Technical Overview

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Analysis of NIST Antibody on the Agilent ProteoAnalyzer System

Introduction

The National Institute of Standards and Technology (NIST), a branch of the US Department of Commerce, develops reference materials to foster the innovation and standardization of technology in various domains. One of these materials is a monoclonal antibody (mAb) called NISTmAb, which has been thoroughly characterized by multiple analytical techniques [1,2,3]. The antibody is composed of two heavy chains and two light chains, connected by interchain disulfide linkages. However, lower molecular weight fragment impurities can be generated during manufacturing, storage, and handling, yielding combinations of heavy and light chains that are smaller than the monomer. Electrophoresis is a standard method used to separate and visualize the pattern of these fragments, and provides information about the mAb size, concentration, composition, and purity. For complete characterization of mAb purity and glycan occupancy, a high-resolution separation system is required that can analyze the mAb under both reduced and nonreduced conditions.

Due to its extensive characterization, the NISTmAb is a well-established standard within the biopharmaceutical industry and is widely used for evaluating analytical methods for mAb quality assessment. Agilent, a leading provider of solutions for mAb characterization, now offers a novel technology that delivers a fast, automated, and higher-throughput alternative to conventional single capillary CE-SDS methods: The Agilent ProteoAnalyzer system[4]. In this technical overview, the characterization of the NISTmAb assessed with the ProteoAnalyzer was compared to the published NIST data obtained using traditional single capillary CE-SDS technology[1,2].

Experimental

NISTmAb (Sigma p/n NIST8671, aliquot from Reference Material 8671, Lot 14HB-D-002)[1] was prepared in PBS at a concentration of 2,000 ng/µL under both reducing and nonreducing conditions according to the Agilent Protein Broad Range P240 kit (p/n 5191-6640) manual[5]. The samples were covalently labeled by incubating with the supplied reagents at 70 °C for 10 minutes. The reduced and nonreduced antibodies were analyzed across multiple capillaries of an Agilent ProteoAnalyzer system[4] with the ProteoAnalyzer Brand Range Kit LM only method. For nonreduced conditions, the sample injection was decreased to 7 kV 6 seconds for optimal results. Analysis of the samples from the ProteoAnalyzer was compared to the known specifications of the NISTmAb from datasheets and published results[1,2].

Results and discussion

Visual representation of NISTmAb

Characterization of the NISTmAb with traditional single capillary CE-SDS is described in the published NIST datasheet[1]. The results of the NISTmAb analyzed with the ProteoAnalyzer were thus compared to the NIST datasheet and published results[2] to evaluate the instrument.

Figure 1 shows representative examples of the published nonreduced NISTmAb compared to the results achieved by the ProteoAnalyzer. Analysis with either method uses a marker for sample alignment and quantitation (single-capillary CE-SDS: 10 kDa peak; ProteoAnalyzer: 6 kDa Lower Marker (LM)). The main peak, or monomer, of the NISTmAb and several smaller fragment peaks can be identified with both the single capillary CE-SDS method and the ProteoAnalyzer. The smaller peaks that can be visualized in the nonreduced sample include the light chain (L or LC), heavy chain (H or HC), and combinations of the two (HC:LC, HC:HC, HC:HC:LC). Although not described in the NISTmAb datasheet,

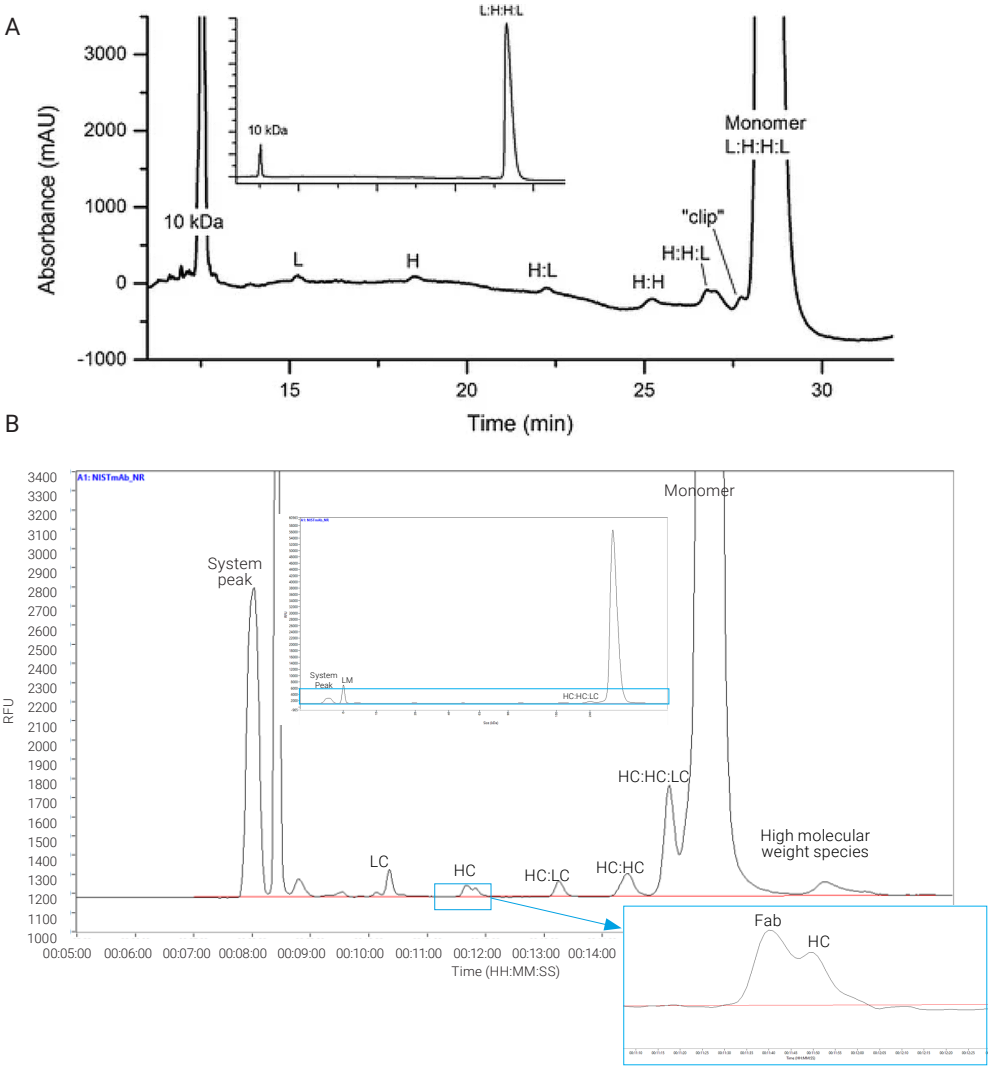

Figure 1. Nonreduced NISTmAb. A) This figure has been reproduced from Turner et al.[2] B) Results of the nonreduced NISTmAb using the Agilent ProteoAnalyzer system.

Turner, A., Yandrofski, K., Telikepalli, S. et al. Development of orthogonal NISTmAb size heterogeneity control methods. Anal Bioanal Chem 410, 2095–2110 (2018). https://doi.org/10.1007/s00216-017-0819-3

the HC when analyzed on the ProteoAnalyzer partially resolves two peaks, indicative of a disulfide-linked fragment antigen binding (Fab) region[6]. Additionally, high molecular weight (HMW) aggregates, when present, are visualized to the right of the monomer and have previously been identified as multimers[6].

The same NISTmAb sample was also run under reduced conditions on the ProteoAnalyzer, and the electropherogram compared to the standards from the NIST datasheet and publications[1,2] (Figure 2). Both systems were able to easily visualize the light chain and heavy chain. The smaller peak to the left of the heavy chain is representative of the nonglycosylated heavy chain (NGHC), and to the right is the thioether peak (or nonreduced species, NRS). Analysis of the reduced NISTmAb with the ProteoAnalyzer used the optional Upper Marker (UM) for better alignment and thus higher sizing precision of the sample peaks. Assessment of the NISTmAb by the ProteoAnalyzer provides highly comparable results to the traditional single capillary CE-SDS, providing users with the confidence to use either system for their analytical methods.

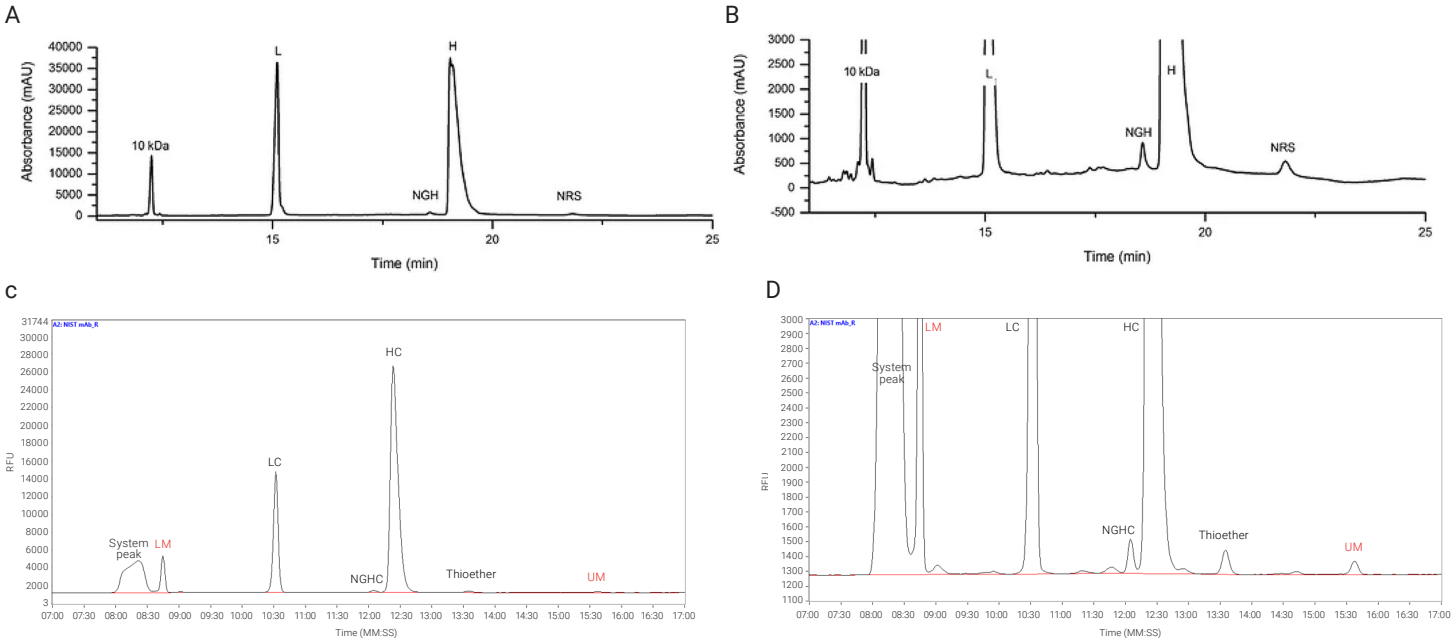

Figure 2. Reduced NISTmAb. (NRS = nonreduced species). A,B) This figure has been reproduced from Turner et al.[2] C,D) Results of the reduced NISTmAb using the Agilent ProteoAnalyzer system.

Turner, A., Yandrofski, K., Telikepalli, S. et al. Development of orthogonal NISTmAb size heterogeneity control methods. Anal Bioanal Chem 410, 2095–2110 (2018). https://doi.org/10.1007/s00216-017-0819-3

Table 1. Critical Quality Attributes of purity for the NISTmAb were determined by the Agilent ProteoAnalyzer system and compared to the published NIST datasheet[1]. ProteoAnalyzer: Nonreduced n = 11; Reduced n = 33.

	NIST Datasheet[1]		ProteoAnalyzer system	
	Size heterogeneity (%)	Combined standard uncertainty (%)	Size heterogeneity (%)	%CV
Monomeric Purity (nonreduced)	98.47	1.03	98.18	0.09
Thioether (reduced)	0.30	0.03	0.40	6.35
Glycan Occupancy (reduced)	99.39	0.07	99.30	0.02

Critical quality attributes of NISTmAb

A crucial aspect in determining the quality and efficacy of monoclonal antibodies is the Critical Quality Attributes (CQAs) of monomeric purity, glycan occupancy, and percent thioether (NRS). Accurate and reliable analysis is required for appropriate assessment of biopharmaceutical products. To evaluate the ProteoAnalyzer system, the monomeric purity, glycan occupancy, and percent thioether CQAs of the NISTmAb were calculated using established equations (Figure 3) and compared to the published NISTmAb data using single capillary CE-SDS[1,2].

$$\text{Monomeric Purity}(\%) = \frac{\text{Conc}_{\text{monomer}}}{\text{Conc}_{\text{monomer}} + \sum\text{Conc}_{\text{fragments}}} \times 100$$

$$\text{Glycan Occupancy}(\%) = \frac{\text{Conc}_{\text{HC}}}{\text{Conc}_{\text{HC}} + \text{Conc}_{\text{NGHC}}} \times 100$$

$$\text{Thioether}(\%) = \frac{\text{Conc}_{\text{thioether}}}{\text{Conc}_{\text{LC}} + \text{Conc}_{\text{HC}} + \text{Conc}_{\text{NGHC}} + \text{Conc}_{\text{thioether}}} \times 100$$

Figure 3. Equations used for calculating the Critical Quality Attributes of purity for the NIST mAb. (Conc = the concentration (ng/ul) of the designated peak as determined by the Agilent ProteoAnalyzer system and ProSize data analysis software.)

A measurement of nonreduced protein quality is the purity of the monomer peak. The published datasheet for the NISTmAb reports a monomeric purity of 98.47%[1]. In contrast, analysis with the ProteoAnalyzer results in a monomeric purity of 98.18%, only a 0.29% difference from the reported single capillary CE-SDS data (Table 1).

For the reduced form, two CQAs are displayed in the published datasheet: the thioether amount, which is 0.30%, and the glycan occupancy, at 99.39%[1]. Analysis of the NISTmAb with the ProteoAnalyzer results in a thioether measurement of 0.40% and a glycan occupancy of 99.30% (Table 1). The thioether measurement is comparable to the published data, while the glycan occupancy is incredibly close, with only a 0.09% difference between the two sources.

A crucial aspect of glycan occupancy assessment is the ability to resolve the NGHC and HC fragments from each other. As shown in Figure 2D, the ProteoAnalyzer offers excellent resolution of the NGHC and HC peaks, with an average R value of 1.60, meeting the kit specifications of an NGHC/HC R ≥ 1 for NISTmAb under reduced conditions. This is highly reproducible between capillaries, with 4.5%CV (n = 33). Together, this data and a comparison to the published NIST datasheet highlight that the ProteoAnalyzer allows for excellent separations and CQA assessment of both the nonreduced and reduced NISTmAb.

Conclusion

The well-characterized NISTmAb is used as a model protein for analysis of new and emerging analytical technologies. In this technical overview, the previously published single capillary CE-SDS separations and purity calculations using previously established formulas[1,2] were compared to the Agilent ProteoAnalyzer system. The system automates sample analysis and allows for higher throughput, running up to 12 samples in parallel, and has the ability to program up to eight runs at a time without user intervention. Data is displayed as either a digital gel or electropherogram, along with a peak table supplying information about the concentration of each fragment within the sample. Evaluation of the NISTmAb demonstrated that the ProteoAnalyzer provides robust separations and percent purity values that correlate well with the published reference data and can be used with confidence in analytical workflows.

References

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