Performance Comparison of Two LabChip Microfluidic Platforms for Protein, DNA, and RNA Analyses

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Introduction

The analysis of any mixture, including complex biological material, includes its separation into single components and an assessment of their quantities in the sample. For biological samples, measurements of nucleic acids (both DNA and RNA) and proteins are the predominant assays. However, the sample complexity in these materials necessitates the combination of a highly sensitive detection method with an efficient separation method. While chromatographic methods have been successful in the separation of small molecules both in the gas and liquid phase, nucleic acids and proteins are easier to separate using electrophoretic methods. The most successful approach to separating proteins and nucleic acids has been gel electrophoresis, where the biopolymers migrate through a gel with a reticulate structure. Although widely practiced today, the disadvantages of slab gel electrophoretic methods include relatively large sample and reagent requirements, a low degree of automation, and long analysis times on the order of hours per analysis.

The development of electrophoretic microchip platforms in the 1990s has allowed these separations to take place on smaller scales and in shorter times. Automated electrophoresis systems combining Caliper Life Sciences' innovative LabChip microfluidic separation with detection such as UV-Vis or fluorescence provide rapid and detailed information about sample quality and quantity. Sample run times on the order of hundreds of seconds are routine and parallel processing may be performed.

In this technical note, we describe the performance of a variety of assays available with the Experion™ automated electrophoresis system from Bio-Rad Laboratories, Inc. We compare the performance of the Experion assays to the performance of the assays of another microfluidics system, the 2100 Bioanalyzer from Agilent Technologies. The assays were analyzed in terms of accuracy, reproducibility, and sensitivity for both systems.

Methods

General

Samples and microfluidic chips were prepared and loaded according to the manufacturers' protocols described in the instruction manuals for each kit. The sample concentrations used in the analysis of the different kits were independently determined using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Inc.).

Protein

Immunoglobulin G (IgG, bovine, Sigma) and carbonic anhydrase (CA, bovine, Sigma) were dissolved into 1× phosphate-buffered saline (PBS). Ovalbumin (Ova, Sigma) was dissolved into proteomics-grade water. *E. coli* protein lysate prepared using a Bio-Rad ReadyPrep™ protein extraction kit was dissolved into 10 mM Tris-HCl. Concentrations were independently determined using UV spectroscopy. The Experion system and Pro260 analysis kit are products of Bio-Rad Laboratories. The 2100 Bioanalyzer, 2100 Expert software (B.02.06.SI418) and Protein 230 assay kit were purchased from Agilent Technologies.

DNA

NoLimits DNA fragments (Fermentas Life Sciences) were diluted into diethylpyrocarbonate (DEPC)-treated water (Bio-Rad). Concentrations were independently determined using UV spectroscopy. The DNA 1K analysis kit, and DNA 12K analysis kit are both products of Bio-Rad Laboratories. The DNA 1000 assay kit and DNA 12000 assay kit were purchased from Agilent Technologies.

RNA

Rat brain total RNA and human skeletal muscle total RNA (both from Life Technologies) were dissolved into DEPC-treated water (Bio-Rad). The Experion RNA StdSens and RNA HighSens analysis kits are products of Bio-Rad. The RNA 6000 Nano assay kit and RNA 6000 Pico assay kit were purchased from Agilent Technologies. For the evaluation of the 25–500 ng samples, only samples showing an RQI (or RIN) of >8.0 were included.

Protein Assay Results and Discussion

Molecular Weight Determination

Protein molecular weight is a fundamental parameter in many biochemical protein applications and processes and, often used to confirm protein identity. In a previous study (Nguyen and Strong 2005), it was shown that the Experion Pro260 assay resulted in highly reproducible results (%CVs of ≤1) in the sizing of two sets of protein standard ladders. The Experion Pro260 assay also was accurate to within 8% of the expected molecular weight for proteins as small as 10 kD. This earlier study used the Protein 200 Plus assay from Agilent to provide similar data, though the Experion assay showed better reproducibility across a broader range of samples. For the current study, we chose to evaluate two separate protein samples (IgG, 150 kD, and Ova, 44.3 kD) at a concentration of approximately 500 ng/µl. The Agilent Protein 230 kit provides the same response as the previously used Protein 200 Plus, but with an enhanced dynamic range. The results of these studies are shown in Table 1. Experion sizing measurements were shown to be consistent within 1.5 and 3.6 %CV for IgG and Ova, respectively. The Agilent Protein 230 assay was similarly reproducible, with 1.1 and 3.2 %CV for the same samples. The sizing accuracies of each system were also within 4% of the expected molecular weight. These results indicate that protein sizing over a wide molecular weight range is quick and accurate on both LabChip platforms.

Quantitation

Numerous assays in protein studies rely on a determination of sample concentration. The Experion Pro260 assay uses a single-point calibration by default, relative to an internal upper protein marker at a known concentration. This option provides a relative concentration value of the protein of interest in the sample. Additionally, the Experion software has an option for generating a calibration curve based on a dilution series of the protein of interest, thus providing a value for the absolute concentration of the analyte (Wu and Strong 2007). These capabilities are also present in the Agilent system, but only the Experion system allows one-point calibration against a user-chosen internal standard and the application of this internal standard in conjunction with the calibration curve. As discussed previously (Nguyen and Strong 2005), this calibration curve approach can significantly increase the accuracy of the quantitation measurement. For the current study, we evaluated IgG and Ova samples in terms of accuracy and reproducibility for relative quantitation. The results of the experimental data are summarized in Table 1. Analysis of an IgG solution at a concentration of 505 ng/µl as measured with UV spectroscopy resulted in an average reported concentration of 514 ng/µl (%CV of 8.03%, accuracy 1.9%) using the Experion system. The 437 ng/µl average reported by the Protein 230 assay was much less accurate, representing a larger error (%CV of 16.3%, accuracy –13.4%). The reasons for quantitation inaccuracies lie in differences in protein staining efficiency relative to the upper marker in each system. Quantitation can be improved by experimentally generating a calibration curve for absolute accuracy for a single protein (Nguyen and Strong 2005). The %CV using the Agilent kit was also greater than the Bio-Rad kit and this spread of results can best be illustrated when comparing the Ova samples. A 438 ng/µl sample of this protein was prepared and analyzed on each system. The %CV of the Experion Pro260 assay is 12.8% compared to 17.6% for the Agilent assay. For this particular protein (ovalbumin), both systems underestimated the concentration when using the default method of quantitation (Figure 1), with the Experion Pro260 assay kit being more accurate across all wells. Each system provides an acceptable reproducibility of the data.

Table 1. Comparison of reproducibility* and accuracy of protein quantitation.** IgG and Ova were analyzed at the given concentrations. The Pro260 analysis kit was used with the Experion system and the Protein 230 assay was used with the Bioanalyzer.

		Experion				2100 Bioanalyzer				
		Sizing		Quantitation		Sizing		Quantitation		
Sample	N	%CV	Accuracy, %	%CV	Accuracy, %	%CV	Accuracy, %	%CV	Accuracy, %	N
IgG (505 ng/µl)	30	1.5	3.7	8.03	1.9	1.1	0.8	16.3	–13.4	30
Ova (438 ng/µl)	30	3.6	–0.5	12.8	–12.5	3.2	3.3	17.6	–47.1	20

* Reproducibility was evaluated using the coefficient of variation (%CV) as a statistical measure.

** Accuracy is reported as the percent difference between the expected, independently determined quantity and value reported by the systems. Negative values represent underestimation of the value. Accuracy values reported are the average of N individual measurements.

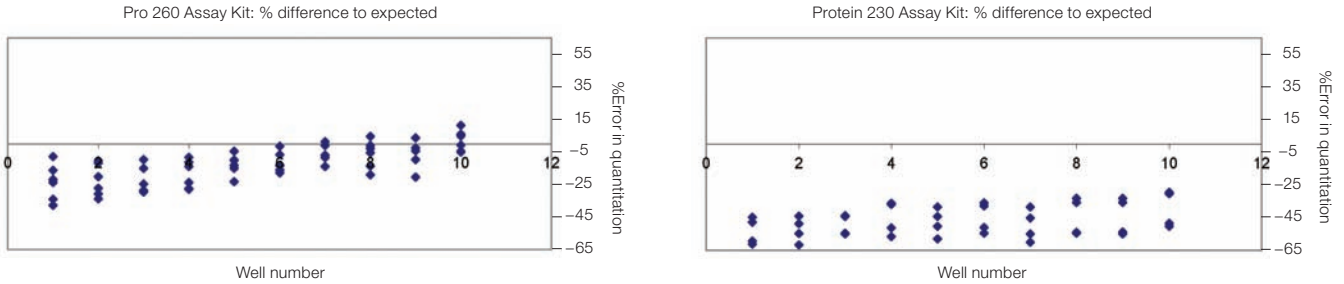

Fig. 1. Scatter plot comparison of quantitation accuracy. Graphical representation of interchip data for ovalbumin. Left panel, data generated by the Pro260 analysis kit using the Experion system; right panel, data on the same samples using the Protein 230 assay on the Bioanalyzer system. Each dot is a separate individual sample and each column relates to the well position on the chip.

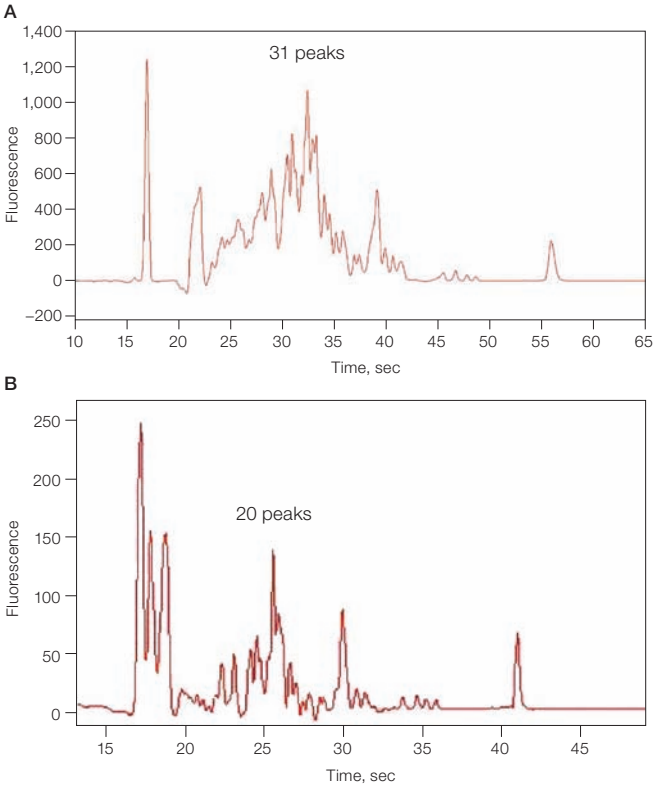

Fig. 2. Resolution of protein separation. Comparison of electropherograms of an *E. coli* protein lysate. Using the Experion Pro260 analysis kit (**A**) and the Agilent Protein 230 assay kit (**B**), the Experion system reveals a larger number of peaks.

Resolution

A higher separation resolution allows for separating proteins with similar molecular weights and in turn allows for better accuracy in both molecular weight measurement and quantitation. The Experion Pro260 assay provides higher resolution than the Agilent assay as evidenced by a larger number of peaks being visible on the electropherogram of an *E. coli* protein lysate in Figure 2. This greater resolution may also be due, in part, to the higher sensitivity exhibited by the Experion system since peaks are detected relative to a threshold signal level. In this example, 31 peaks were detected using the Experion Pro260 kit, while the Agilent kit only identified 20 individual peaks. The Experion Pro260 assay kit shows better resolution of complex protein samples across a wide molecular weight range.

Sensitivity

Limits of detection are what define the utility of a particular assay. If an insufficient linear range of detection exists, then identifying low-abundance analytes or determining impurity levels are compromised. This is illustrated in Figures 2a and 2b with the fluorescence intensity from the Experion Pro260 assay being much greater (5-fold or more) than that of the Protein 230 assay. In a previous report it was shown that the limit of detection using the Experion Pro260 assay kit was superior to that of the Agilent Protein 200 Plus assay. In that study, for a 2.5 ng/µl sample of CA the Experion system easily detected the analyte with an S/N (signal to noise) ratio of 20.5 (Nguyen and Strong 2005). In this experiment, we prepared samples of CA at 2.5 ng/µl and ran a series of these assays on the two systems. The Experion Pro260 assay again had no difficulty detecting the CA with a calculated average S/N of 64. In contrast, the Bioanalyzer was not able to detect the protein at this concentration. For comparison, 50 ng/µl solutions of CA and BSA were prepared and analyzed following each manufacturer's instructions. The results are shown in Table 2. For BSA, the Experion system reported a mean S/N of 377 for the 50 ng/µl sample while the Agilent Bioanalyzer detected the BSA with a mean S/N of only 62. The 50 ng/µl CA provided a full log unit better S/N on the Experion system.

Table 2. Comparison of sensitivity of protein detection. The average signal-to-noise (S/N) of BSA or CA were compared at the given concentrations. The Bioanalyzer Protein 230 assay kit was not able to produce a detectable peak for CA at 2.5 ng/µl.

	Mean Signal-to-Noise	
Sample	**Experion**	**2100 Bioanalyzer**
BSA (50 ng/µl)	377	62
CA (50 ng/µl)	2300	212
CA (2.5 ng/µl)	64	Not detected

Optimal conditions should favor full loading of the protein upon electrokinetic injection. Regardless of these factors, it is apparent that the Experion Pro260 assay provides a more sensitive measurement under identical sample conditions.

DNA Assay Results and Discussion

Separation, sizing, and quantitation of DNA fragments were one of the first marketed applications of the microfluidic electrophoresis platforms. The initial kits were designed for smaller DNA (up to about 500 base pairs (bp)). Since then, the range of sizing options has increased greatly. In this study, two mixtures, each containing three different DNA fragments mixed in equal amounts to a final DNA concentration of 30 ng/µl (10 ng/µl each fragment), were used. The mixtures were analyzed on the Experion and Bioanalyzer systems to compare accuracy and variance in sizing. Additionally, the concentrations of the individual components of the sample were measured and the performances of both systems were compared. These comparisons were done on at least four separate chips for each system.

Estimation of Size (bp)

For the DNA 1K assay, DNA fragments of 150, 300, and 850 bp were mixed as described and assayed. A representative electropherogram is shown in Figure 3. The comparison between the two systems is shown in Table 3. Both systems provide accurate sizing measurements. The Experion DNA 1K assay was accurate to within 3.2% of the expected sizes of the three fragments with minimal variance (1.2 %CV). The Bioanalyzer DNA 1000 assay was also accurate, being within 2.1% of the expected sizes. The same trends were observed in the DNA 12K assay. Three fragments (850, 3000 and

Table 3. Comparison of reproducibility and accuracy of DNA quantitation. DNA fragments of the indicated sizes were mixed in a 1:1:1 ratio (total concentration of 30 ng/µl) and assayed. Both sets of data were obtained across four different chips and run on their respective systems.

		Experion				2100 Bioanalyzer				
		Sizing		Quantitation		Sizing		Quantitation		
Sample	N*	%CV	Accuracy%	%CV	Accuracy%	%CV	Accuracy%	%CV	Accuracy%	N*
150, 300, 850 bp	48	1.2	–3.2	3.5	2.0	0.8	2.1	8.8	2.9	48
850, 3000, 7000 bp	48	4.6	–2.5	6.4	–1.5	2.0	5.6	7.8	6.1	48

* 12 samples were run on four different chips.

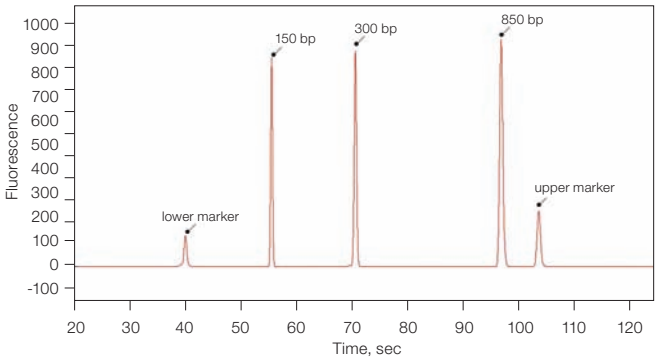

Fig. 3. Analysis of DNA fragments on the Experion automated electrophoresis system. Representative electropherogram of three DNA fragments separated on the Experion system using the DNA 1K analysis kit.

7000 bp) were separated using both systems. The Experion system was accurate to within 2.5% of the expected size while the Bioanalyzer was within 5.6%. The CVs of all of the sizing assays, less than 5%, were very good.

Quantitation

For the quantitation studies, the individual components were present at 10 ng/µl each. Samples were run on at least four separate chips. Table 3 shows the results using the Experion or the Bioanalyzer systems and kits. Both the Experion DNA 1K and DNA 12K assays are excellent in their determinations of concentration. There is a 2% or less difference between the expected and the determined concentrations for the Experion kits. Additionally, when compared to the Bioanalyzer data, the Experion system provides more reproducible results (%CV <6.4%) for both sets of analytes.

RNA Assay Results and Discussion

The purity and integrity of an RNA sample is critical for the overall success of gene expression analyses (Taylor et al. 2009; Bustin et al. 2009). Quick determination of quantity and quality are needed in order to provide confidence in the results of a gene expression study. In this work, we looked at the performance of the different RNA analysis assays using rat brain and human skeletal tissue total RNA. For the Experion HighSens and Bioanalyzer Pico chips, a single concentration (100 pg/µl or 1,000 pg/µl) was analyzed multiple times in order to look at the reproducibility of the two systems. Two data sets are reported: (1) a single concentration of human skeletal tissue total RNA (at 100 ng/µl) to measure reproducibility, (2) a dilution series of rat brain total RNA, ranging from 5 to 500 ng/µl, to determine assay accuracy and variability.

The Bio-Rad suite of RNA chips allows one to assay samples ranging from 100 pg/µl up to 500 ng/µl. We prepared solutions of rat brain total RNA at 1,000 pg/µl and human skeletal muscle total RNA at concentrations of 100 and 1,000 pg/µl. These concentrations are below the useful level of UV spectroscopy. Accuracies are reported, based upon the assumption of nominal concentrations of 100 or 1,000 pg/µl. The results are from three separate chips and

Table 4. Comparison of reproducibility and accuracy of quantitation of picogram to nanogram per microliter levels of total RNA. Total RNA at the given concentrations were analyzed using the appropriate kits and their respective automated system.

		Experion		2100 Bioanalyzer		
		RNA HighSens Assay Kit		RNA Pico 6000 Assay Kit		
Total RNA, pg/µl	N	%CV	Accuracy%	%CV	Accuracy%	N
Rat Brain						
1000	33	15.90	7.25	13.00	64.30	33
Human Skeletal Tissue						
1000	33	12.95	22.00	8.60	48.80	33
100	33	13.60	–26.70	18.90	80.60	33
Total RNA, ng/µl		**RNA StdSens Assay Kit**		**RNA Nano 6000 Assay Kit**		
Rat Brain						
5	15	27.73	–3.79	46.83	31.70	18
25	15	7.33	3.05	12.70	1.52	17
100	19	7.06	15.45	8.33	–2.05	23
500	19	3.18	35.92	8.04	20.42	23
Human Skeletal Tissue						
100	59	8.60	6.68	11.28	7.17	69

data using the Bioanalyzer system and kits are shown for comparison in Table 4. Both systems exhibited little variance within and between chips, thus providing confidence in the data. As the concentrations of RNA decrease below 200 pg/µl, the accuracy and reproducibility of the Experion RNA HighSens assay maintains its linearity, while the Bioanalyzer kit results drop below the useful range of quantitation. The accuracy of the Experion RNA HighSens analysis kit appears to be better than the competing assay across a wider range for these samples.

Results from the StdSens assay kit are shown in Table 4. The performance of the Experion system was strong, as shown with analysis of human skeletal tissue at 100 ng/µl. The concentration of RNA in samples could be determined to within 7% of the expected value with CVs of less than 9%. The assessment of RNA integrity has been discussed elsewhere (Denisov et al. 2008) with both the Experion software and Bioanalyzer software producing comparable numbers for the same samples.

The range of usefulness of the assay is indicated when considering the rat brain total RNA dilution series. A set of four concentrations within the quantitative range for both system of this sample were prepared and analyzed on a minimum of four chips using at least three wells per chip. The Experion system performs well in this regard. For samples in the range of 25–500 ng/µl, the %CV for quantitation reproducibility is less than 10%, being closer to 3% for the more concentrated samples. The variability begins to increase at the lower limit of the chip as the 5 ng/µl sample showed CVs of almost 28%. Still the Experion system and kits outperformed the Bioanalyzer products when one considers reproducibility of results.

Conclusions

Protein Analysis

In total, the Experion Pro260 assay kit performed as well or better than the Bioanalyzer Protein 230 kit in all the studies presented here. The results from the Experion system display little variance over a broad range of molecular weight determinations enabling accurate and confident sizing. Relative quantitation of complex samples is possible due to the enhanced resolution and sensitivity of the Experion system and kit. The Pro260 assay kit provides good data quality from an automated electrophoresis system.

DNA Analysis

Nucleic acid analysis is important in a variety of biotechnology processes. The Experion system has two kits which together can cover a range between 15 and 17,000 bp, measure as little as 0.1 ng of sample, and are applicable across a wide range of studies. The DNA 1K and DNA 12K analysis kits provide excellent results in regard to sizing and quantitation. When compared to the Bioanalyzer data, both systems perform quite well. There is a noticeable advantage using the Experion kits and system when considering quantitation. The results were within 2% of the known value and showed reproducibility of better than 7% CV. The Experion system can be included in any workflow that requires rapid separation of DNA fragments and oligonucletides, including restriction fragment analysis, DNA sequencing, and PCR-based genotyping such as STR or single nucleotide polymorphism (SNP) analyses.

RNA Analysis

The accuracy of gene expression data is influenced by both the quantity and quality of starting RNA. Assays such as quantitative RT-PCR (RT-qPCR) and cDNA microarray analysis require accurate qualitative and quantitative assessment of RNA samples. The Experion RNA StdSens and RNA HighSens analysis kits allow for accurate, reproducible determinations of RNA quality and concentration using only 1 µl of sample in a short amount of time. The StdSens analysis kit was accurate over the 5–500 ng/µl range with good reproducibility down to the 5 ng/µl samples. The associated Bioanalyzer kit also provides accurate data in the 5–500 ng/µl range; however, the CVs begin to drop on the lower end. The RNA HighSens kit provided more accurate, reproducible results even at 100 pg/µl. Both systems offer an objective, standardized RNA quality assessment number (Experion RQI, Bioanalyzer RIN) that helps RT-qPCR researchers adhere to the new MIQE (minimum information for publication of quantitative real-time PCR experiments) guidelines (Bustin et al. 2009; Taylor et al. 2009). Overall, when considering a breadth of concentrations over the manufacturers' suggested working range, the Experion system provides accurate data with generally greater reproducibility between wells and chips than the Bioanalyzer system. This conclusion has been independently confirmed (Pfaffl et al. 2008).

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