based on the principle that live cells have intact membranes and these membranes exclude Trypan Blue:
- Live or viable cells are unstained and appear light
- Dead or nonviable cells uptake the dye and appear dark

Though other assays exist, Trypan Blue Exclusion is the one that is used by BioProfile FLEX and most other cell counting instruments because it is an automated version of the manual counting method that utilizes:
- A manual hemocytometer slide with appropriate grid spacing
- A microscope
- A well-trained operator
Results

Cell Density/Viability

Figure 1. TOTAL DENSITY over Analyzer Operating Range BioProfile FLEX Cell Density Module vs. CEDEX

Figure 2. VIABLE DENSITY over Analyzer Operating Range BioProfile FLEX Cell Density Module vs. CEDEX
Example of Cell Density Image

Figure 3. BioProfile FLEX sample: Hybridoma

Figure 4. CEDEX Sample: Hybridoma
Results (Continued)

**Osmolality**

**Figure 5.** OSMOLALITY over Evaluation Range BioProfile FLEX Osmometer Module vs. Advanced Instruments 210

**Figure 6.** OSMOLALITY over Anticipated Cell Culture Range BioProfile FLEX Osmometer Performance vs. Advanced Instruments 210
Example of Chemistries

**Figure 7.** pH Performance over Evaluation Range
BioProfile FLEX vs. BioProfile 400

**Figure 8.** NH4+ Performance over Evaluation Range
BioProfile FLEX vs. BioProfile 400

**Figure 9.** Gluc Performance over Evaluation Range
BioProfile FLEX vs. BioProfile 400

**Figure 10.** Gln Performance over Analytical Range
BioProfile FLEX vs. BioProfile 400

BioProfile FLEX incorporates the following measurement technologies (continued):

**Osmometer Module:**
Advanced Instruments: Freezing Point Depression Method

**Gas Electrolyte and Nutrient Metabolite Modules:**
These modules include both potentiometric and amperometric sensors. pH, PCO2, NH4+, Na+, K+, Ca++ are potentiometric sensors while PO2, Glutamine, Glutamate, Glucose, and Lactate are amperometric sensors. Ion Selective electrodes (ISE) selectively measure the activity of the ion of interest. When the ISE is contacted with a sample, a potential is developed. This potential is proportional to the logarithm of the ionic activity and is measured versus a reference electrode. The enzyme biosensors combine an immobilized enzyme membrane and an electrochemical sensor to measure nutrients and metabolites. A constant potential is applied to these electrodes and the current produced by the reactions at the surface of the electrode is linearly related to the concentration of nutrient or metabolite in the sample.

**BioProfile 400 Measurement Technology**
This analyzer includes both potentiometric and amperometric sensors. pH, PCO2, NH4+, Na+, K+ are potentiometric sensors while PO2, Glutamine, Glutamate, Glucose, and Lactate are amperometric sensors.
BioProfile FLEX incorporates the following measurement technologies (continued):

**CEDEX Measurement Technology** Trypan Blue Exclusion Assay for Cell Viability
Advanced Instruments **Model 210 Technology** Freezing Point Depression Method

**Cells and Media**

HPCHO cells were cultured in a 50/50 v/v mixture of BD CHO Medium without L-Glutamine and Ex-Cell ACF CHO Medium supplemented with 1mM sodium pyruvate, 100 mg penicillin-streptomycin, 6mM L-glutamine, and 5mL pluronic acid. IB4 (hybridoma) cells were cultured in BD Cell MAb Medium Quantum Yield supplemented with 1mM sodium pyruvate, 100 mg penicillin-streptomycin, 2mM L-glutamine, 10 mL pluronic acid, and 10% fetal bovine serum. MethA (cancer) cells were cultured in RPMI 1640 medium supplemented with 2.5 g/L glucose, 2 g/L sodium bicarbonate, 0.87 g/L L-glutamine, 1mM sodium pyruvate, 100 mg penicillin-streptomycin, and 10% fetal bovine serum.

**Conclusions**

The BioProfile FLEX instrument can process cell culture samples and measure cell concentration, cell viability, and osmolality in addition to nutrient and metabolite concentrations. BioProfile FLEX uses an improved sample dilution system which is different from BioProfile 400. This difference can account for some degree of variance between the measurements of the two instruments. The integrity of the nutrient and metabolite measurements was maintained in BioProfile FLEX as compared to BioProfile 400, with R2 values for pH, ammonium, glutamine, and glucose ranging from 0.99 – 0.94. See Figures 7 thru 10. The correlation between cell concentration measurements between BioProfile FLEX and a dedicated cell counter (CEDEX) were also very good, (R2=0.99, 0.98). See Figures 1 and 2. This suggests that the change in sample positioning methodology (settling and moving flowcell as opposed to flowing sample past camera) did not negatively affect measurements. The correlation of osmolality measurements was R2=0.93 in the sample range of 305 – 360 mOsm/Kg. See Figure 5. Most of the sample points are in the narrow range of 306 – 340 mOsm/Kg. If the anticipated cell culture range is considered 200 – 600 mOsm/Kg using controls, a higher correlation coefficient of R2 = 0.99 was obtained. See Figure 6.

**Future Work**

BioProfile FLEX will further expand its measurement capability to include a module to measure phosphate and it will incorporate an autosampler to allow for direct connection to reactors. The autosampler will allow BioProfile FLEX to perform scheduled sampling without an operator present.

**References**


**Acknowledgements**

The authors wish to thank Nova Biomedical (Waltham, MA) for the loan of the BioProfile FLEX instrument and for support, especially Jon Scott and Nicole Pouliot. The authors are also grateful to Kathy Hufford and Tracie Saunders for their contribution of cell culture samples and to Michael Hong for his help with sampling during the course of this study.