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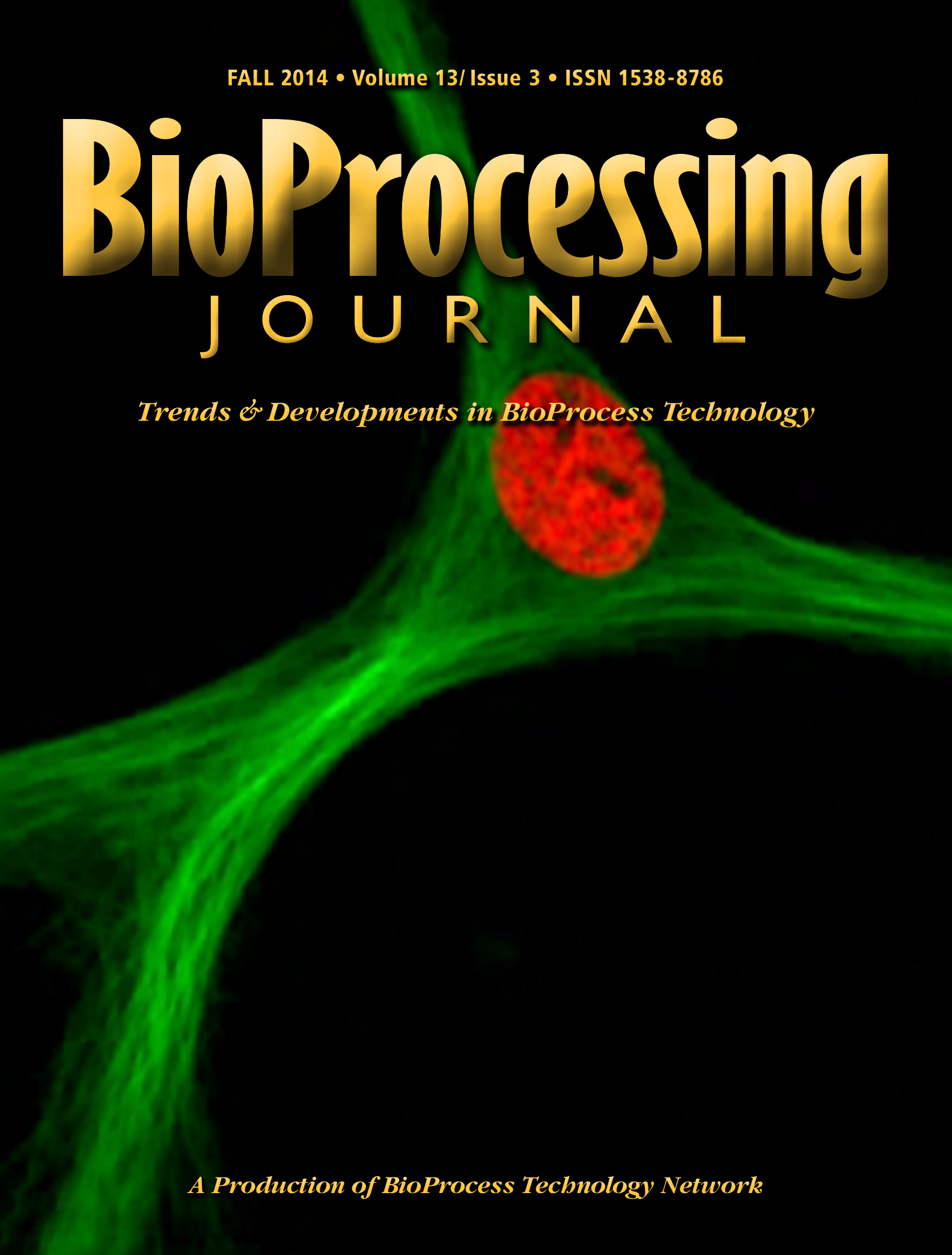

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Which Factors to Consider When Selecting an Analytical Method for Cell Culture Fermentation: *A Comparison of Four Different Metabolic Analyzer Instruments*

By CAITLIN KURTZ and JOHN PAUL SMELKO

Abstract

Mammalian cell culture processes require an in depth understanding of inputs and outputs in order to maximize productivity, efficiency, and product quality. Daily monitoring of essential metabolites, nutrients, and protein titer using at-line analyzers are the building blocks of drug manufacturing process development and characterization. In order to better understand how these instruments perform, a comparative analysis was conducted using two different classes of metabolic-sensing technologies, membrane-based technology (MBT) and absorption photometric-based technology (APBT). Four commercially available instruments were examined using various samples and maintenance conditions in order to emphasize the following testing criteria: specificity, linearity, range, accuracy, and precision. Samples included standard solutions with known metabolite concentrations, cell culture supernatant, and supernatant liquid spiked with additional metabolite solutions.

The results from our testing indicated that the photometric analyzers yielded more accurate and consistent results than the membrane-based analyzers and were easier and less time-consuming to maintain. Ultimately, these studies summarized the capabilities and limitations for both types of analyzers and provide a critical summary for instruments used in everyday bioprocess monitoring.

Introduction

Typical development laboratory workflows involve processing a large quantity of time-sensitive samples used to monitor, understand, and develop drug manufacturing processes. With so many commercially available metabolite analyzers on the market, it is often difficult to determine which ones are going to be accurate, consistent, and robust enough for everyday use. In addition, to enable efficient cell culture process development, industrial biotechnology laboratories generally contain many different analytical instruments within the same lab due to the diversity of nutrients, metabolites, and protein titer measurements requiring at-line monitoring. Implementation of a robust platform at-line analyzer would be ideal, helping to eliminate the inconsistencies that are commonly observed when comparing data across multiple different metabolite instruments.

The intent of this study was to demonstrate which of these at-line metabolic-sensing technologies would give the most accurate and reproducible results when operated under a controlled testing environment as well as a "normalized" day-to-day laboratory setting. The four instruments evaluated were the Cedex Bio HT (Roche Custom Biotech), CuBiAn HT-270® (Optocell Technology), BioProfile® FLEX (Nova Biomedical), and the YSI 2950 (YSI Life Sciences). These instruments were chosen because each provides near real-time at-line nutrient, metabolite, and/or protein titer analysis making them all competitive analyzer options for bioprocess

monitoring. The studies performed examined the accuracy, precision, linearity, specificity, and range characteristics of each instrument in order to further expand on the prior results generated by Andrew Bawn *et al.* in "Metabolic-sensing characteristics of absorption-photometry for mammalian cell cultures in biopharmaceutical processes."[1] Building upon the results from Bawn *et al.*, which compared a Cedex Bio, a BioProfile 400, and a Waters® 2695 HPLC (high-pressure liquid chromatography), this study compared four instruments (Table 1), addresses additional analytes, and expands the concentration ranges. We feel this information is crucial in determining which metabolic detection technologies on the market are robust enough to be implemented for every day cell culture monitoring and development.

Materials and Methods

Technology Overview

Metabolite concentration profiles are commonly generated using at-line bioprocessing analyzers in order to monitor the physiological state of the culture throughout the different stages of the cell culture process. Two different classes of metabolic-sensing technologies, membrane-based technology (MBT) and absorption photometric-based technology (APBT) were assessed to demonstrate comparability and suitability for the intended purpose of analyte measurement. For the measurement of glucose, lactate, glutamine, and glutamate concentration, the MBT analyzers employ biosensor technology in which the reaction of a substrate with the enzyme produces a current that can be readily measured by the instrument.[2] The immobilized enzymes present on the membrane tip of the amperometric electrodes are highly specified for their target analyte, which allows for fast, accurate measurements to be made.[3] Ammonium, along with other electrolytes (*i.e.*, sodium and potassium) typically monitored during a cell culture process, are measured by potentiometric-based electrodes which have ion-selective membranes.[2] While all commercial MBT analyzers utilize a similar method of measurement for the metabolites examined (glucose, lactate, glutamine, glutamate, and ammonium), there are a few significant differences between the physical features of these two MBT instruments that are important to consider. For example, both MBT instruments advertise small sample volume requirements as a major feature for conserving cell mass and end-product.[2] However, some MBT instruments require up to a 1.0 mL sample volume while others require as little as 10–60 µL of sample volume depending on the number of metabolites being tested. Sample volumes for at-line analysis have recently become a topic of interest with the growing advancement and application of new microscale fermentation systems such as the ambr15™ (TAP Biosystems/Sartorius Stedim) with a working volume of only 10–15 mL.[4]

In contrast to the amperometric and potentiometric technology that the MBT analyzers use, the APBT analyzers apply the principle of photometric assay measurement to determine metabolite (glucose, lactate, glutamine, glutamate, and ammonia) concentrations. For APBT measurements, the sample of interest is mixed with a set of reagents that are stored within the temperature-controlled unit. The absorption levels generated by the enzymatic reaction taking place are measured by the photometric measurement unit to calculate the metabolite concentrations. Commercially available APBT instruments have recently gained interest by the biotechnology industry for their sensitive, precise, and accurate analytical data that guarantees high-quality monitoring of the fermentation process.[5] The APBT instruments are designed for high-throughput applications in process development, and some instruments are capable of performing up to 320 tests per hour.[5] In addition to the high-throughput of sample analysis, a unique capability of the APBT instruments is the automatic dilution function, which not only extends the assay measurement range, but also decreases variability associated with manual operator dilutions while reducing operational time.[5]

TABLE 1. APBT vs. MBT instrument comparison overview.

Instrument	Company	Technology
Cedex Bio HT	Roche Custom Biotech	Absorption Photometric (APBT)
CuBiAn HT-270	Optocell Technology	Absorption Photometric (APBT)
BioProfile FLEX	Nova Biomedical	Immobilized Enzymatic Membrane (MBT)
YSI 2950	YSI Life Sciences	Immobilized Enzymatic Membrane (MBT)

NOTE: All four instruments are excellent products, which is why they are in use at Biogen Idec. In this study, one of the APBT instruments is referred to as APBT 1 and one is APBT 2. Likewise, one of the MBT instruments is called MBT 1 and the other, MBT 2. This has been done in an effort to maintain a level of impartiality.

Testing Criteria

Following the directive for the validation of analytical procedures reported by the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use[6], the following validation characteristics were emphasized during the studies: precision, specificity, linearity, range and accuracy.

The precision characteristics of each instrument was the most widely examined criteria and is present in all aspects of this multi-analyzer evaluation. As outlined in the ICH guidelines, there are three distinct elements associated with overall concept of instrument precision: (1) repeatability; (2) intermediate precision; and (3) reproducibility. Each of these elements provides a unique evaluation tool that is essential in characterizing the variation that exists amongst each individual analyzer for the series of testing samples.

The repeatability component of each of the four instruments was investigated using triplicate samples of standards prepared from commercially available YSI Life Science analyte solutions. Six concentrations spanning a wide range for five analytes were chosen to represent the metabolite range commonly observed over the course of a cell culture process. This experimental matrix exceeded the "minimum of nine determinations covering the specified range for the procedure (*e.g.*, three concentrations/three replicates each),"[6] as detailed in the ICH guidelines, in order to evaluate instrument performance over a broad range of metabolite concentrations.

Testing for intermediate precision took place over a two-month time period in conjunction with the examination of the other validation elements highlighted. The intent of the intermediate precision assessment was to "establish the effects of random events on the precision of the analytical procedure"[6] for each of the four metabolite analyzers over a period of ten test days. To capture these effects, the ten test days were chosen at random, and samples were not run in triplicate in order to simulate typical sampling activities. The goal for the intermediate precision study was to further evaluate which instrument(s) would generate the most consistent results with the least amount of variation over a two-month testing period. In order to capture the effects of day-to-day variation, a "normalized" analyzer maintenance schedule was incorporated in order to simulate typical laboratory operations. Quality control samples were performed once a week to ensure the integrity of the MBT instrument membranes and the APBT on-board reagents.

In order to demonstrate reproducibility, data was generated from two different Biogen Idec locations, Research Triangle Park (RTP), North Carolina, and Cambridge (Camb), Massachusetts, with different operators to depict what types of variations might exist during inter-laboratory operations using identical instruments. It is important to note that the initial APBT/MBT multi-analyzer comparison was performed prior to the cross-site study. Data generated from the multi-analyzer assessment indicated that the APBT 1 instrument had the greatest potential for accurate and robust cell culture monitoring. To further investigate this instrument, the second location (Camb) and operator was chosen to evaluate the reproducibility of results generated during the multi-analyzer investigation. Samples were prepared in bulk in the RTP laboratory, frozen to −80°C, and shipped (dry ice) to the Camb laboratory where the analysis was completed in order to compare the results from two locations.

Specificity is defined as "the ability to assess unequivocally the analyte in the presence of components which may be expected to be present."[6] In order to evaluate the performance of the four analyzers for the ability to recognize only one analyte of interest, a spiking study was conducted using filtered cell culture supernatant samples from two different production bioreactors. The cell culture supernatant samples were spiked with high and low concentrations of glucose, lactate, glutamate, and ammonium that were purchased from YSI Life Sciences. Samples were run on each instrument prior to the addition of spiking solutions in order to obtain baseline metabolite readings. In the interest of minimizing the variability between individual testing samples, spiking standards were added to bulk solutions of liquid supernatant (25 mL) and mixed before being dispensed into sample cups for each instrument.

Finally, linearity, range, and accuracy were also incorporated within these studies in order to maintain compliance with the ICH validation guidelines. Linearity characterization requires a minimum of five concentrations as well as a mathematical analysis of the regression lines and deviations between samples. All of these specifications were incorporated into the multi-analyzer comparison in which triplicate samples were tested across six concentrations and were mathematically evaluated using JMP® software (SAS Institute Inc.). Metabolite range and instrument accuracy were accounted for in almost every aspect of these specialized studies so that the overall evaluation of the four chosen metabolite-sensing technologies was thorough and comprehensive.

Standard Samples with Known Concentrations

Commercially available standard solutions purchased from YSI Life Sciences for glucose, lactate, glutamine, glutamate, and ammonium were used to evaluate the accuracy and precision of the APBT and MBT instruments. Standard samples for each analyte were prepared with

TABLE 2. Metabolite matrix from high to low concentration.

Glucose (g/L)	Lactate (g/L)	Glutamine (mM)	Glutamate (mM)	Ammonium (mM)
15.00	5.00	6.00	9.00	15.00
5.00	2.50	3.00	5.00	10.00
2.50	1.25	1.50	2.50	5.00
1.25	0.63	0.75	1.25	2.50
0.20	0.20	0.45	0.25	0.25
0.03	0.01	0.20	0.10	0.05

YSI standard solutions diluted with a phosphate buffered saline (PBS) solution to obtain the desired analyte concentrations outlined in Table 2. Each prepared solution was 100 mL in volume. All were vortexed in order to ensure homogeneity, dispensed into eppendorf tubes, and then stored at −80°C until analyzed in triplicate.

Cell Culture Supernatant Samples

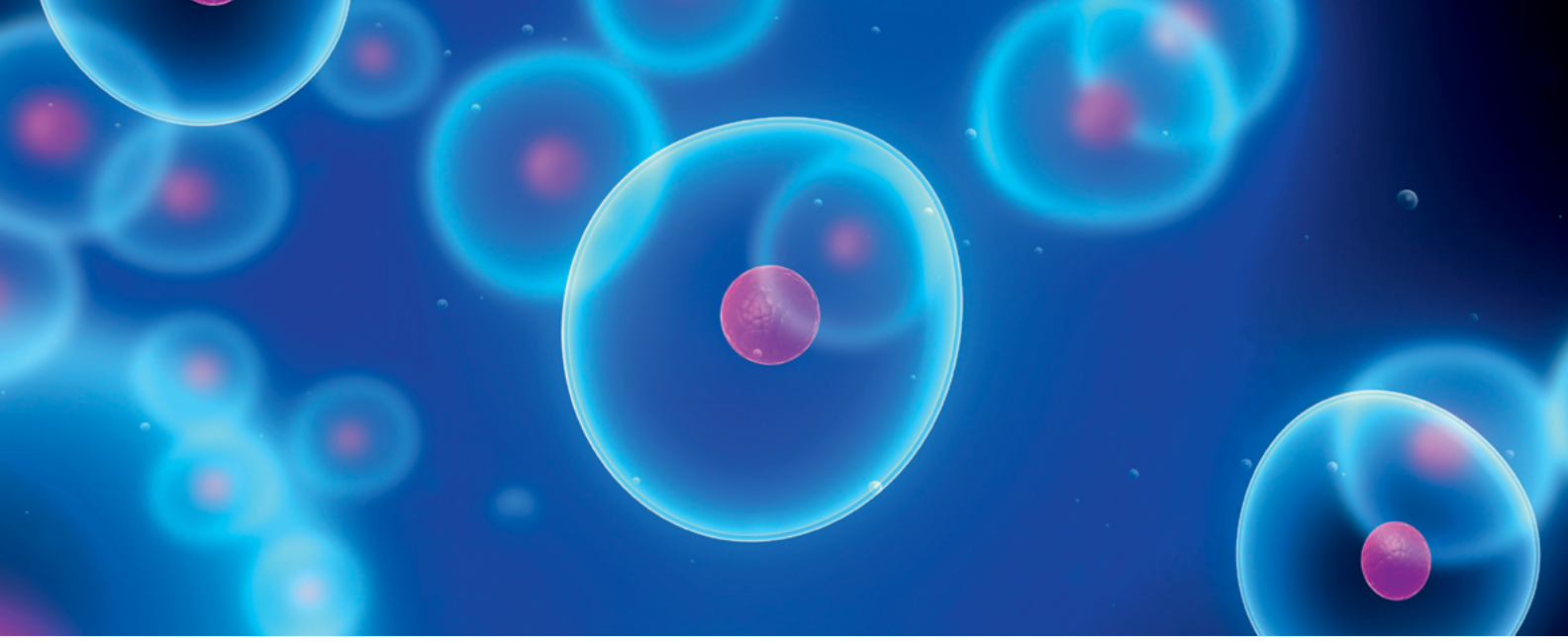
The cell culture samples were obtained from four different programs derived from a Chinese hamster ovary (CHO) host cell line. To eliminate any interference from particulates and cell debris, all cell culture samples were centrifuged at 3,000 rpm for ten minutes after being collected from the stainless steel bioreactors. After centrifugation, the supernatant was vacuum filtered using a Millipore Express® PLUS 0.22 µm membrane and stored at −80°C. Two of the programs were used for evaluating the intermediate precision element of the analyzers while the other two program samples were used in the spiking study to assess the analyte-specific recovery of each instrument.

Quality Controls/Calibrations

Calibration specifications are a large differentiator between the MBT and APBT instruments when considering required resources. A summary of vendor-recommended calibration schedules is outlined in Table 3. Due to the numerous sample runs necessary for this study, the MBT instruments required frequent calibrations in order to

TABLE 3. Calibration intervals required for each metabolite-sensing instrument.

Instrument	Analyte	Calibration Interval
APBT 1	Glucose	Each new lot (or 90 days)
	Lactate	Each new lot (or 90 days)
	Glutamine	4 days
	Glutamate	14 days
	Ammonia	Each new lot (or 90 days)
APBT 2	Glucose	New lot (or 4 weeks)
	Lactate	New lot (or 4 weeks)
	Glutamine	New lot (or 2 weeks)
	Glutamate	New lot (or 4 weeks)
	Ammonia	New lot (or 2 weeks)
MBT 1	Glucose	Automatic calibration recommended every 2 hours
	Lactate	
	Glutamine	
	Glutamate	
	Ammonium	
MBT 2	Glucose	Automatic calibration recommended every 5 samples (~15 minutes)
	Lactate	
	Glutamine	
	Glutamate	
	Ammonium	



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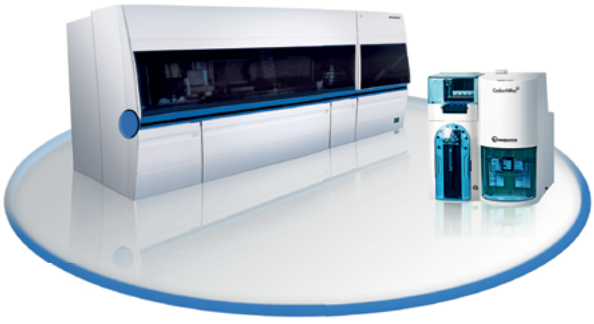

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maintain membrane integrity and minimize drift. The frequent calibration intervals added a significant amount time to obtain results.

Quality controls for each instrument were run the same morning of sample testing for each investigation discussed in this study, except for the intermediate precision element. For the two-month intermediate precision study, the goal was to capture natural variation over the course of ten testing days. However, for the spiking study and the multi-analyzer comparison, quality controls were run prior to testing in order to ensure that all instruments were operating under ideal conditions. For the MBT instruments, membranes were exchanged if quality controls did not pass, and for the APBT instruments, reagents were re-calibrated and controls run again until all analyzers passed within ±1 standard deviation of the control value. Quality control results over the course of the entire study are shown in Figure 1 (A–D). Red bars on the graph indicate the range of acceptable recovery of quality control standards.

Statistical Analysis

A statistical comparison of mean estimates was conducted using JMP software to determine if significant differences existed between instruments and technologies within a series of measurements. Since we are comparing means for more than two groups of interest, we can use the analysis of variance (ANOVA) technique in order to determine if there is a significant difference between triplicate sample values. When the probability values (*p*-value) based on the *F*-test are less than 0.05, it can be concluded that there exists some significant difference between the average values reported. *P*-values greater than 0.05 support the idea that there is no difference between the mean values reported by each of the four instruments or the two different technologies. For every ANOVA in which a significant difference was identified, Tukey-Kramer honestly significant difference (HSD) test was employed as a follow-up to the initial ANOVA conducted. The Tukey-Kramer

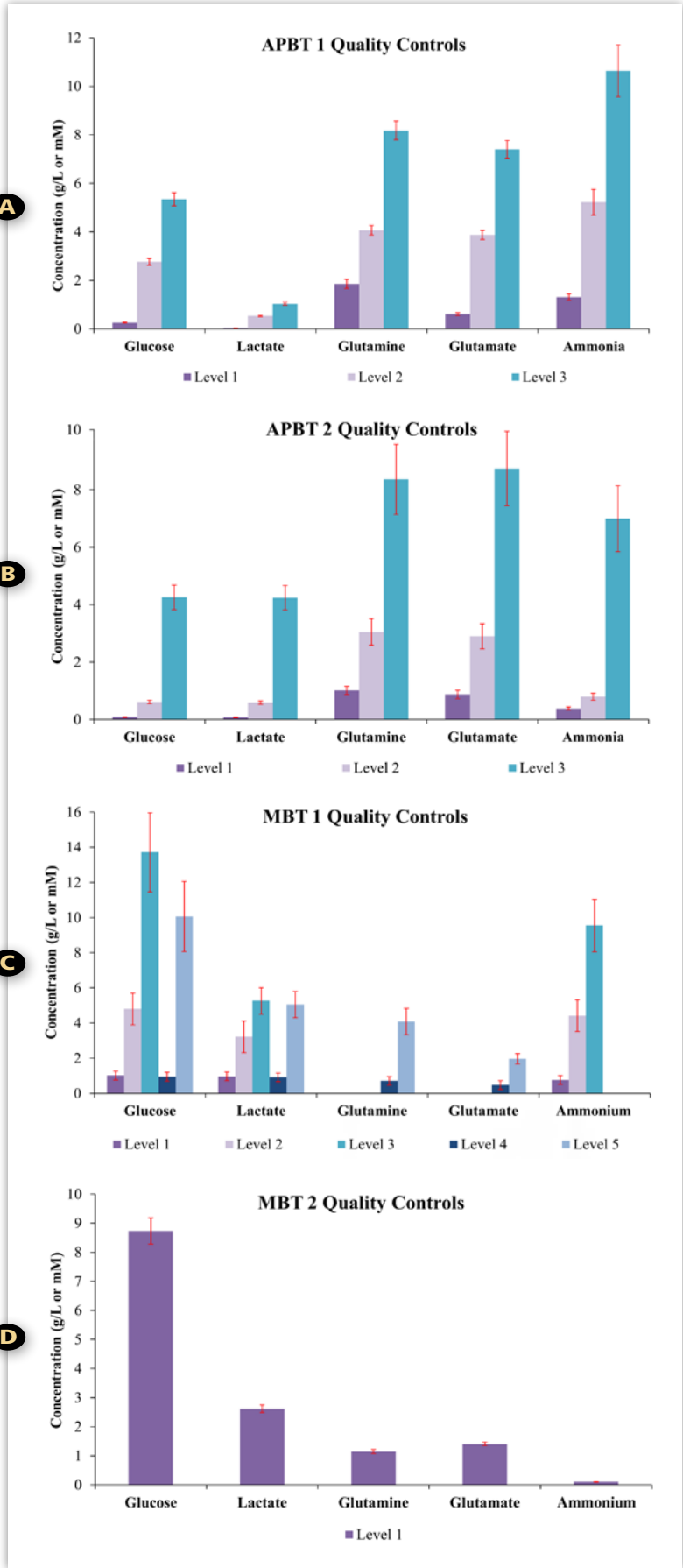


FIGURE 1. Quality control values for: **(A)** APBT 1; **(B)** APBT 2; **(C)** MBT 1; and **(D)** MBT 2. Red bars indicate acceptable range of quality control recovery.

HSD test is a means comparison method that compares the actual difference between group means with the difference that would be significantly different.[7] By using this range test, we could determine which of the four instruments was reporting significantly different measurements from the control values, as well as which technologies (MBT or APBT) were reporting sample measurements that were significantly different from each other.

Experimental Outline

Analyzer Maintenance

Vendor specifications for complete analyzer functionality and accuracy include an array of maintenance operations, calibrations, and quality controls that must be performed prior to obtaining cell culture sample data. Maintaining the metabolite analyzers per vendor recommendations may not always be possible when operating in a busy lab setting and is therefore an important aspect to evaluate when choosing which at-line instrument to implement as the platform bioprocessing analyzer. In order to determine how the MBT and the APBT instruments would perform under different maintenance schedules, ideal maintenance and simulated weekly lab maintenance conditions were employed during the study.

Ideal operating conditions consisted of ensuring that all of the analyzers had been properly calibrated and passed all quality control requirements for each testing analyte prior to running any of the samples. For the MBT instruments, all analyte membranes were replaced to ensure that membrane age was not a factor for poor results. Establishing that both the MBT and APBT analyzers were operating at optimal conditions was a key factor in assessing performance under a best-case scenario.

A simulated weekly maintenance routine for the analytical equipment was examined during the intermediate precision study. It was determined that during normal laboratory operations, the APBT instruments would only be calibrated when it was required by the instrument and quality controls would be run once a week. The MBT analyzers have calibrator packs that are stored within the instrument, so calibrations were regulated by the MBT instrument and quality control standards were also only run once a week.

Multi-Analyzer Comparison

For the multi-analyzer comparison, six concentrations of each analyte were chosen to represent the metabolite matrix commonly observed during some mammalian cell culture processes. In order to encompass the precision element required by the ICH validation guidelines, the aforementioned matrix values (as outlined in Table 2) for the metabolites were run in triplicate. This process is not typically performed during a cell culture process due to time and resource constraints, but for the purpose of this analytical technology assessment, attaining information about an instrument's ability to repeat homogenous samples and report values with a low coefficient of variance is essential. Mean (μ), standard deviation (σ), relative

standard deviation (RSD), and percent recovery are summarized in Table 4 (A–E) for all metabolites and their specified concentration range. Observed standard deviations for each concentration were very low for both APBT and MBT instruments since the samples being tested in triplicate were simple, known solutions. One would expect to see less variability among the standard solutions (YSI solutions diluted with PBS) versus cell culture supernatant samples

TABLE 4. Mean (μ), standard deviation (σ), relative standard deviation (RSD), and recovery (%) for: **(A)** glucose (g/L); **(B)** lactate (g/L); **(C)** glutamine (mM); **(D)** glutamate (mM); and **(E)** ammonium (mM).

A

Glucose (g/L)							
Instrument		**15.00**	**5.00**	**2.50**	**1.25**	**0.20**	**0.03**
APBT 1	μ	15.76	5.06	2.53	1.27	0.20	0.03
	σ	0.07	0.02	0.00	0.02	0.01	0.01
	RSD	0.45	0.30	0.00	1.36	2.84	21.65
	Recovery (%)	105.04	101.13	101.20	101.60	101.67	88.89
APBT 2	μ	15.50	5.34	2.69	1.33	0.18	0.01
	σ	0.11	0.01	0.04	0.01	0.00	0.00
	RSD	0.74	0.14	1.46	0.83	0.31	4.68
	Recovery (%)	103.33	106.79	107.40	106.13	92.17	41.11
MBT 1	μ	14.94	5.16	2.59	1.25	0.08	N/A
	σ	0.38	0.17	0.01	0.01	0.01	N/A
	RSD	2.56	3.34	0.22	0.46	6.93	N/A
	Recovery (%)	99.60	103.13	103.47	99.73	41.67	N/A
MBT 2	μ	13.37	4.50	2.27	1.13	0.17	N/A
	σ	0.15	0.03	0.02	0.01	0.01	N/A
	RSD	1.14	0.68	0.88	0.51	3.52	N/A
	Recovery (%)	89.11	90.07	90.80	90.67	83.17	N/A

B

Lactate (g/L)							
Instrument		**5.00**	**2.50**	**1.25**	**0.63**	**0.20**	**0.01**
APBT 1	μ	5.11	2.48	1.26	0.65	0.20	0.01
	σ	0.01	0.01	0.00	0.00	0.00	0.00
	RSD	0.00	0.23	0.44	0.63	0.43	0.53
	Recovery (%)	102.20	99.33	100.80	103.17	100.00	100.00
APBT 2	μ	4.93	2.38	1.29	0.63	0.19	0.01
	σ	0.08	0.02	0.01	0.00	0.00	0.00
	RSD	1.69	0.80	0.94	0.51	0.61	16.67
	Recovery (%)	98.53	95.16	103.28	100.37	94.67	60.00
MBT 1	μ	4.82	2.35	1.23	0.43	N/A	N/A
	σ	0.46	0.05	0.02	0.01	N/A	N/A
	RSD	9.46	2.01	1.70	1.35	N/A	N/A
	Recovery (%)	96.33	94.13	98.13	67.72	N/A	N/A
MBT 2	μ	4.51	2.15	1.17	0.59	0.18	0.01
	σ	0.04	0.01	0.02	0.00	0.00	0.00
	RSD	0.84	0.27	1.97	0.34	0.31	10.19
	Recovery (%)	90.27	85.87	93.87	93.49	92.17	56.67

C

Glutamine (mM)							
Instrument		**6.00**	**3.00**	**1.50**	**0.75**	**0.45**	**0.20**
APBT 1	μ	6.06	3.15	1.61	0.76	0.45	#N/A
	σ	0.01	0.01	0.01	0.00	0.02	#N/A
	RSD	0.10	0.37	0.36	0.00	3.42	#N/A
	Recovery (%)	100.94	104.89	107.11	101.33	99.26	#N/A
APBT 2	μ	5.94	3.12	1.44	0.49	0.15	0.03
	σ	0.03	0.02	0.11	0.04	0.01	0.01
	RSD	0.55	0.72	7.72	8.77	5.00	22.42
	Recovery (%)	99.05	103.99	96.21	65.51	33.93	15.67
MBT 1	μ	6.30	3.27	1.49	0.46	0.09	N/A
	σ	0.07	0.03	0.02	0.00	0.01	N/A
	RSD	1.14	0.81	1.03	0.00	6.19	N/A
	Recovery (%)	105.00	109.00	99.11	61.33	20.74	N/A
MBT 2	μ	5.45	2.89	1.49	0.71	0.44	0.03
	σ	0.13	0.01	0.03	0.02	0.00	0.00
	RSD	2.32	0.20	1.69	2.39	0.80	6.44
	Recovery (%)	90.89	96.22	99.11	94.84	97.19	16.17

D

Glutamate (mM)							
Instrument		**9.00**	**5.00**	**2.50**	**1.25**	**0.25**	**0.10**
APBT 1	μ	9.06	5.03	2.54	1.25	0.23	0.08
	σ	0.02	0.02	0.02	0.01	0.01	0.01
	RSD	0.25	0.41	0.82	0.46	2.47	6.93
	Recovery (%)	100.70	100.67	101.47	99.73	93.33	83.33
APBT 2	μ	9.24	5.01	2.47	1.19	0.22	0.08
	σ	0.02	0.13	0.01	0.00	0.00	0.00
	RSD	0.27	2.55	0.30	0.30	0.70	0.70
	Recovery (%)	102.70	100.19	98.91	95.44	87.73	82.33
MBT 1	μ	8.52	5.57	2.92	1.49	0.17	0.01
	σ	0.11	0.37	0.01	0.01	0.00	0.01
	RSD	1.33	6.70	0.34	0.39	0.00	86.60
	Recovery (%)	94.70	111.40	116.80	119.47	68.00	13.33
MBT 2	μ	8.29	4.82	2.39	1.16	0.19	0.03
	σ	0.08	0.05	0.02	0.02	0.00	0.00
	RSD	0.92	1.04	0.87	1.31	2.45	6.44
	Recovery (%)	92.07	96.40	95.47	93.07	74.80	32.33

E

Ammonium (mM)							
Instrument		**15.00**	**10.00**	**5.00**	**2.50**	**0.25**	**0.05**
APBT 1	μ	14.31	9.57	4.79	2.35	0.22	0.05
	σ	0.08	0.07	0.05	0.01	0.01	0.03
	RSD	0.55	0.76	0.96	0.34	2.74	60.33
	Recovery (%)	95.40	95.70	95.73	94.17	88.13	94.00
APBT 2	μ	10.53	8.30	4.50	2.28	0.19	0.05
	σ	0.05	0.07	0.03	0.03	0.01	0.00
	RSD	0.47	0.87	0.77	1.47	4.92	5.75
	Recovery (%)	70.22	83.02	90.08	91.33	75.47	92.00
MBT 1	μ	16.09	9.83	4.64	2.21	0.30	0.14
	σ	0.10	0.03	0.05	0.02	0.01	0.01
	RSD	0.64	0.27	0.99	0.78	1.95	4.03
	Recovery (%)	107.24	98.30	92.80	88.40	118.67	286.67
MBT 2	μ	14.87	10.07	5.68	3.32	1.16	1.00
	σ	0.21	0.06	0.02	0.02	0.03	0.04
	RSD	1.40	0.57	0.37	0.63	2.64	3.95
	Recovery (%)	99.11	100.67	113.67	132.93	462.67	1995.33

Samples represented in this table were prepared from standard solutions purchased from YSI Life Sciences and diluted with a phosphate buffered saline solution. Mean (μ) and standard deviation (σ) were generated from triplicate sample measurements of standard solutions at each concentration. Recovery (%) was determined by comparing the reported mean value of each instrument to the desired theoretical sample concentration listed along the top line of each table.

due to their matrix differences, which is why using the standard solutions provides justifiable results reflecting the intra-assay precision of each analyzer.

From viewing the results shown in Table 4, it is clear that the APBT instruments most consistently match up with the known concentrations of the standard YSI Life Sciences solutions and have a percent recovery very close to 100% across the testing range. Concentrations that did not reach into the lower limits of the assay range were recovered very well by the MBT instruments, but the MBT analyzers were not capable of recovering the lower limit concentration values as accurately or precisely as the APBTs, and often suffered from poor recovery values. The APBT assay limit of detection for each metabolite was typically lower than the MBT analyzers, which accounts for the lowest concentration of each metabolite sample not displaying a percent recovery value in both Table 4 (A–E) and Figure 2 (A–E). The APBT instruments were able to recover concentrations across the entire pre-determined range except for APBT 1 whose lower limit of detection for glutamine is 0.41 mM, and therefore did not display a percent recovery value for concentrations below this. A visual representation of the metabolite recovery data presented in Table 4 is shown in Figure 2 (A–E).

Metabolite recovery was typically the most accurate for the three highest concentrations of each analyte examined and declined as concentrations approached the assay lower limit of detection. Out of the five metabolites examined, the ammonium standards showed the worst recovery and greatest variability for all four instruments. Both APBT analyzers reported values that were lower than the standards used across the entire testing range. The MBT analyzers, however, had acceptable recovery for the highest two concentrations tested but displayed values very different from the standards used for the four lower concentrations investigated. The recovery values calculated for the lower ammonium concentrations were so high that the graph (Figure 2E) could not be extended enough to show them. Possible reasons for the extremely high recovery values given by the MBT instruments has yet to be determined, and further studies should be done to examine the large discrepancies that exist between the two types of technologies for the measurement of ammonium.

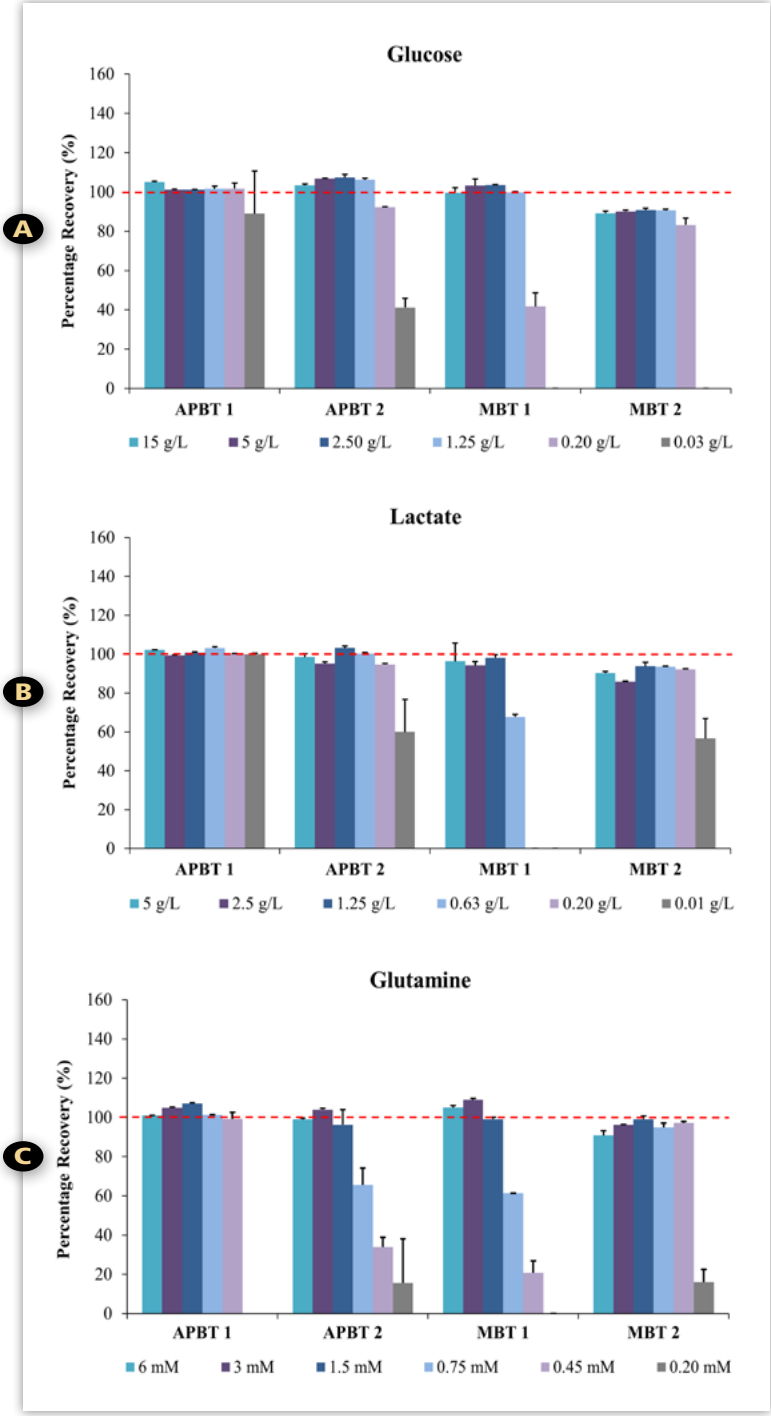
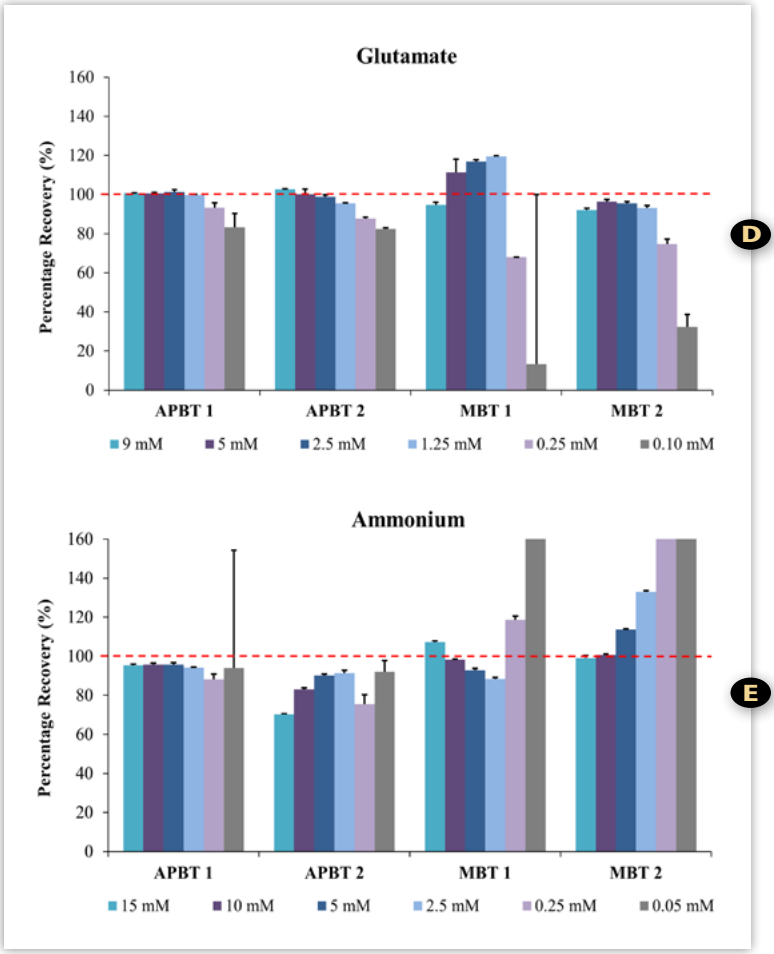


FIGURE 2 (A–C). Metabolite recovery for standard samples. Calculated recovery (%) was determined by comparing the reported mean value of each instrument to the desired theoretical sample concentration listed along the top line of Table 4 (A–C). Recovery bars are not displayed for values which extend lower than the instrument assay range. The error bars indicate the relative standard deviation calculated from the triplicate sample measurements.



The *p*-values from the Tukey-Kramer HSD test are recorded in Table 5. The *p*-values indicate which instruments reported values that were statistically comparable to the actual standard concentrations prepared using the YSI Life Sciences standards. The comparison outlined in the table (instrument vs. control) refers to the theoretical value of the prepared standards in relation to the mean value reported by each instrument. From the table, it is clear that APBT 1 reported values that were most equivalent with the theoretical value of the prepared solutions. Not including the ammonium *p*-values, APBT 1 only reported two other statistically different values: lactate (0.63 g/L) and glutamate (0.45 mM). As previously stated, all instruments performed poorly with the ammonium standards which is further emphasized by the *p*-values of each instrument

FIGURE 2 (D and E). Metabolite recovery for standard samples. Calculated recovery (%) was determined by comparing the reported mean value of each instrument to the desired theoretical sample concentration listed along the top line of Table 4 (D and E). Recovery bars are not displayed for values which extend lower than the instrument assay range. The error bars indicate the relative standard deviation calculated from the triplicate sample measurements.

TABLE 5. ANOVA results between triplicate sample measurements of standard samples for each metabolite.

A

Glucose *p*-values					
Instrument vs. Control	15.0 g/L	5.0 g/L	2.5 g/L	1.25 g/L	0.2 g/L
APBT 1	0.5139	0.8969	0.4203	0.1791	0.9276
APBT 2	0.0603	0.0025*	<0.0001*	<0.0001*	0.0180*
MBT 1	0.9948	0.1814	0.0028*	0.9932	<0.0001*
MBT 2	<0.0001*	0.0001*	<0.0001*	<0.0001*	<0.0001*

B

Lactate *p*-values					
Instrument vs. Control	5.0 g/L	2.5 g/L	1.25 g/L	0.63 g/L	0.2 g/L
APBT 1	0.9631	0.8924	0.9166	0.0004*	1.000
APBT 2	0.9916	0.0005*	0.032*	0.794	0.0055*
MBT 1	0.8126	0.0001*	0.3638	<0.0001*	<0.0001*
MBT 2	0.0960	<0.0001*	0.0006*	<0.0001*	<0.0001*

C

Glutamine *p*-values					
Instrument vs. Control	6.0 mM	3.0 mM	1.5 mM	0.75 mM	0.45 mM
APBT 1	0.8309	0.3101	0.1527	0.9745	0.9855
APBT 2	0.8309	<0.0001*	0.7087	<0.0001*	<0.0001*
MBT 1	0.0019*	<0.0001*	0.9973	<0.0001*	<0.0001*
MBT 2	<0.0001*	<0.0001*	0.9973	0.2108	0.3316

D

Glutamate *p*-values					
Instrument vs. Control	9.0 mM	5.0 mM	1.5 mM	0.75 mM	0.45 mM
APBT 1	0.7360	0.9993	0.0632	0.9837	0.0009*
APBT 2	0.0057*	1.0000	0.2093	<0.0001*	<0.0001*
MBT 1	<0.0001*	0.0189*	<0.0001*	<0.0001*	<0.0001*
MBT 2	<0.0001*	0.7312	<0.0001*	<0.0001*	<0.0001*

E

Ammonium *p*-values					
Instrument vs. Control	15.0 mM	10.0 mM	5.0 mM	2.5 mM	0.25 mM
APBT 1	0.0001*	<0.0001*	<0.0001*	<0.0001*	0.1760
APBT 2	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0033*
MBT 1	<0.0001*	0.0205*	<0.0001*	<0.0001*	0.0203*
MBT 2	0.6074	0.5752	<0.0001*	<0.0001*	<0.0001*

*P-values less than 0.05 indicate a significant difference between the instrument measurement and the control sample concentration.

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being consistently lower than 0.05. Poor results for ammonium that were reflected in Table 4 (A–E) and Figure 2 (A–E) are further confirmed in this statistical examination of the triplicate samples. Only the APBT 1 (0.25 mM) and the MBT 2 (15.0 mM and 10.0 mM) instruments reported probability values that were greater than 0.05, but overall instrument precision was the most inconsistent for this metabolite than any other using the prepared standard solutions. Further studies investigating the precision and accuracy of ammonium measurements over an extensive range would need to be conducted in order to determine which instrument is capable of most consistently quantifying this important metabolite.

Intermediate Precision Analysis

The prepared standard solutions (YSI solutions diluted with PBS) allowed for a variability baseline to be determined for each instrument since lower sample variability was expected in comparison to cell culture samples. However, the intended purpose of metabolite-sensing technologies is to accurately measure the composition of cell culture samples and must therefore be the primary focus when evaluating multiple instruments. Therefore, the next specialized study emphasized the intermediate precision element outlined in the ICH guidelines. The experimental design, as previously described for the intermediate precision study, was to examine cell culture supernatant samples over a period of ten chosen testing days. Choosing ten test days at random not only shows the precision capabilities of the instrument but also highlights the equipment variability that may be present when the machine is operating on a normal day-to-day schedule.

Figure 3 (A–D) shows the average values reported for glucose (g/L), lactate (g/L), glutamate (mM), and ammonium (mM) over ten sample days for each instrument. The error bars shown

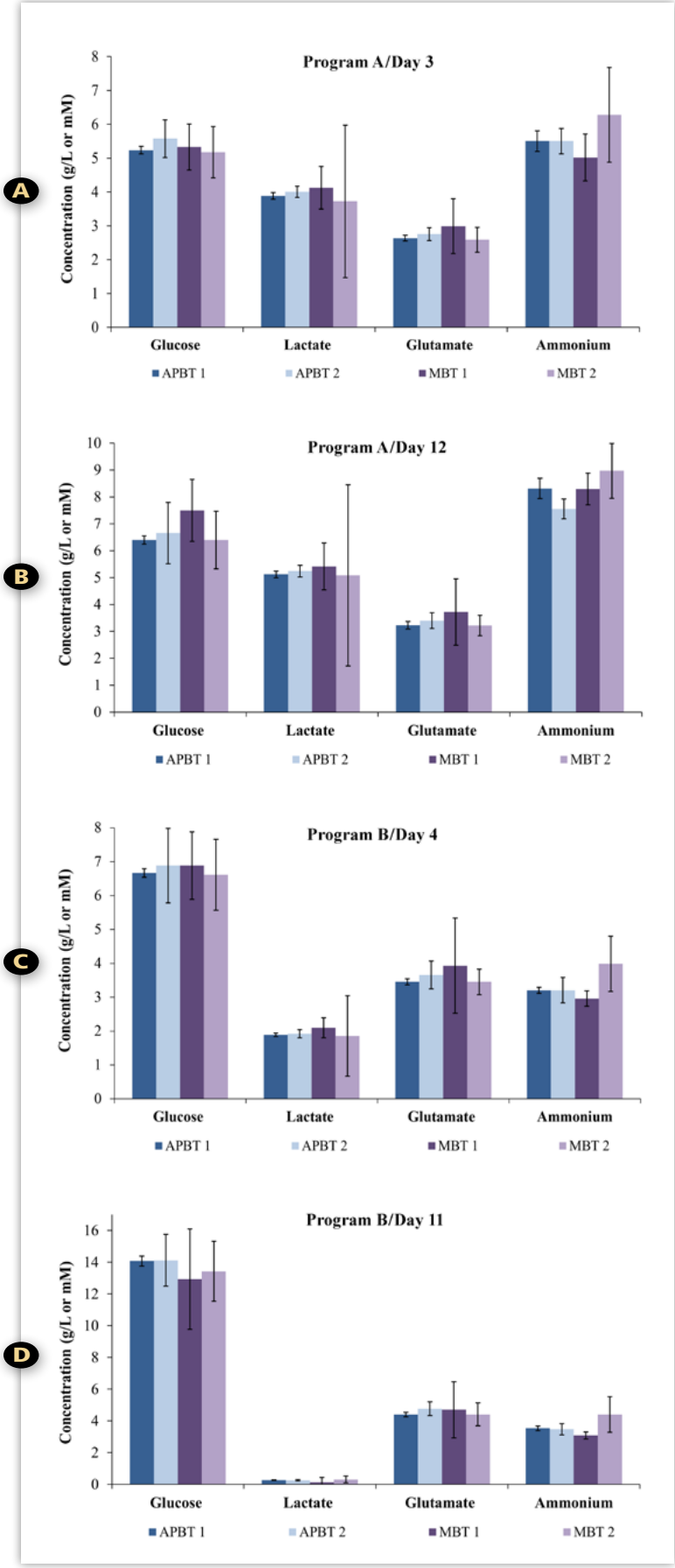


FIGURE 3 (A–D). Intermediate precision results for two CHO-based cell culture programs (A and B) for beginning and late stage supernatant samples. Reported concentration values represent the mean measurement over a period of ten testing days. The included error bars represent three standard deviations from the mean and indicate which instruments had the greatest observed variation between supernatant measurements over the course of ten days.

represent three calculated standard deviations from the reported mean value and captures the variability observed for each instrument over the course of the ten days. The APBT analyzer results are displayed in shades of blue while the purple bars represent reported averages from the MBT analyzers. From the error bars alone, it is clear that the MBT instruments demonstrated greater variation among sample measurements than the APBTs. Possible reasons for this observed difference may be due to the degradation of the membranes that occur over time. While membranes for each of the MBT instruments were replaced based on vendor instructions, it appears that there was significant drift in the values reported, such as glutamate by MBT 1, and lactate and ammonium by MBT 2. The glucose measurements for the four supernatant samples also had large variation for APBT 2, MBT 1, and MBT 2 despite the fact that the mean suggested the samples were within measurement capabilities of the glucose assays. On the other hand, APBT 1 seemed to report the least amount of variation between samples over the course of the study for each of the metabolite assays. The results from the intermediate precision analysis suggest that APBT 1 might provide the best and most consistent results for samples during a production-stage cell culture process.

Further statistical analysis was conducted using JMP software in order to compare results generated by each of the two technologies (absorption photometric and membrane-based). The APBT 1 and APBT 2 mean values over ten days were compared and analyzed for significant differences in their reported sample measurements of the liquid supernatants. The mean values reported by the MBT 1 and MBT 2 instruments were also examined for significant differences. Table 6 displays the *p*-values between the two technologies for two different CHO-based programs (A and B) for each of the metabolites. The "mean" column indicates the overall average of each metabolite over the ten testing days that was then averaged for all four instruments. The *p*-values indicate whether or not a significant difference existed between the values produced by the MBT and APBT analyzers.

Table 6 reveals that there was a large difference between the values reported by the MBT instruments for the four metabolites examined. Out of the 16 analyte tests performed by each analyzer, the MBT instruments reported significantly different means eight times (50%), while the APBT instruments were only significantly different once (Program A/Day 3: Glucose). It is clear that for the liquid supernatant samples, the APBT analyzers demonstrated better overall performance in reporting comparable measurements throughout the course of the study. The four metabolite assays with the APBT analyzers examined in this study exhibited the consistency and the precision capabilities that are preferable for a platform cell culture instrument being used for daily measurements, or to develop next-generation, real-time models for advanced process control.

TABLE 6. Probability values between the two technologies for two different CHO-based programs (A and B) for each of the metabolites.

Cell Culture Supernatant	Component	Instruments	Mean	*p*-value
Program A Day 3	Glucose	MBTs	5.33	0.2211
		APBTs		0.0041*
	Lactate	MBTs	3.95	0.2217
		APBTs		0.9178
	Glutamate	MBTs	2.75	<0.0001*
		APBTs		0.4087
	Ammonium	MBTs	5.42	<0.0001*
		APBTs		0.3962
Program A Day 12	Glucose	MBTs	6.66	<0.0001*
		APBTs		0.8538
	Lactate	MBTs	5.23	0.7027
		APBTs		0.9781
	Glutamate	MBTs	3.42	0.0009*
		APBTs		0.4372
	Ammonium	MBTs	11.92	1.0000
		APBTs		0.4482
Program B Day 4	Glucose	MBTs	6.77	0.2288
		APBTs		0.4456
	Lactate	MBTs	1.95	0.1356
		APBTs		0.9899
	Glutamate	MBTs	3.65	0.0047*
		APBTs		0.3996
	Ammonium	MBTs	3.24	<0.0001*
		APBTs		0.7061
Program B Day 11	Glucose	MBTs	13.64	0.4893
		APBTs		0.9989
	Lactate	MBTs	0.33	<0.0001*
		APBTs		0.9517
	Glutamate	MBTs	4.60	0.5019
		APBTs		0.0962
	Ammonium	MBTs	3.52	<0.0001*
		APBTs		0.8291

The APBT and MBT instruments were compared to one another using JMP software to evaluate which technology reported the most consistent values over the course of ten days. **P*-values for each less than 0.05 indicate a significant difference in the mean values reported.

Multi-Analyzer Spiking Study

In order to provide a more in-depth analysis of the four instruments being examined, a spiking study was conducted using liquid supernatant from cell culture samples that had been pulled from production-stage bioreactors. Supernatant was collected for two different processes derived from the CHO host cell lines in order to evaluate instrument recovery over a broad range of metabolite concentrations. Approximately 300 mL of sterile cell culture was taken from each production stage bioreactor, centrifuged

for ten minutes at 3,000 rpm, filtered with a 0.22 µm vacuum filter, and then spiked with the desired metabolite in a biosafety cabinet. Prior to the addition of spiking solutions, the unspiked supernatant liquid (or "blank") was run in triplicate on each instrument in order to obtain a baseline value for each metabolite. The average of the triplicate "blank" samples was then used as the initial concentration in the final calculation of recovery (%). In order to minimize the variability between individual testing samples, spiking standards of glucose, lactate, glutamate, and ammonium were added to bulk solutions of liquid supernatant (25 mL) and then mixed before being dispensed into sample cups for each instrument. Spiked samples were run in triplicate in order to capture the variability between samples from the same bulk solution as well as to capture the element of instrument repeatability for this study. After measuring the spiked samples on each instrument, the recovery of each metabolite at low and high concentrations was calculated using the following equation:

$$Recovery = \frac{C_3(V_1 + V_2)}{(C_1 \times V_1) + (C_2 \times V_2)} \times 100\%$$

Table 7 defines each of the variables present in the equation for calculating instrument recovery (%). It is important to note that values for C1 and C3 are an average taken from the three reported values for both the original "blank" supernatant samples as well as the final spiked supernatant solution. It was necessary to calculate average baseline values for every analyte on each instrument in order to correctly quantify the recovery of individual analyzers. Using initial concentrations from only one instrument would not allow for the correct assessment of specificity for all other instruments since each instrument reported slightly different initial concentrations. A summary of the initial metabolite concentrations for Programs C and D are outlined in Table 8 and an overview of the final low and high level concentrations for each metabolite are listed in Table 9.

TABLE 7. Spiking study recovery calculation (C) variables (V).

Variables	C1	C2	C3	V1	V2
Description	Average initial concentration of triplicate supernatant samples prior to addition of spiking solution.	Concentration (g/L or mM) of spiking solution.	Average final concentration of triplicate spiked supernatant samples after addition of spiking solution.	Volume of bulk supernatant sample prior to addition of spiking solution (25 mL)	Volume of spiking solution added (mL)

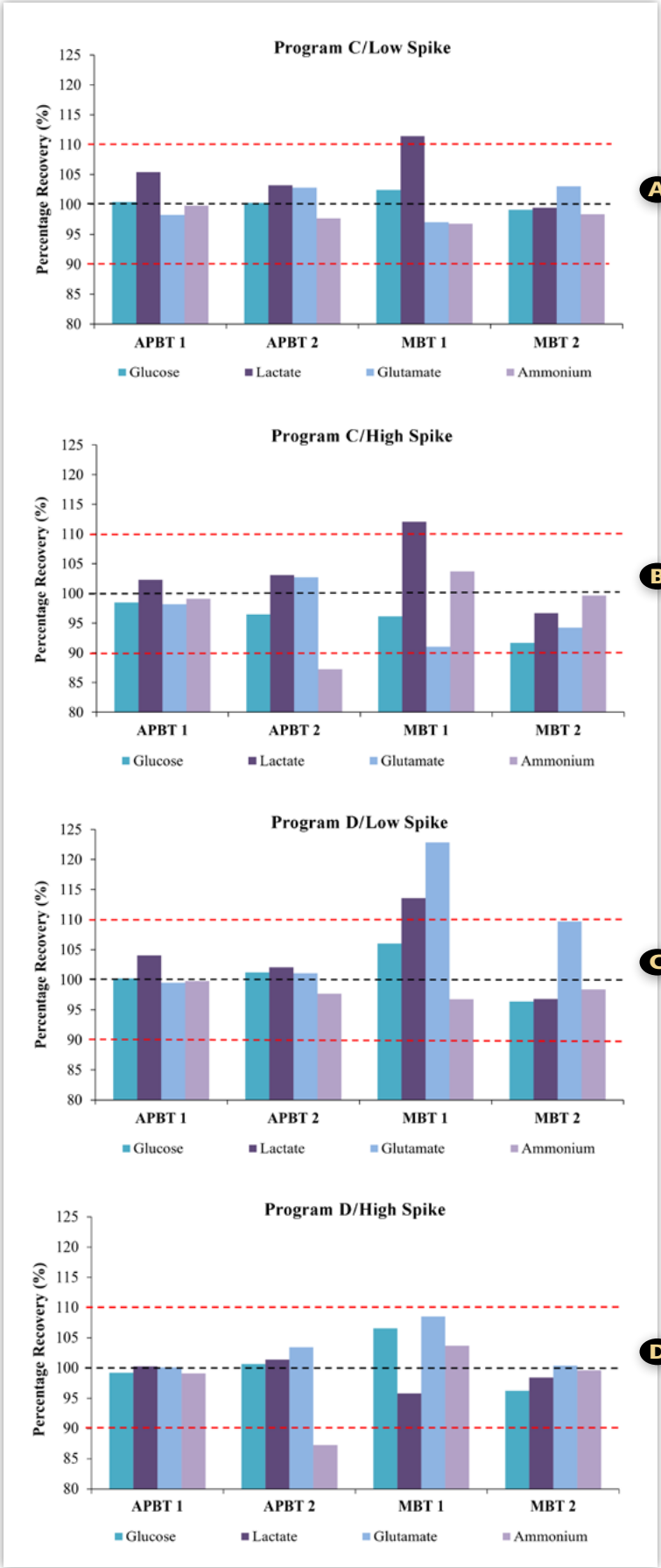
TABLE 8. Average initial metabolite concentrations for two CHO-based programs (C and D).

Program C				
Instrument	**Glucose (g/L)**	**Lactate (g/L)**	**Glutamate (mM)**	**Ammonium (mM)**
APBT 1	4.50	1.01	2.85	4.11
APBT 2	4.79	1.03	2.91	4.02
MBT 1	4.70	1.07	3.35	3.62
MBT 2	4.41	0.99	2.65	5.15
Program D				
Instrument	**Glucose (g/L)**	**Lactate (g/L)**	**Glutamate (mM)**	**Ammonium (mM)**
APBT 1	1.58	1.91	6.00	4.24
APBT 2	1.66	1.88	6.26	4.15
MBT 1	2.28	2.00	5.96	3.83
MBT 2	1.50	1.69	5.57	4.87

Baseline concentrations for each metabolite were determined prior to the addition of spiking solutions in order to calculate the analyte recovery (%) for each instrument. Supernatant samples were run in triplicate to obtain the average initial concentration values displayed in the table.

TABLE 9. Desired low and high concentrations of each metabolite after the addition of spiking solutions.

Program C								
Instrument	**Glucose (g/L)**		**Lactate (g/L)**		**Glutamate (mM)**		**Ammonium (mM)**	
	Low	*High*	*Low*	*High*	*Low*	*High*	*Low*	*High*
APBT 1	4.71	12.28	1.59	4.61	3.28	7.67	4.56	9.49
APBT 2	4.99	12.15	1.57	4.66	3.48	8.05	4.40	8.29
MBT 1	5.01	12.07	1.73	5.11	3.61	7.25	4.10	9.54
MBT 2	4.57	11.40	1.49	4.34	3.28	7.31	5.19	10.33
Program D								
Instrument	**Glucose (g/L)**		**Lactate (g/L)**		**Glutamate (mM)**		**Ammonium (mM)**	
	Low	*High*	*Low*	*High*	*Low*	*High*	*Low*	*High*
APBT 1	1.78	6.52	2.51	4.72	6.46	9.50	4.78	9.64
APBT 2	1.86	6.67	2.45	4.75	6.79	9.85	4.63	8.50
MBT 1	2.47	7.52	2.77	4.59	7.94	10.29	4.00	9.88
MBT 2	1.65	6.27	2.26	4.45	6.72	9.47	5.60	9.88



Recovery results for low and high concentration spiking solutions for each metabolite are displayed in Figure 4 (A–D). Figure 4 (A and B) shows the recovery of glucose, lactate, glutamate, and ammonium for the low and high levels of Program C. Similarly, Figure 4 (C and D) displays recovery results for low and high levels of metabolites for Program D. For the purpose of this study, a value within the range of ±10% (90–110%) was determined to be acceptable metabolite recovery. From Figure 4 (A and C), it is clear that APBT 1, APBT 2, and MBT 2 recovered the low level spike for Programs C and D of each metabolite successfully. MBT 1 showed good recovery of glucose, glutamate, and ammonium, but reported poor recovery of lactate for three out of the four spiked supernatant samples examined (Figure 4, A–C). Possible reasons for this were not investigated further but may be due to incorrect initial concentration measurements or membrane degradation. APBT 2 showed consistent, acceptable recovery of glucose, lactate, and glutamate for both low and high levels of Programs C and D, but was unable to recover the high level of ammonium for either successfully. While APBT 2 reported poor recovery for these two sets of triplicates, it is believed that these results might be the consequence of an error made when configuring the ammonium assay prior to using the instrument for this study. Further ammonium studies should be performed in order to correctly assess the instrument's performance characteristics. Overall, the APBT instruments exhibited higher sensitivity and precision than the MBT instruments for both low and high concentrations of every analyte.

Cross-Site Comparison

Data collected from the multi-analyzer comparison indicated that the absorption photometric-based technology exceeds the membrane-based technology in precision, accuracy, and metabolite sensitivity. To incorporate the reproducibility of the results generated in this study, a second APBT 1 instrument was chosen to rerun the standard samples previously outlined in Table 2

FIGURE 4 (A–D). Spiking study recovery rates for two CHO-based programs (C and D). Recovery values within ± 10% (90–110%) were determined to be acceptable metabolite recovery.

and the spiked supernatant samples outlined in Table 9. Previously described prepared standard solutions for glucose, lactate, glutamine, glutamate, and ammonia, as well as the spiked liquid supernatant samples, were frozen to –80°C and shipped (dry ice) from RTP to Camb. In order to ensure consistency after sample shipment, both sites recorded calibration and quality control values for each analyte prior to testing. Samples were run in triplicate once it was determined that both locations had calibrations within range, and quality control values for each analyzer had passed within ±1 standard deviation.

Figure 5 (A–E) shows the relationship between the two APBT 1 instruments for the standard prepared solutions. From the graphs and coefficients of determination (R^2 value) shown, it is clear that the two instruments are extremely comparable for the metabolite solutions of glucose, lactate, glutamine, glutamate and ammonia displayed. The error bars representing three standard

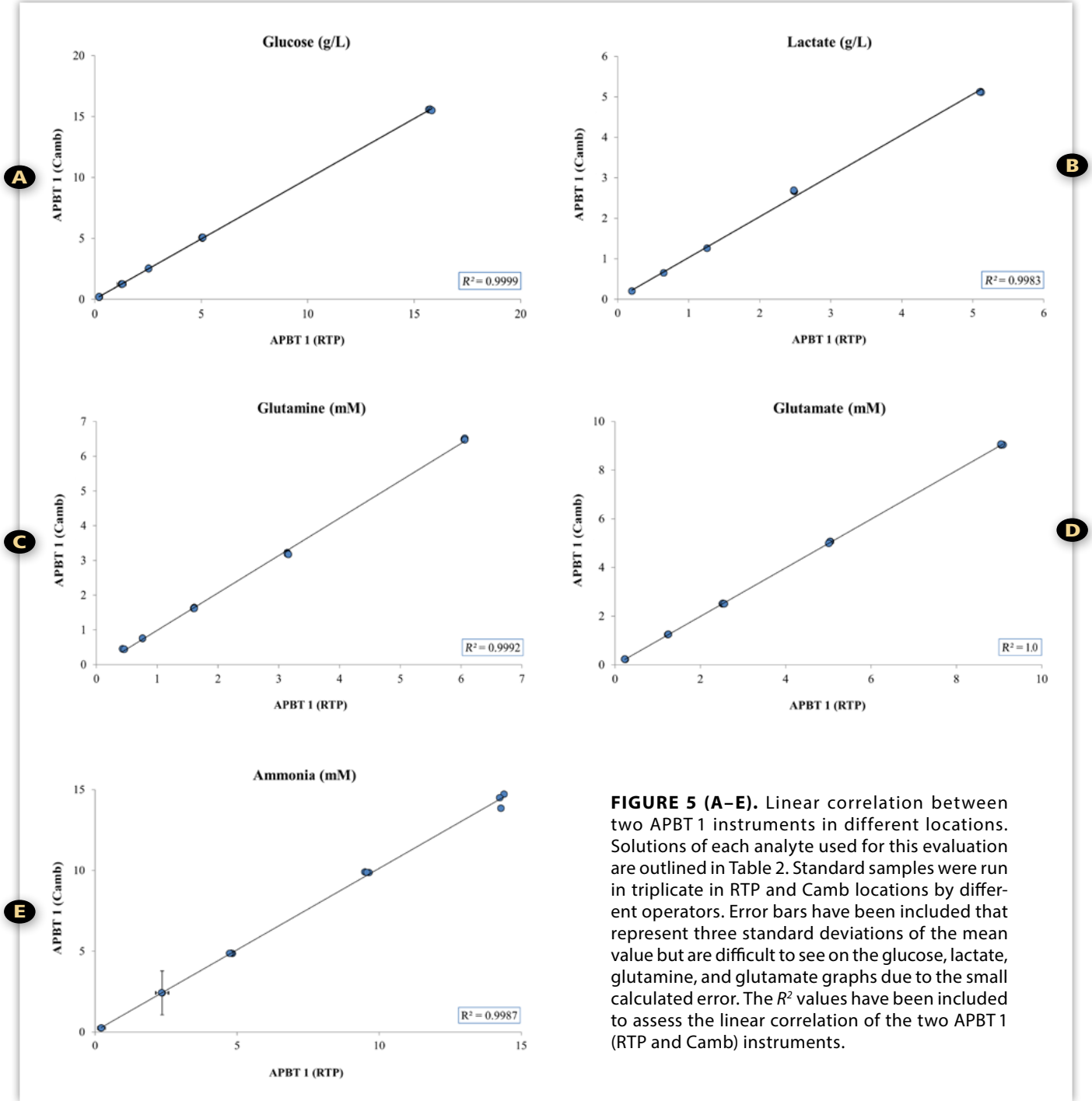
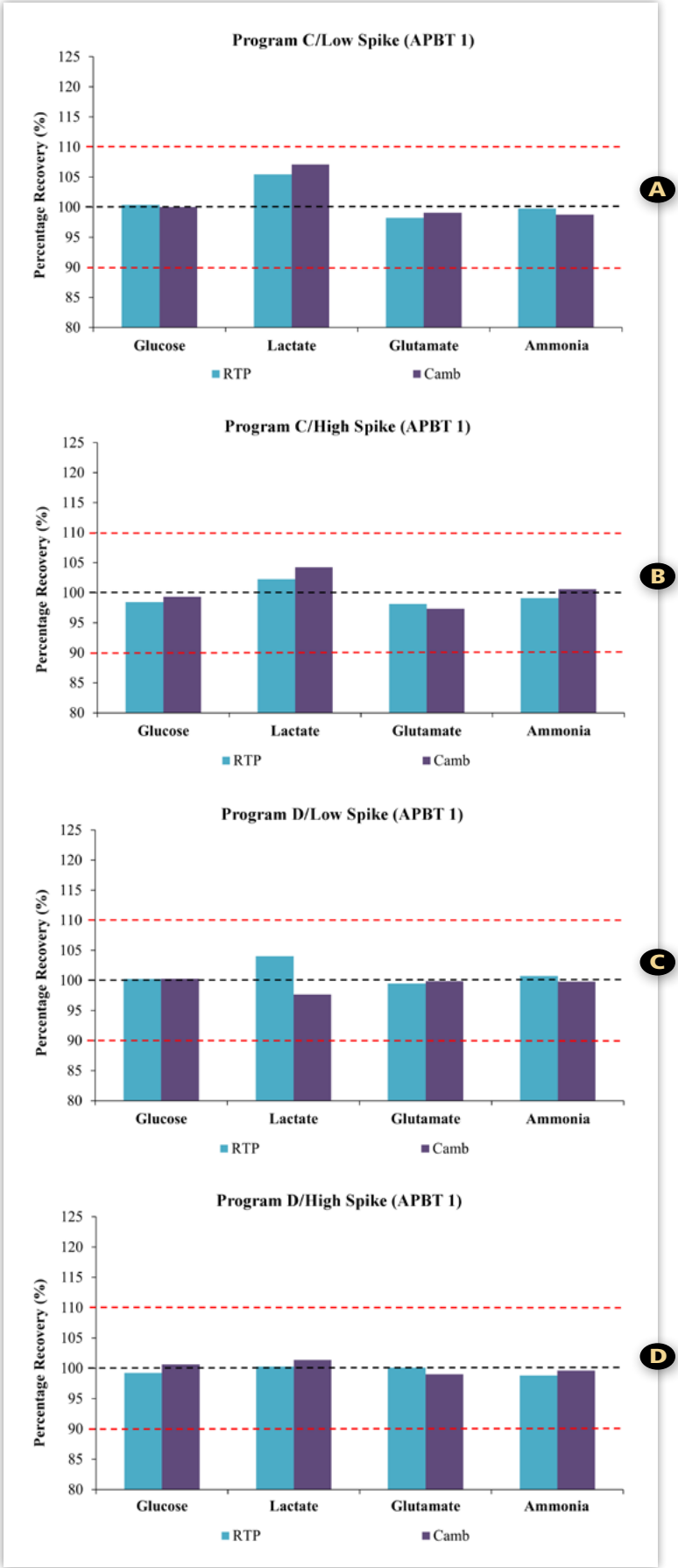


FIGURE 5 (A–E). Linear correlation between two APBT 1 instruments in different locations. Solutions of each analyte used for this evaluation are outlined in Table 2. Standard samples were run in triplicate in RTP and Camb locations by different operators. Error bars have been included that represent three standard deviations of the mean value but are difficult to see on the glucose, lactate, glutamine, and glutamate graphs due to the small calculated error. The R^2 values have been included to assess the linear correlation of the two APBT 1 (RTP and Camb) instruments.



deviations for each metabolite are extremely small for all of the graphs except ammonia and are difficult to see. As stated earlier, one would expect to see less variability among the standard solutions (YSI solutions diluted with PBS) than cell culture samples due to their matrix differences, which explains why such small sample measurement variation was observed.

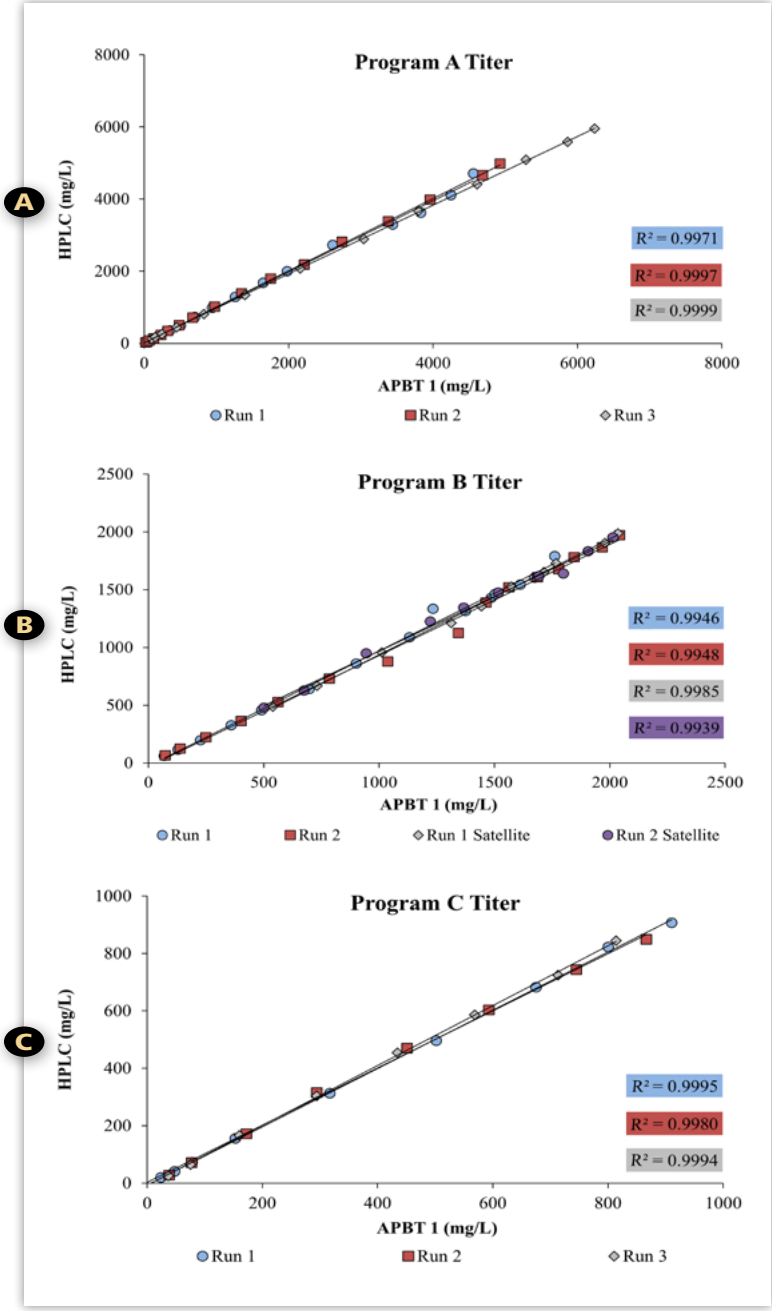
Figure 6 (A–D) shows the spiked supernatant sample recovery results for APBT 1 (RTP) and APBT 1 (Camb). For the spiked supernatant samples, a value within the range of ±10% (90–110%) was determined to be an acceptable metabolite recovery. The two instruments showed acceptable recoveries of every metabolite tested for low and high-level spiked Programs C and D supernatants. The cross-site comparison of these two instruments demonstrated that the results initially generated by the APBT 1 (RTP) were highly reproducible on APBT 1 (Camb) and showed that both APBT 1 instruments performed comparably across two locations.

Future Perspective

At-line monitoring of essential metabolites accurately and precisely is not the only advantage to implementing APBT analyzers into the development process of cell culture. The APBT instruments also come equipped with robust assays for monitoring protein titer. While HPLC instruments provide extremely accurate and consistent protein titer measurements due to the strict nature of HPLC assay protocols, the process requires more time, effort, and resources to obtain the results for these high priority samples. Preliminary protein titer data comparing the linear relationship between the HPLC protein G assay and the APBT 1 IgG assay results has been collected for three different CHO-based programs shown in Figure 7 (A–C). Preliminary results indicate strong linear correlations between HPLC and APBT 1 protein titer measurements. It's important to

FIGURE 6 (A–D). Spiking study recovery rates of two CHO-based programs (C and D) between two APBT 1 instruments in different locations. Spiking samples used are outlined in Tables 8 and 9. Recovery values within ± 10% (90–110%) were determined to be acceptable metabolite recovery.

FIGURE 7 (A–C). Preliminary data to compare linearity between APBT and HPLC for the measurement of protein titer. Multiple runs for three different CHO-based programs (A, B, and C) have shown that the APBT 1 and HPLC have a strong correlation between their measurements of protein titer.



note that for the APBT 1 instrument, no special correction factors were used for any data shown, only the stock-standard assay parameters.

Conclusion

Qualitative

A qualitative assessment of the four instruments is shown in Table 10. The table gives an overview of the performance validation characteristics previously discussed as well as user input from instrument maintenance and functionality over the course of the study.

Summary

The intent of this study was to evaluate the accuracy, precision, specificity, linearity, and range characteristics of two APBT and two MBT instruments commonly used for at-line metabolite monitoring during cell culture processes. Standard samples with known concentrations and cell culture supernatant samples were used during this evaluation in order to provide a comprehensive analysis of the four instruments. Results from this study confirm the generalizations made by Bawn *et al.*, which state, "APBT demonstrates great potential for complementing, or replacing, the existing technology for monitoring the metabolites in off-line or at-line manners."[1] The results generated from the APBT analyzers establish that the absorption-photometric technology is precise and robust enough to handle a wide range of metabolite concentrations and is capable of maintaining measurement accuracy over a long testing period. The APBT instruments not only meet all of the validation requirement as outlined in the ICH guidelines[6] but also improve daily laboratory activities by reducing required operator maintenance. Implementation of a robust at-line metabolite analyzer should be adopted for all existing cell culture processes in order to eliminate inconsistencies, improve the quality of the daily sample measurements, and increase efficiency of daily lab practices.

TABLE 10. Qualitative summary of multi-analyzer comparison study.

Criteria	APBT 1	APBT 2	MBT 1	MBT 2
Accuracy (standard samples)	✓✓	✓	✗	✓
Precision (supernatant samples)	✓✓	✓	✓	✗
Specificity (spiking study)	✓✓	✓	✗	✓
Range (assay range)	✓✓	✓✓	✓	✓
Ease of Use	✗	✓	✓	✓✓
Footprint	✗	✗	✓✓	✓✓
Maintenance Frequency	✓✓	✓✓	✓	✗✗
Calibration and Control Frequency	✓✓	✓✓	✓	✗✗
Software Interface	✓	✓✓	✓	✓✓
Sample Volume	✓	✓	✗	✓✓

✓✓ Excellent ✓ Average ✗ Below Average ✗✗ Poor

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References

[1] Bawn A, Lee HW, Downey A, Xu J, Starkey JA, Yoon S. Metabolic-sensing characteristics of absorption-photometry for mammalian cell cultures in biopharmaceutical processes. *Pharm Bioprocess*, 2013; 1:255-66. http://dx.doi.org/10.4155/pbp.13.31.

[2] Nova Biomedical, BioProfile FLEX. (Accessed June 21, 2014, http://www.novabiomedical.com/products/bioprofile-flex/.)

[3] YSI Life Sciences, YSI 2950 Biochemistry Analyzer. (Accessed June 21, 2014, http://www.ysilifesciences.com/index.php?page=ysi-2950-biochemistry-analyzer.)

[4] TAP Biosystems, single-use ambr microbioreactors. (Accessed June 22, 2014, http://www.tapbiosystems.com/tap/cell_culture/ambr.htm.)

[5] Advertorial: Using Roche Cedex Bio Analyzers for in-process monitoring in biotech production. *Genet Eng Biotechnol News*, 2013. (Accessed June 21, 2014, http://www.genengnews.com/gen-articles/advertorial-using-roche-cedex-bio-analyzers-for-in-process-monitoring-in-biotech-production/4780/.)

[6] International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use. ICH-harmonized tripartite guideline. *Validation of Analytical Procedures: Text and Methodology*, Q2(R1); November 2005. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf.

[7] SAS Institute Inc. 2009. *JMP® 8 Introductory Guide, Second Edition*. Cary, NC: SAS Institute Inc. http://www.jmp.com/support/downloads/pdf/jmp8/jmp_introductory_guide.pdf.

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