Quality Control for the Agilent 2100 Bioanalyzer Protein 200 Plus LabChip Kits

Technical Overview

Proteomics

Authors

Tobias Preckel, Marc Valer, and Martin Kratzmeier
Agilent Technologies, Inc.
Waldbronn, Germany

Introduction

Lab-on-a-Chip (LoaC) technology has had a major impact on the automation of protein analysis. Traditionally, SDS-PAGE gels are run for sizing and quantitation of proteins. Microfluidic protein analysis is now beginning to replace this traditional method. In August 2002, the Food and Drug Administration (FDA) announced a significant new initiative, pharmaceutical-current Good Manufacturing Practices (GMPs) for the 21st Century, to enhance and modernize the regulation of pharmaceutical manufacturing and product quality. With the increasing focus on proteins as pharmaceutical drugs and diagnostic tools, there is a strong demand for standardized and reproducible protein analysis methods that comply with GLP (good laboratory practices), GMP, and 21CFR Part 11 requirements. Here, LoaC technology can offer a benefit. For protein analysis, it integrates sample handling, separation, staining, destaining, detection, and digital data processing. In addition, due to the integration of several individual procedures, an increase in throughput and reproducibility can be achieved. However, the quality of the reagents and consumables is crucial to obtain high quality and reliable data. This technical note describes some of the typical quality control tests and specifications that apply to the Protein 200 Plus LabChip kits.

Protein 200 Plus Assay Specifications

The specifications of the bioanalyzer Protein 200 Plus assay are shown in Table 1. These were conservatively set, and compliance of the assay with these specifications was demonstrated across a large number of instruments and sample conditions (such as salt content and individual users). With regard to the accuracy of sizing protein samples, the results are typically within 20% C.V. of the expected value. However, it is important to keep in mind that specific proteins or modified proteins (such as glycosylated proteins) can migrate differently than would be expected from their molecular weight (MW) depending on their structure and modification [1].

Table 1. Protein 200 Plus Assay Specifications

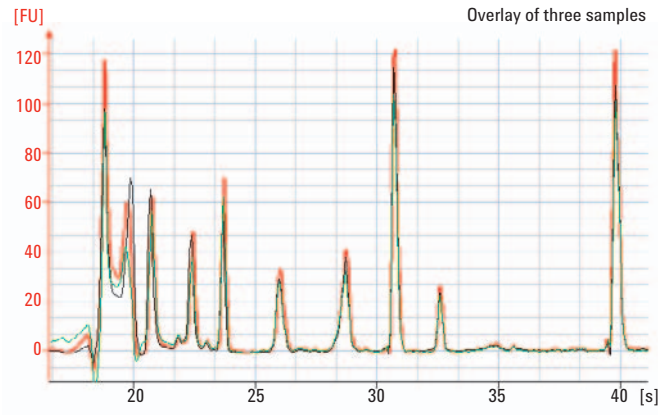
Sizing range	14–200 kDa
Typical sizing accuracy	20% C.V.*
Sizing reproducibility	10% C.V.*
Sizing resolution	10% difference in MW
Minute detectable concentration	
PBS	20 µg/mL (BSA)
0.5 M NaCl	40 µg/mL (BSA)
Max. protein concentration	4 mg/mL
Linear dynamic range	20–2000 µg/mL
Quantitation reproducibility	20% C.V.
Sample buffer myosin calibration	HSA standard curve slope: 0.8–1.2
Ladder quality	No major impurities, minor impurities at 18 and 25 kDa
Ladder quantity	Mean peak height >100 FU
Physical specifications	
Samples per chip:	10 + ladder
Time to first result:	5 min
Time to last result:	25 min
Minimum volume of sample required:	4 µL

*C.V. (coefficient of variation) is defined as standard deviation × 100/average of measured values.

Sizing Accuracy and Reproducibility

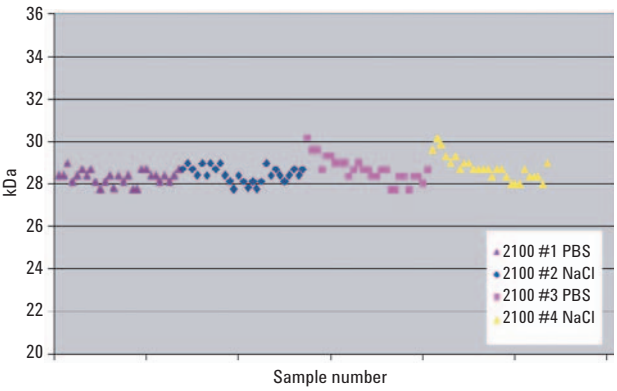
Figure 1A highlights the resolution of individual protein bands. The overlay of 3 individual runs (one chip) indicates excellent measurement reproducibility. The table below Figure 1A shows the accuracy and reproducibility of the sizing data obtained with the Protein 200 Plus assay for the individual protein bands in the standard. In this case, sizing accuracy was within 90% or better of the expected size. In Figure 1B, accuracy and reproducibility of sizing was investigated for carbonic anhydrase on four instruments and two different buffer conditions. The average size of 28.6 kDa compares well with the mass-spectrometry-determined size of the protein [2]. The graph shows that sizing does not depend on the salt concentration.

A) Sizing accuracy and reproducibility
(Bio-Rad CE-SDS protein size standard, Order no. 148-2015, non-catalog item)



Expected	**14**	**21**	**31**	**45**	**66**	**97**	**116**	**kDa**
Average	13.3	20.5	28.5	44.5	67.8	94.2	118.5	kDa
SD	0.5	0.5	0.5	0.5	1.3	0.8	0.5	
CV	3.9	2.7	1.9	1.2	2.0	0.8	0.5	

B) Sizing reproducibility
Carbonic anhydrase, Sigma order no. C-2273, 100 µg/mL in PBS or 0.5 M NaCl



Average: 28.6 kDa
CV: 1.7%

Figure 1. A) Sizing Resolution. A protein standard (BioRad CE-SDS Protein Standard, order no. 148-2015) was diluted 1:10 in phosphate buffered saline (PBS). Two µL were mixed with 4 µL of sample buffer (supplied in kit) and were run on a Protein 200 Plus LabChip (27 samples on three different instruments). A representative overlay of individual samples of one chip is shown. B) Sizing Reproducibility. One hundred µg/mL carbonic anhydrase (Sigma order no. C-2273) in either PBS or in 0.5 M NaCl were run on several chips on four instruments. The experimentally determined size of the protein is plotted against the sample number, and each dot represents an individual measurement. The MS determined size is 29.000 Da [2]. Twenty-seven samples were run on a total of 18 chips by two independent users. Two reagent batches and two chip batches were used.

Sizing Resolution

Figure 2 shows the quality of sizing resolution of a self-mixed protein standard for two independent runs from two different validation tests. ß-lactoglobulin (18.7 kDa) and trypsin inhibitor (20.8 kDa), which differ in molecular weight (MW) by 10%, were well separated in both runs. Also, carbonic anhydrase I was separated from carbonic anhydrase II (28.3 versus 31.0 kDa; 8.7% difference in MW). With the exception of trypsinogen (MW 26.7 kDa), which could not be completely separated from carbonic anhydrase II (MW 28.3 kDa; 5.6% difference in MW), all other proteins could be separated with baseline separation.

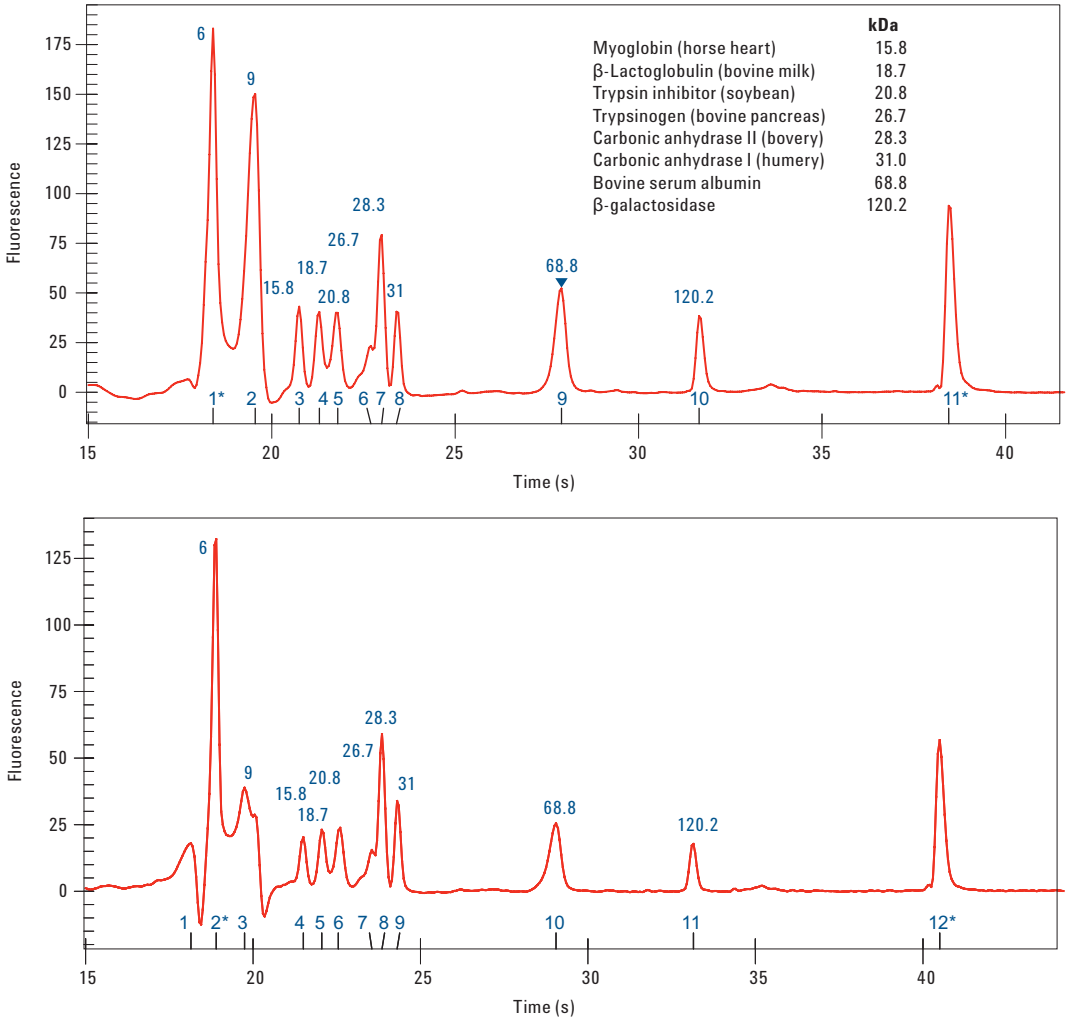


Figure 2. Sizing resolution. A self-made protein mixture of eight different proteins in a concentration of approximately 65 µg/mL each in PBS was prepared and analyzed with the Protein 200 Plus LabChip kit.

Detection Limit

To assess the minimal detectable protein concentration of the assay bovine serum albumin (BSA), samples in PBS (Figure 3A) and in 0.5 M NaCl (Figure 3B) were tested. Samples were run in triplicate on each chip (n = 45, 15 individual chips). Three representative electropherograms are overlaid. BSA (20 µg/mL) in PBS (upper panel) was detected with a good signal/noise ratio (S/N) of 6, whereas 40 µg/mL BSA in 0.5 M NaCl (lower panel) could be detected with a high S/N ratio of 8. The data demonstrates that the specified detection limit of 20 and 40 µg/mL BSA, respectively, is easily reached by the assay.

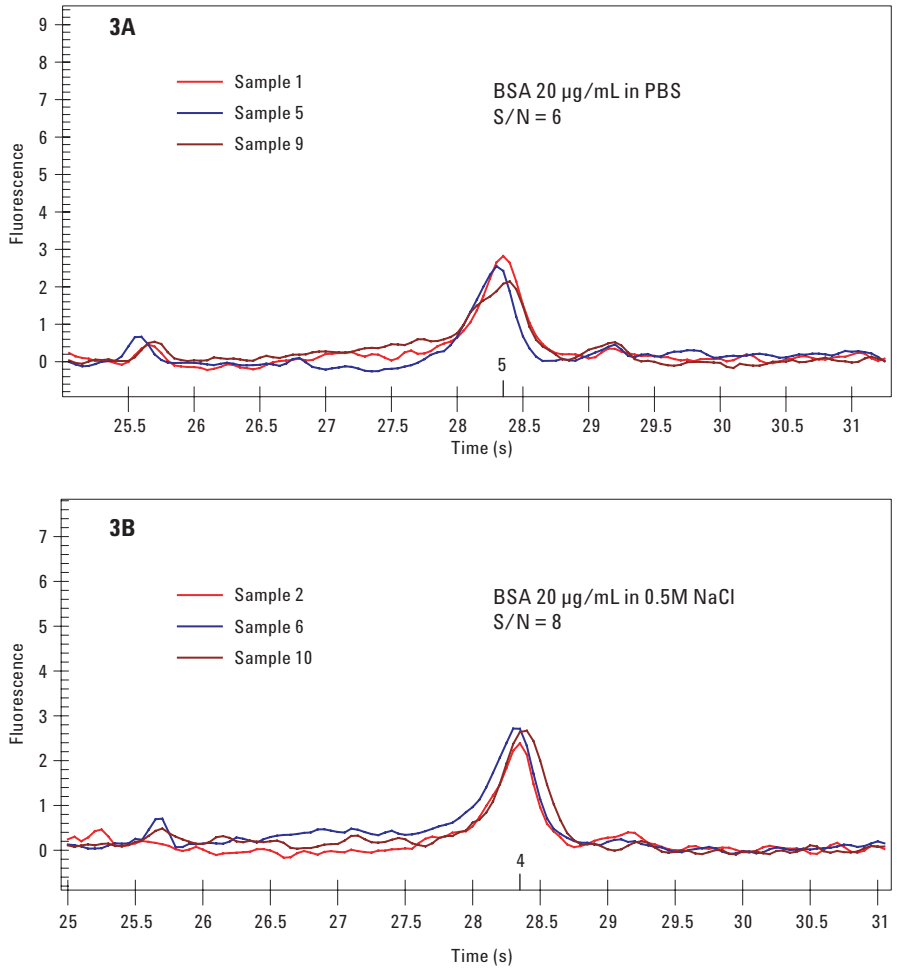


Figure 3. Sensitivity (minimal detectable concentration). BSA (20 µg/mL) in PBS (upper panel) and 40 µg/mL BSA in 0.5 M NaCl (lower panel) were run in triplicate on a single protein chip. Individual runs were overlaid.

Linear Dynamic Range

The linear dynamic range of the Protein 200 assay is specified for 0.02–2 mg/mL of protein. Using a human serum albumin (HSA) standard (Figure 4), the quality of quantitation across the specified dynamic range was assessed. Each of the five concentrations was measured 16 times with a mean C.V. of 10.5%. The slope of the curve indicates the batch-to-batch reproducibility, with 1 and no uncertainty being perfect reproducibility, of the upper marker concentration calibration procedure within the sample buffer. The external HSA standards used have a highly reproducible protein concentration. Thus, the slope of the HSA curve is a critical quality parameter. Expected for this calibration curve is a slope between 1.0 ±0.2. This means that the relative concentration that is calculated (for example HSA 1000) should always be between 800 and 1200 µg/mL. In this representative measurement it is 0.995, and it is well inside the specified value of 0.8–1.0.

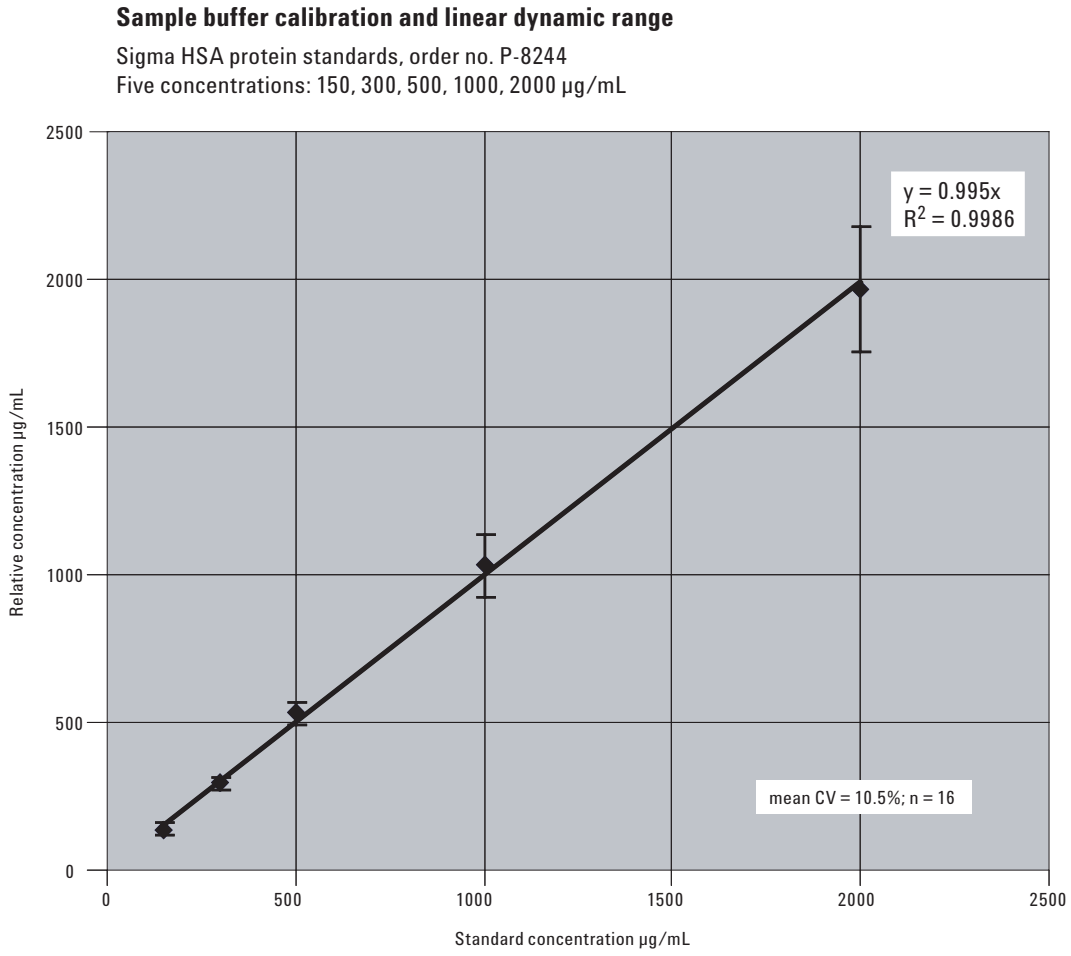


Figure 4. Sample buffer calibration and linear dynamic range. An HSA protein standard (Sigma order no. P-8244) was run at five different concentrations (0.15, 0.3, 0.5, 1, 2 mg/mL). For each concentration, 16 individual runs were performed.

Area Reproducibility

Agilent specifies an area reproducibility of 20% C.V. relative to the upper marker. Figure 5 shows data obtained with 100 µg/mL BSA in 10-mM Tris/75-mM NaCl, which was run as a sample across several chips and 11 instruments. Whereas there is some area variability between instruments and chip runs due to either slightly different optical setups or differences in electrokinetic sample injection, the average area reproducibility for an individual instrument is 15.1%. For determination of concentration, the area of each protein peak is correlated to the area of the upper marker in each individual sample. Therefore, area differences between instruments or individual chip runs are accounted for and do not affect quantitation.

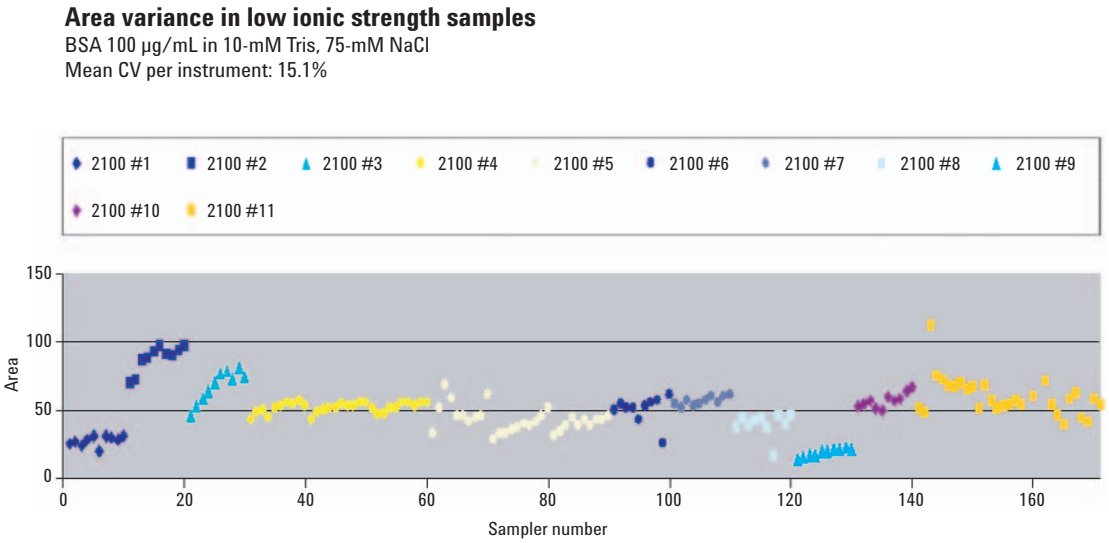


Figure 5. **Area variance in low ionic strength samples. BSA (100 µg/mL) in 10-mM Tris/75-mM NaCl was run as a sample across several chips and instruments. Each dot represents an individual measurement. Area is in arbitrary units. Overall 170 samples (17 chips with 10 samples each) were measured across 11 instruments by six independent users.**

Conclusion

Here we show representative tests to assess compliance of the Protein 200 Plus LabChip kit with the factory specifications. Specifications such as resolution and minimal detectable concentration were analyzed by different individual users on a variety of bioanalyzer instruments. Typical and representative data was well within or exceeded specifications in all cases.

References

1. L. Kelly and P. Barthmaier, Glycoprotein Sizing on the 2100 Agilent Bioanalyzer, Agilent Technologies, publication 5989-0332EN, www.agilent.com/chem
2. Puschett et al. (2003) Molecular effects of Volume Expansion on the renal sodium Phosphate cotransporter, *Am. J. Med. Sci.* **326:1.**

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