Protein Quantitation: A Comparative Analysis Using the Experion™ Automated Electrophoresis System, Bradford and Modified Lowry Assays, and SDS-PAGE

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Introduction

Protein quantitation is a routine procedure used in all research laboratories working with protein samples. Among other applications, protein quantitation is used to determine the appropriate amount of sample to use in protein separations and analyses and to calculate the purity, yield, or percent recovery of purified proteins. A number of methods are available for protein quantitation, including ultraviolet (UV) spectroscopy at 280 nm, colorimetric dye-based assays, such as Bradford (Bradford 1976) and Lowry (Lowry et al. 1951) assays, and SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

Each protein quantitation method has its benefits and drawbacks. UV spectroscopy works well for quantitating purified proteins with known extinction coefficients or for tracking relative amounts of protein through a purification or modification process; however, this method is limited by its relatively poor sensitivity and by the fact that nonprotein species in the sample buffer can also absorb at 280 nm. Colorimetric dye-based assays are sufficiently sensitive for most applications and offer good dynamic range, excellent reproducibility, and higher throughput through microplate protocols; however, these assays require the generation of a standard curve each time a protein is quantitated, are often incompatible with commonly used buffer constituents (for example, detergents or reducing agents), and can deliver different responses for different proteins, thereby compromising accuracy (Bio-Rad Laboratories 2003, Bradford 1976, Lowry et al. 1951). The separation step of SDS-PAGE affords sizing and purity information and is virtually unaffected by buffer constituents; however, traditional SDS-PAGE is laborious, requires several hours to complete, and requires specialized imaging equipment and analysis software for quantitation.

The Experion automated electrophoresis system, based on microfluidic separation technology, provides a revolutionary method for protein quantitation (Nguyen and Strong 2005). It is rapid (requiring ~30 min), accurate, and reproducible. It requires minimum amounts of samples and reagents, and generates results that are displayed in real time and stored in a digital format. The Experion Pro260 analysis kit, which is used for protein analysis, includes a built-in standard (the 260 kD upper marker) in the sample buffer for use in relative concentration estimates (relative quantitation). However, Experion software allows substitution of the upper marker with any user-defined standard for this purpose, and Pro260 analysis also allows absolute quantitation through use of a standard curve generated by a purified protein standard. Moreover, like SDS-PAGE but unlike dye-based assays, the Pro260 analysis kit provides sizing and purity information.

In this tech note, we examine the accuracy and reproducibility of both relative and absolute quantitation achieved with microfluidics-based systems (Experion system using the Pro260 analysis kit, and Agilent 2100 bioanalyzer using the protein 200 plus LabChip kit), Bradford-based assays, modified Lowry assays, and SDS-PAGE coupled with a gel documentation and analysis system.

Methods

Protein Samples

Purified bovine serum albumin (BSA, 66 kD), bovine erythrocyte carbonic anhydrase (CA, 29 kD) (Sigma-Aldrich), and bovine plasma γ-globulin (IgG, 150 kD) were used. Each protein was dissolved and diluted in proteomics-grade water, and each dilution was stored in 100 µl aliquots at –20°C. Concentrations of the three proteins were independently determined using UV spectroscopy and extinction coefficients for 1 mg/ml solutions of 0.667 (at 279 nm) for BSA concentrations of 200 ng/µl and higher, and 1.73 and 1.38 (at 280 nm) for CA and IgG concentrations of 100 ng/µl and higher, respectively.

Experion Pro260 and Protein 200 Plus LabChip Analyses

The Experion Pro260 analysis kit was used for protein quantitation with the Experion system, and the protein 200 plus LabChip kit was used with the Agilent 2100 bioanalyzer (Agilent Technologies). The Experion Pro260 and protein 200 plus LabChip kits each include a protein ladder, sample buffer, gel solution, fluorescent stain, spin filters, and microfluidic

chips. Samples (4 µl) were mixed with 2 µl sample buffer either with β-mercaptoethanol (BSA and CA samples) or without β-mercaptoethanol (IgG samples), heated at 95°C for 5 min, diluted with proteomics-grade water, and loaded onto chips primed according to the protocols provided with each kit.

For relative quantitation two chips were run, and the four samples (50, 200, 750, and 1,250 ng/µl) were each run in five wells (n = 5). For absolute quantitation five chips were run, each with 25, 100, 250, 500, 1,000, and 2,000 ng/µl samples as standards for generating the calibration curve and the same four sample concentrations mentioned above (n = 5). Protein concentrations (total protein or primary peak) were taken directly from the data reported by Experion or Agilent 2100 Expert software; statistical analysis was performed using JMP version 5.1 software (SAS Institute, Inc.).

Bradford Assays

The Bio-Rad protein assay dye reagent concentrate and Coomassie (Bradford) protein assay kit reagent (Pierce Biotechnology) were both evaluated using microplate protocols. To generate calibration curves, an ultrapure water blank and 11 standard concentrations of each protein (2.5, 10, 50, 100, 250, 500, 750, 1,000, 1,500, 1,750, and 2,000 ng/µl) were loaded in triplicate on separate plates. For analysis, each protein at four different concentrations was loaded in five replicate wells (n = 5) containing diluted dye reagent (the Bio-Rad reagent required 10 µl protein and 200 µl diluted dye reagent, and the Pierce assay required 5 µl protein and 250 µl dye reagent). The samples and reagent were then incubated for 5 min at room temperature, and the absorbance at 595 nm of each well was measured using a Benchmark™ Plus microplate reader.

Modified Lowry Assays

The performance of the *DC*™ protein assay was evaluated using the microplate protocol (96-well format). An ultrapure water blank and 11 standard concentrations of each protein (2.5, 10, 50, 100, 250, 500, 750, 1,000, 1,500, 1,750, and 2,000 ng/µl) were loaded in triplicate on separate plates to generate calibration curves. Each protein (5 µl) at four different concentrations was loaded in five replicate wells (n = 5) and mixed with 25 µl *DC* reagent A and 200 µl *DC* reagent B. After incubation for 15 min at room temperature, the absorbance at 750 nm was measured using a Benchmark Plus microplate reader.

Similarly, the BCA protein assay from Pierce Biotechnology was performed using the microplate protocol, wherein 25 µl sample was mixed with 200 µl BCA working reagent, incubated at 37°C for 30 min, and cooled to room temperature, and the absorbance measured at 562 nm. Calibrations were performed as described above for the *DC* protein assay.

SDS-PAGE

SDS-PAGE was performed using the Criterion™ cell and 7.5%, 10%, and 15% Criterion Tris-HCl precast gels to separate IgG, BSA, and CA, respectively. Protein sample (4 µl) was mixed with 4 µl 2x Laemmli sample buffer either with 5% β-mercaptoethanol (BSA and CA samples) or without β-mercaptoethanol (IgG samples), heated at 95°C for 5 min, and loaded onto the gel. Electrophoresis was performed at a constant 200 V for 55 min. Gels were stained for 1 hr with Bio-Safe™ Coomassie G-250 stain, destained in water overnight, scanned on a GS-800™ densitometer, and analyzed using Quantity One® software. Five gels were run for each protein (n = 5), and on each gel a calibration curve was generated by plotting either the combined signal densities of all bands (total protein) or the signal density of the primary band versus the known concentration for six standards (25, 100, 250, 500, 1,000, and 2,000 ng/µl); concentrations (total protein or primary band) of four samples (50, 200, 750, and 1,250 ng/µl) were determined based on these curves.

Results and Discussion

Three purified proteins (BSA, CA, and IgG) were subjected to quantitation by microfluidics-based electrophoresis systems, Bradford and modified Lowry assays, and SDS-PAGE. The protein concentrations derived from these approaches were compared to those obtained by UV spectroscopy. As a measure of reproducibility, the coefficient of variation (%CV) was calculated as the standard deviation/mean x 100. Accuracy was measured by the % difference between the measured concentration and the spectroscopically determined (UV-based) value.

Microfluidics-Based Systems

The microfluidics-based systems examined here automatically determine the relative concentration of a protein using a single-point calibration, wherein the peak area of the protein is compared to that of an internal marker protein present in each sample at a known concentration. Inclusion of an internal standard provides the added benefit of allowing automatic correction of any sample-to-sample differences in injection or separation (for example, those caused by differences in the concentration of salt or other buffer constituents), as all the proteins in the sample are affected by these differences to the same extent.

With these systems, the user also has the option of using known concentrations of the purified protein to create a calibration curve on the chip. Such absolute quantitation takes into account inherent differences in the efficiency of protein-dye binding that can generate protein-to-protein variations in quantitation accuracy in any dye-based assay (Bio-Rad Laboratories 2003, Bradford 1976, Lowry et al. 1951). The Experion Pro260 analysis kit, for example, simplifies this process by allowing the user to create a standard curve by selecting the wells to be used for generating the plot and entering their concentrations into the corresponding fields.

In these experiments, the concentrations of each protein were determined by relative and absolute quantitation approaches (Table 1). For relative quantitation with the Experion Pro260

Table 1. Accuracy* and reproducibility of relative and absolute quantitation by the Experion Pro260 analysis kit.** BSA, CA, and IgG were separated with the Experion Pro260 analysis kit and the Experion system. Peak and total protein concentrations are shown compared to those obtained by UV spectroscopy.

		Relative Quantitation			Absolute Quantitation		
Sample	UV-Based Conc. (ng/µl)	Measured Conc. (ng/µl)	Accuracy	Reproducibility	Measured Conc. (ng/µl)	Accuracy	Reproducibility
Total protein							
BSA	1,460	1,549.8	6.1%	3.8%	1,442.6	–1.2%	4.3%
	886	932.1	5.2%	3.0%	869.3	–1.9%	4.1%
	212	209.6	–1.1%	2.8%	205.1	–3.3%	6.4%
	53	42.0	–20.8%	14.8%	41.2	–22.3%	9.6%
CA	1,055	1,385.2	31.3%	10.4%	1,267.1	20.1%	5.6%
	689	1,075.3	56.1%	8.5%	955.1	38.6%	3.6%
	189	474.3	151.0%	13.7%	438.9	132.2%	4.0%
	50	123.1	146.2%	6.9%	110.0	120.1%	4.4%
IgG	1,319	1,038.0	–21.3%	3.1%	1,008.1	–23.6%	4.2%
	716	669.3	–6.5%	3.9%	621.2	–13.2%	2.9%
	192	169.6	–11.6%	3.4%	168.4	–12.3%	2.4%
	50	33.6	–32.8%	13.0%	32.8	–34.4%	4.6%
Peak protein							
BSA	1,460	1,307.7	–10.4%	5.2%	1,442.0	–1.2%	2.2%
	886	798.4	–9.9%	4.3%	883.8	–0.2%	3.9%
	212	192.1	–9.4%	0.8%	227.7	7.4%	3.1%
	53	37.0	–30.2%	11.3%	50.9	–4.0%	8.9%
CA	1,055	1,307.6	23.9%	10.4%	705.0	–33.2%	9.5%
	689	1,039.6	50.9%	8.6%	513.7	–25.4%	4.8%
	189	460.3	143.5%	14.3%	142.6	–24.5%	10.0%
	50	119.1	138.3%	8.3%	<0	<0%	<0%
IgG	1,319	970.4	–26.4%	3.1%	1,176.3	–10.8%	4.5%
	716	626.5	–12.5%	4.2%	722.6	0.9%	5.2%
	192	164.7	–14.2%	3.5%	189.3	–1.4%	3.9%
	50	30.0	–40.0%	7.4%	24.3	–51.4%	21.2%

* Calculated as % difference relative to expected.
** Calculated as %CV.

analysis kit, each protein sample was loaded into five wells on the same chip. The peak area of each sample was compared to the peak area of the 260 kD internal upper marker, and the relative concentration of each sample was reported by Experion software. For absolute quantitation, each protein sample was analyzed on five chips. Each chip contained six known concentrations of protein, which were used to generate calibration curves, as well as four sample concentrations, whose concentrations were reported by Experion software. The average r^2 values of the calibration curves (not shown) were 1.0 for BSA, 0.965 for CA, and 0.995 for IgG, indicating excellent linear fit.

In these experiments, the concentrations of both the primary peak and the total protein per well were recorded, the latter for comparison with the other colorimetric methods and the former for comparison with SDS-PAGE. The capability of the microfluidics-based systems to report both peak and total protein concentrations also facilitates determination of the percentage of total protein for any species detected. As expected, the peak and total protein concentrations determined by absolute quantitation showed higher accuracy in most cases than those determined by relative quantitation (Table 1).

The same samples were also separated on the Agilent 2100 bioanalyzer system using the protein 200 plus LabChip kit. The average r^2 values were 0.971 for BSA, 0.936 for CA, and 0.938 for IgG. Lower reproducibility (higher %CV) and lower accuracy (higher % difference to UV) were observed with this system than with the Experion system (not shown; see Nguyen and Strong 2005 for a detailed comparison of the two systems). Taking the total protein concentrations of the 200 ng/µl IgG sample as an example, relative quantitation generated a %CV of 3.4% for the Experion system as compared to 15.9% for the 2100 bioanalyzer system, and the % differences to UV measurements were –11.6% and 35.7% for the Experion and 2100 bioanalyzer systems, respectively. Using absolute quantitation, the %CV was 2.4% for the Experion system and 18.2% with the 2100 bioanalyzer system, and the % differences to UV measurements were –12.3% and 39.1% for the Experion and 2100 bioanalyzer systems, respectively.

Bradford Assays

The Bio-Rad protein assay kit, which has a linear range of 50–500 ng/µl, was used to perform this assay. The r^2 values of the calibration curves generated by BSA, CA, and IgG in this range were 0.963, 0.981, and 0.999, respectively, and the values for the linear fit over the 0–2,000 ng/µl range were 0.919, 0.784, and 0.953. However, when a second-order polynomial fit was used in the same range, the r^2 values increased to 0.994, 0.957, and 1.00. Equations derived from either a linear or second-order polynomial fit of the calibration data were used to generate the quantitation data shown in Table 2.

For the data generated with a linear fit, quantitation accuracy at the higher concentrations was low since those concentrations were outside of the linear range of the data.

Table 2. Accuracy* and reproducibility of quantitation with the Bio-Rad protein assay kit.** Total protein concentrations were generated from comparisons with calibration curves fitted with a second-order polynomial fit (using 0–2,000 ng/µl concentrations) or linear fit (using 50–500 ng/µl concentrations) of the data and are shown compared to those obtained by UV spectroscopy.

		Second-Order Polynomial Fit (0–2,000 ng/µl)			Linear Fit (50–500 ng/µl)		
Sample	UV-Based Conc. (ng/µl)	Measured Conc. (ng/µl)	Accuracy	Reproducibility	Measured Conc. (ng/µl)	Accuracy	Reproducibility
BSA	1,460	1,438.6	–1.5%	2.0%	1,006.4	–31.1%	2.0%
	886	892.2	0.7%	1.1%	745.8	–15.8%	1.1%
	212	260.1	22.7%	2.2%	231.5	9.2%	2.2%
	53	23.3	–56.0%	1.3%	<0	<0%	<0%
CA	1,055	1,027.1	–2.6%	1.4%	519.5	–50.8%	1.4%
	689	816.4	18.5%	1.7%	465.0	–32.5%	1.7%
	189	270.4	43.1%	1.4%	205.5	8.7%	1.4%
	50	28.9	–42.2%	1.0%	36.4	–27.2%	1.0%
IgG	1,319	1,197.6	–9.2%	5.5%	919.4	–30.3%	5.5%
	716	746.9	4.3%	2.9%	685.8	–4.2%	2.9%
	192	159.7	–16.8%	2.9%	178.1	–7.2%	2.9%
	50	27.7	–44.5%	1.1%	32.4	–35.1%	1.1%

* Calculated as % difference relative to expected.
** Calculated as %CV.

However, with the second-order polynomial fit, quantitation for these concentrations was considerably closer to the spectroscopically determined concentrations. The 50 ng/µl concentration, which is at the lower limit of the linear range of detection of this assay, generated the most inaccurate concentration estimates, and using the second-order polynomial fit did not make the data more accurate (Table 2).

Similar results were obtained with the Coomassie (Bradford) protein assay kit from Pierce Biotechnology (not shown). The published linear range of this assay is 100–1,500 ng/µl, but for comparison with the Bio-Rad protein assay, the 50–500 ng/µl range was used for the linear fit equations, and the 0–2,000 ng/µl range was used for the second-order polynomial fit. Again, the 50 ng/µl sample generated large variations, and the 750 ng/µl and 1,250 ng/µl samples demonstrated improved accuracy when analyzed with the second-order polynomial fit rather than with the linear fit of the data. Overall, the two assay kits produced similar results for each of the test proteins, with average %CV values within 1%, and accuracy values within 6%.

Modified Lowry Assays

The *DC* protein assay is a modified Lowry assay based on the reaction of proteins with an alkaline copper tartrate solution and Folin reagent. The reported linear range of this protocol is 200–1,500 ng/µl, and a near-perfect linear fit was obtained for calibration curves of each protein when the full range of concentrations (0–2,000 ng/µl) was examined; the r^2 values of the BSA, CA, and IgG curves at this range were 0.999, 0.981, and 0.998. The equations gained from this linear fit were used to calculate the protein concentrations shown in Table 3. With the *DC* protein assay, higher protein concentrations (>200 ng/µl) were measured with greater accuracy than the samples with lower concentrations.

With the Pierce assay, which utilizes the reduction of Cu^{2+} to Cu^{+} by protein in an alkaline medium followed by detection of the cuprous cation with a reagent containing bicinchoninic acid (BCA), the r^2 values of the BSA, CA, and IgG calibration curves (0–2,000 ng/µl) were 0.998, 0.966, and 0.996, respectively. As with the *DC* protein assay, higher concentrations (>200 ng/µl) were quantitated with greater accuracy than the samples with lower concentrations (not shown). The average %CV for the three proteins was within 1%, and the % difference vs. the UV-based concentration was within 8% when results for the two assay kits were compared.

Table 3. Accuracy* and reproducibility of quantitation with the *DC* protein assay.** Total protein concentrations were generated from comparisons with calibration curves fitted with a linear fit of the data (0–2,000 ng/µl) and are shown compared to those obtained by UV spectroscopy.

Sample	UV-Based Conc. (ng/µl)	Measured Conc. (ng/µl)	Accuracy	Reproducibility
BSA	1,460	1,743.3	19.4%	2.8%
	886	1,085.3	22.5%	3.1%
	212	325.3	53.4%	2.3%
	53	73.3	38.3%	5.1%
CA	1,055	1,096.3	3.9%	1.9%
	689	686.8	–0.3%	1.3%
	189	145.8	–22.9%	3.9%
	50	1.5	–97.0%	3.4%
IgG	1,319	1,259.2	–4.5%	1.7%
	716	728.2	1.7%	0.3%
	192	165.2	–14.0%	1.2%
	50	20.2	–59.6%	1.4%

* Calculated as % difference relative to expected.
** Calculated as %CV.

SDS-PAGE

IgG, BSA, and CA samples were analyzed by SDS-PAGE using 7.5%, 10%, and 15% Tris-HCl precast gels, respectively. Each gel also contained separations of six standard concentrations (25–2,000 ng/µl), which were used to generate calibration curves. The intensities of all bands detected by Quantity One software were exported to Excel software for determination of concentration against the calibration curves. As with the microfluidics-based analyses, both the primary band intensity and the sum of all bands (total protein) per lane were used, since with BSA and IgG, more than one band was detected in lanes loaded with high protein concentrations.

The average r^2 values were 0.999 for BSA and IgG, and 0.973 for CA, indicating excellent linear fit. As shown in Table 4, higher protein concentrations were quantitated with higher accuracy; 50 ng/µl samples were quantitated with the lowest reproducibility and the poorest accuracy.

Comparison of Quantitation Methods

Quantitation results generated by SDS-PAGE (Table 4) were similar to those presented for the Experion system (Table 1). However, for traditional SDS-PAGE, samples were loaded on gels, separated by electrophoresis, stained, destained, scanned, and analyzed with Quantity One and Excel software in a process that typically required 3–4 hr to complete. The Experion automated electrophoresis system generated comparable results for up to ten samples in only 30 min.

To further examine the differences in the reproducibility of quantitation by the microfluidics- and gel-based methods, all data were plotted in a scatter plot (Figure 1). The Pro260 assay and SDS-PAGE data displayed similar clustering patterns, and both sets of data displayed tighter clustering (higher reproducibility) than the data from the 2100 bioanalyzer system (Figure 1).

The data were also evaluated using the %CV (Figure 2A). Generally, the Bradford and modified Lowry assays generated the most reproducible results, likely because no protein separation was involved. Samples with the lowest protein concentrations (50 ng/µl) displayed poorer quantitation reproducibility than those with higher concentrations; this was particularly prominent in the SDS-PAGE data (Figure 2A). The Experion Pro260 assay, in most cases, had a %CV of <20% for all three proteins (Figure 2A), indicating good reproducibility.

The accuracy of quantitation for all methods was also compared, and the % differences were plotted for three of the four concentrations examined (Figure 2B). (For all three proteins, the 50 ng/µl samples showed the least accuracy; therefore, these data were excluded.) The Bradford and modified Lowry assays generated comparable results, and most data fell within 20% of the UV-based concentrations. The Experion Pro260 assay performed similarly to SDS-PAGE and the dye-based assay kits, and was slightly better than the 2100 bioanalyzer system. For BSA and IgG, the peak and total protein concentrations determined by the Experion Pro260 analysis were very similar, or the peak values showed slightly higher accuracy. For CA, the accuracy using the microfluidics-based quantitation methods was not as good as the other methods when total protein concentrations were examined; this is likely due to differences in protein staining

Table 4. Accuracy* and reproducibility of quantitation by SDS-PAGE and Quantity One analysis.** BSA, CA, and IgG were separated by SDS-PAGE and analyzed with Quantity One software. Concentrations were determined from comparisons with calibration curves fitted with a linear fit of the data (25–2,000 ng/µl). Primary band and total protein concentrations are shown compared to those obtained by UV spectroscopy.

		Primary Band			Total Protein		
Sample	UV-Based Conc. (ng/µl)	Measured Conc. (ng/µl)	Accuracy	Reproducibility	Measured Conc. (ng/µl)	Accuracy	Reproducibility
BSA	1,460	1,448.1	–0.8%	7.0%	1,476.6	1.1%	3.7%
	886	933.7	5.4%	7.0%	923.1	4.2%	3.7%
	212	226.3	6.7%	5.1%	230.7	8.8%	7.9%
	53	23.2	–56.1%	43.6%	43.0	–18.9%	24.0%
CA	1,055	814.3	–22.8%	4.3%	842.8	–20.1%	8.7%
	689	591.0	–14.2%	5.3%	585.7	–15.0%	6.8%
	189	221.8	17.4%	3.3%	221.4	17.1%	3.5%
	50	3.2	–93.6%	104.2%	7.6	–84.8%	81.5%
IgG	1,319	1,383.9	4.9%	6.3%	1,300.8	–1.4%	5.9%
	716	842.0	17.6%	5.6%	761.5	6.4%	6.0%
	192	125.0	–34.9%	17.6%	160.4	–16.5%	9.2%
	50	<0	<0%	<0%	27.8	–44.4%	67.9%

* Calculated as % difference relative to expected.

** Calculated as %CV.

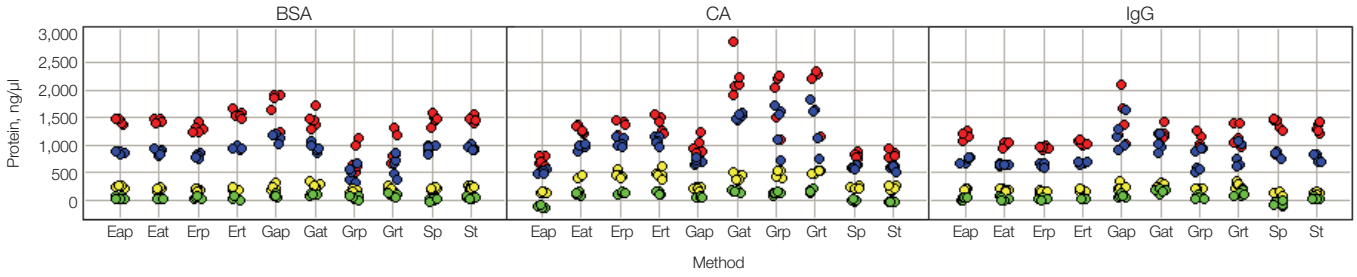

Fig. 1. Scatter plot comparison of quantitation reproducibility of microfluidics- or gel-based methods. Quantitation data from the Experion Pro260 analysis are shown as: Eap = absolute peak concentration; Eat = absolute total concentration; Erp = relative peak concentration; Ert = relative total concentration. Data from the Agilent protein 200 plus LabChip kit are shown as: Gap = absolute peak concentration; Gat = absolute total concentration; Grp = relative peak concentration; Grt = relative total concentration. Data generated by SDS-PAGE are shown as: Sp = primary band concentration; St = total concentration. Each dot represents data recorded from a single well or lane, and each color represents a different protein concentration: green = 50 ng/µl; yellow = 200 ng/µl; blue = 750 ng/µl; red = 1,250 ng/µl.

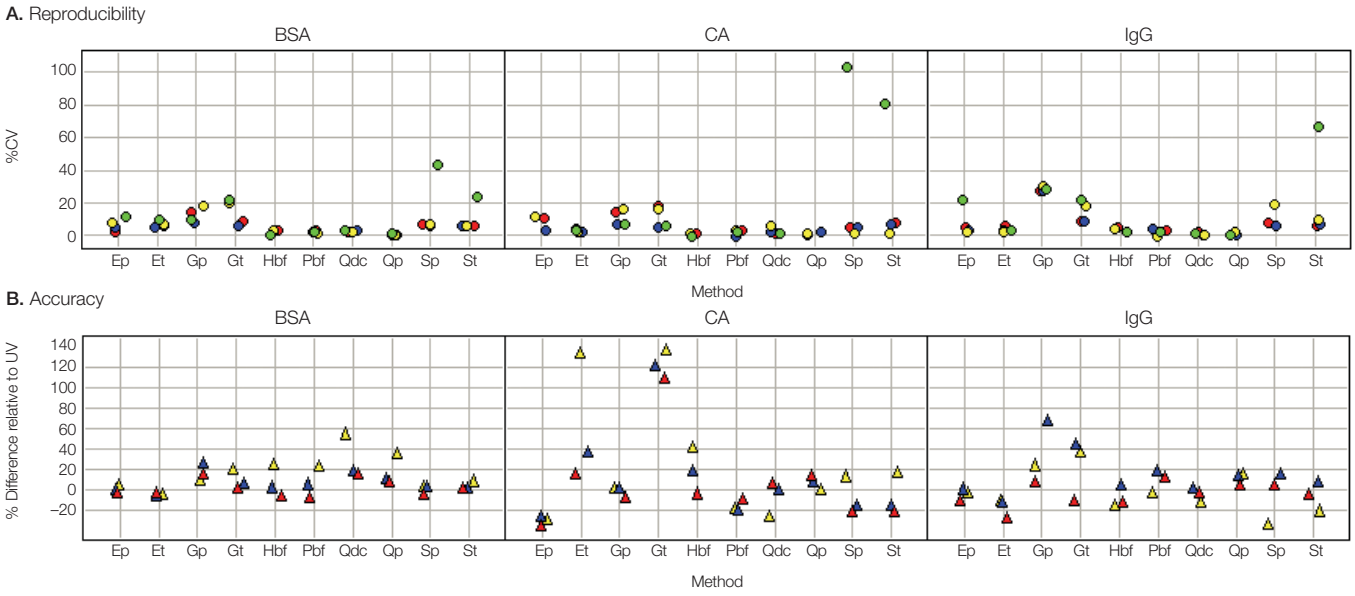

Fig. 2. Scatter plot comparison of quantitation reproducibility and accuracy. Data from the Experion Pro260 analysis using absolute quantitation are shown as: Ep = peak concentration; Et = total protein concentration. Data from the Agilent protein 200 plus LabChip kit using absolute quantitation are shown as: Gp = peak concentration; Gt = total protein concentration. Data from the Bio-Rad protein assay (Hbf), Pierce Coomassie (Bradford) assay (Pbf), *DC* assay (Qdc), and Pierce BCA assay (Qp), as well as SDS-PAGE (Sp = primary band concentration and St = total protein concentration) are also shown. Data for both Bradford assays were obtained using the polynomial fit. Data from 50 ng/µl samples were excluded from B due to the large variations in accuracy observed with these samples with all methods. Each color represents a different protein concentration: green = 50 ng/µl; yellow = 200 ng/µl; blue = 750 ng/µl; red = 1,250 ng/µl.

efficiency between CA and the upper marker (Nguyen and Strong 2005). However, a dramatic improvement in quantitation accuracy was observed with absolute quantitation of peak protein concentrations. Overall, some of the most accurate measurements generated in this study were those made with the Experion system (Figure 2B).

Conclusions

The Experion system and Pro260 protein analysis kit offer rapid and reliable protein quantitation. As shown here, this method provides comparable reproducibility and slightly higher accuracy than other traditional protein quantitation methods, such as SDS-PAGE, Bradford assay, and Lowry assay, yet this automated system covers a wider range of linearity, requires lower sample and reagent volumes, offers significantly reduced time-to-results and hands-on time, and decreases exposure to hazardous chemicals. In addition, the Experion Pro260 assay provides both the primary peak and total protein concentrations, whereas other dye-based colorimetric methods only determine total protein concentrations. Therefore, for mixed protein samples, the Experion Pro260 assay is an excellent choice for either relative or absolute quantitation of all protein components within a sample.

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