High throughput and high resolution glycan analysis by capillary electrophoresis on Applied Biosystems DNA sequencers

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ABSTRACT
Here, we report the simple and rapid GlycanAssure™ workflow that combines high-throughput and high resolution glycan analysis of 96 samples in 7-9 hours using Applied Biosystems™ 3500L, 24-capillary electrophoresis system. The process eliminates vacuum drying and highly toxic cyanobromofluoride in the labeling reaction. Use of Dynabeads™ magnetic beads for glycan purification post dephosphorylation and removal of free dye after labeling streamlines the process for automation. Two proprietary fluorescent dyes provide faster labeling and better resolution than conventional APTS. Newly developed software can finish data analysis in 1 hour, providing glycan profile, relative quantities, %CV, and trending of relative quantities of specific glycans from samples from different conditions.

Figure 1. GlycanAssure WorkFlow, 7-9 hours to process and finish CE analysis of 96 samples with no vacuum centrifugation, hands on time ~2 hr 40 min; data analysis ≤ 1 hr.

MATERIALS AND METHODS
Glycan standards were obtained from Qbiobio, V-Lab and Prozyme. The purified human serum APTs was obtained from Invitrogen (P/N 27102). Sample preparation, labeling reaction, dye removal, and CE runs were performed as described in the user guide (GlycanAssure™ user guide, Thermo Fisher Scientific, Publication Number MA0014008). Capillary electrophoresis was performed using the 3500L, a system configured with a 505 nm solid state laser and laser induced fluorescence detection (Applied Biosystems). Experimental details for this work were as follow:
- Polymer used in CE capillary: POP7 (P/N 4393706)
- Anode Buffer (P/N 4393927); Cathode buffer (P/N 4408256)
- 350L Sample Injection (P/N 4408256) and Cathode buffer (P/N 4408256)
- LIZ Size Standard v2.0 (P/N 4408339) used in every injection
- Injection conditions: 1.6 kV
- Voltage: PuffPrep at 15.5 kV and Run at 19.5 kV
- Run time: 1320 sec
- Capillary temperature: 60°C
- APTS EX 475nm EM 501nm; TURQUOISE™ Dye EX 466nm EM 505nm; TEAL™ EX 493nm EM 520nm

RESULTS
Fig. 3. Precision of glycan detection and separation among 24 capillaries (Figure 2) APTS-labeled mixed glycans prepared from Fetuin and human serum IgG were run from 24 capillaries. The mixture covers very diverse types of glycans. Percentage of peak was calculated by dividing the individual peak area to the summed areas. Average percentage of peak and %CV among 24 capillaries were calculated. Tight precision was also observed among multiple injections (data not shown).

Fig. 4. Overlay of glycan peaks from multiple injections. Mixture of APTS-labeled glycan standards was distributed in a 36-well plate (as shown in Fig. 2) which was injected 3 times to make a total of 288 runs. All glycan peaks were aligned nicely. Some results were observed for complicated set of more than 35 APTS-labeled glycans prepared from human plasma (data not shown).

Fig. 5. TEAL dye provides better glycan separation than APTS 15 glycans were labeled with TEAL dye and APTS and were run under the same conditions. TEAL dye separated A1F, Man5, G2F, and Man9; these cannot be separated by APTS. Turquoise dye could separate A1F and Man9, but not G2F and Man9 (data not shown).

Fig. 6. Responsive of CE detection to increased glycan concentrations 3 mixtures (M1, M2, and M3) of 15 APTS-labeled glycans were created. Concentrations of A2F, Man7, and G2 were increased by 2x in M2, and by 4x in M3. Concentrations of the rest 12 glycans were unchanged. Signals of A2F, Man7, and G2 were increased by 2x in M2 and by 4x in M3 from 3500L CE analysis.

Fig. 7. Robust process produced consistent relative quantities of glycans from wide inputs of 10-100 µg human IgG 8 independent sample preps were performed for each input quantity. Consistent average relative quantities and low %CV were obtained from 10, 50, and 100 µg.

Fig. 8. Data analysis of CE sample results within 1 hour Data analysis program automatically creates a list of time which can rapidly map and calculate relative quantities of same glycans from 96 samples and calculate %CV among selected samples (8A), an analysis example of 16 samples are shown in the figure. Trending display of results provides easy analysis of conditions that produce different glycan profiles (samples 7 and 8 in Fig. 8A and C).

SUMMARY
- Easy and rapid high throughput workflow: Analysis of 96 samples can be completed in 7-9 hours (Fig. 2 and Fig. 9).
- High precision in glycan analysis and reproducible alignment of glycan peaks: Very low variation in relative quantities of glycans among 24 capillaries (≤ 2%) and nice overlay of glycan peaks from multiple injections (Fig. 6).
- Higher resolution by new fluorescent dyes: Availability of these dyes allows selection of a dye for the best resolution of glycans from any particular sample (Fig. 9).
- CE condition detects the proportionality of concentrations of glycans in the sample: Selectively increased concentrations of charged fucosylated and sialylated glycan (ADF), high mannose (ManN), and uncharged complex G0 detected in a mixture with fixed concentration of another 12 glycans (Fig. 6).
- Consistent glycan profile and relative quantities from wide input sample amounts: Replicated 8 analyses of the same input sample produced results with low %CV in relative quantities from ~2% down to <1% (Fig. 7).
- Rapid data analysis of capillary electrophoresis results: The new software allows automatic creation and easy modifications to identify glycan peaks from multiple samples (Fig. 8). Relative quantification and %CV can be easily calculated. The overlay and trending features of the program allows quick identification of samples that produce different glycan profiles for further analysis and the causes. The program can analyze 96 samples in one hour that typically takes a day or longer.
- Same day result from 96 samples: It is now possible to finish glycan analysis of 96 samples in 1 day with the rapid sample prep, high throughput CE, and rapid data analysis.

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