Quality control of antibodies using the 2100 bioanalyzer and the Protein 200 Plus assay

Application

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Abstract

Antibodies are commonly used in diagnostics, as research tools (for example protein arrays) or as biopharmaceutical therapeutics. In all cases the successful production of antibodies requires precise testing and quality control. The key quality criteria include: protein identification, quantitation and the monitoring of purity and stability. Agilent's 2100 bioanalyzer, the first commercial lab-on-a-chip analysis tool, developed in collaboration with Caliper Technologies Corporation, was used to verify the suitability of this technology for antibody quality control. The system allows sizing and analysis proteins of 5 to 200 kDa, depending on the application. In addition, it determines the relative quantitation based on internal standards for each sample, or absolute quantitation based on user-defined calibration standards. The microfabricated chips with distinct microfluidic channels allows antibody analysis under both reducing and non-reducing conditions in a single run. In this Application Note we demonstrate the use of the system for the evaluation of a stress stability test of two different antibodies.

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Experiments and Results

Antibody samples were kindly provided from a customer in the pharmaceutical industry. For the first sample a stress stability test was performed for one month at 40 °C, for the second sample the stress test was done for 12 weeks at 40 °C. The standard samples and the stress test samples were analyzed under reducing conditions on the Agilent 2100 bioanalyzer using a Protein 200 Plus LabChip® kit and the dedicated Protein 200 Plus software assay. All chips were prepared according to the protocol provided with the Protein 200 Plus LabChip kit. The kit includes 25 chips, spin filters and all the reagents needed for the experiments including the Protein 200 Plus ladder as well as the upper and lower marker premixed in the sample buffer.

To validate the performance of the instrument three individual users measured the samples on two separate instruments using two lots of reagents on different days. Thus a total number of twelve chips were run. The samples were

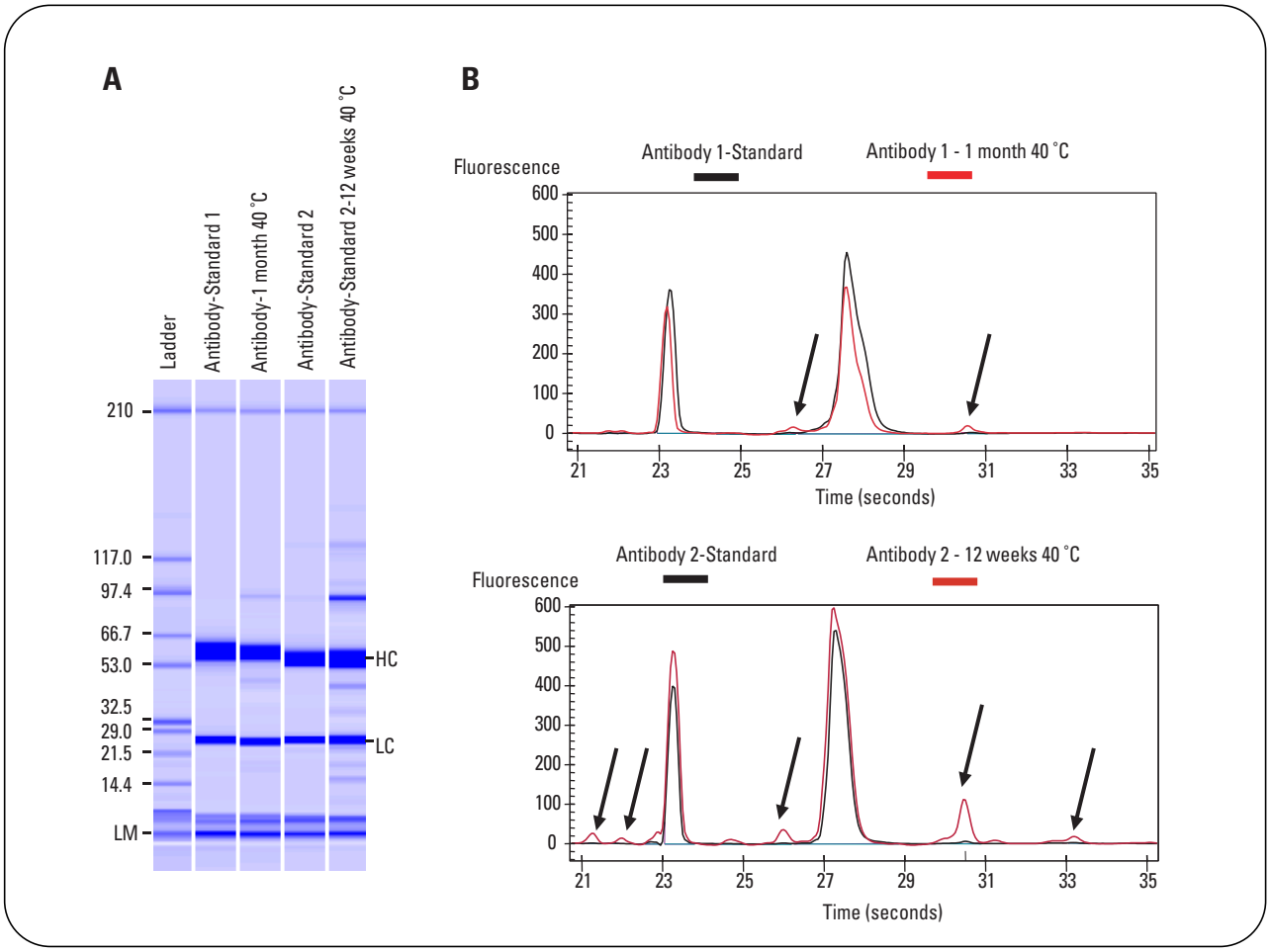

Figure 1
Representative example of the performed validation on the Agilent 2100 bioanalyzer using the Protein 200 Plus assay under reduced conditions. The gel-like image (A) and overlay of the corresponding electropherograms (B) are shown. Incubation of the antibodies at elevated temperature lead to the formation of additional peaks.

diluted 1:3 in PBS buffer (Life Technologies GmbH, Karlsruhe, Germany) in order to adjust the concentration of the samples to approximately 5 mg/ml total protein concentration. Sample preparation was performed individually for each chip. All samples were loaded twice on each chip. Therefore, 24 analyses per sample were evaluated using the following Protein 200 Plus assay settings for peak integration: minimum peak height of 0.1, minimum peak width of 0.1 and slope threshold of 4.0.

An example of the performed analysis is depicted in figure 1. Figure 1A shows the gel-like image of the four different antibody samples (2 standards, 2 test samples) aligned to the ladder sample. The ladder includes proteins of known sizes, was analyzed on each chip and was used for size determination. Figure 1B displays the overlay of the corresponding electropherograms. Both standard samples show almost only two peaks corresponding to the light (LC) and heavy chain (HC) of the antibody respectively. After incubation of the antibodies at elevated temperatures additional peaks appear in the electropherograms (marked with an arrow) due to degradation of the antibody or the formation of aggregates, larger products. Table 1 shows the results of the validation study. On average 98.76 % of standard antibody sample 1 corresponds to the light and heavy chain of the antibody. After incubation for one month at 40°C the two chains represent only 93.78% of the whole protein amount detected. Antibody 2 was incubated for 12 weeks at 40°C and the proportion of light and heavy chain decreases from an average of 98.38 % to 85.34 %. An excellent reproducibility below 2 % CV was achieved

	Average	StDev	CV [%]
Sample 1 - Standard	98.76	0.61	0.62
Sample 1 - 1 month at 40 °C	93.78	0.93	0.99
Sample 2 - Standard	98.38	0.66	0.67
Sample 2 - 12 weeks at 40 °C	85.34	1.48	1.73

Table 1
Results of the validation study. The proportion of the sum of the light and heavy chain from the total protein concentration of the sample was calculated. Average, StDev and relative StDev are displayed (n = 24).

Conclusion

In this study we show that the Agilent 2100 bioanalyzer can be used as an ideal tool to study the stability of antibodies in stress tests with excellent reproducibility. This application is commonly used as a quality control step in QA/QC departments in order to trigger typical degradation and aggregation patterns for a specific antibody. Those patterns will be used in the down stream processes of antibody production and formulations.

Traditional denaturing sodium dodecylsulfate-polyacrylamide gel electrophoreses (SDS-PAGE) still serves as one of the standard methods for the QC of antibodies. SDS-PAGE is labor-intensive, time-consuming and difficult to standardize (1-3). In contrast the Agilent 2100 bioanalyzer provides a fast, standardized method with automated and detailed data analysis.

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