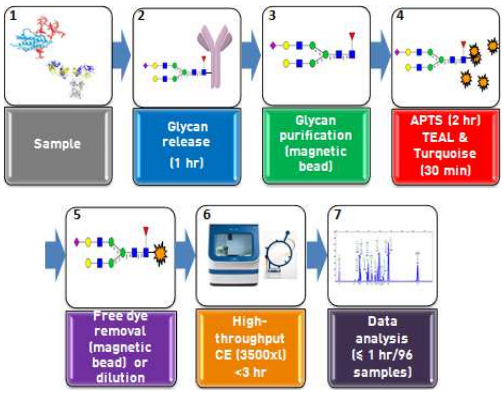
High throughput and high resolution glycan analysis by capillary electrophoresis on Applied Biosystems DNA sequencers

Jenkuei Liu, James Stray, Shaheer Khan, Bharti Solanki, Brian Evans, Steve Gorfien, and Baburaj Kunnummal
Pharma Analytics, BioProduction Division
180 Oyster Point Blvd., South San Francisco, California, 94408

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™ 3500xL 24-capillary electrophoresis system. The process eliminates vacuum drying and highly toxic cyanoborohydride in the labeling reaction. Use of Dynabeads™ magnetic beads for glycan purification post deglycosylation and removal of free dyes 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 of 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.

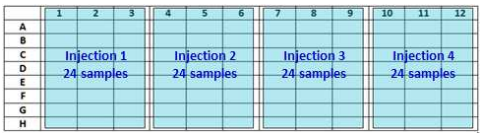


MATERIALS AND METHODS

Glycan standards were obtained from Qabio, V-Lab and Prozyme. The purified human serum IgG 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 MAN0014008). Capillary electrophoresis was performed using the 3500xL, 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:

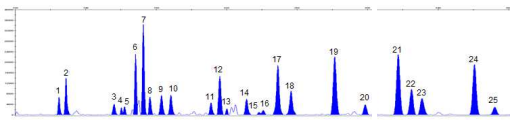
- Polymer used in CE capillary: POP7 (P/N 4393708)
- Anode Buffer (P/N 4393927); Cathode buffer (P/N 4408256)
- 3500xL Genetic Analyzer Capillary Array, 50 cm (P/N 4404689)
- LIZ Size Standard v2.0 (P/N 4408339) used in every injection
- Injection conditions: 1.6 kV
- Voltages: PreRun at 19.5 kV and Run at 19.5 kV
- Run time : 1330 sec
- Capillary oven temperature: 60ºC
- APTS EX 475nm EM 501nm; TEAL™ Dye EX 466nm EM 505nm; TURQUOISE™ Dye EX 493nm EM 520nm

Figure 2. Analysis of 96 samples by 4 injections with 3500xL instrument. 24 samples are analyzed simultaneously from 24 capillaries in each injection. CE electrophoresis of one injection takes about 40 min.



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).



	Average (percentage)	CV%		Average (percentage)	CV%		Average (percentage)	CV%
1	1.8%	1.5%	10	2.7%	0.7%	19	11.0%	0.8%
2	3.9%	2.1%	11	1.7%	2.0%	20	1.8%	1.7%
3	1.4%	1.5%	12	5.4%	1.4%	20	1.8%	1.7%
4	0.6%	3.1%	13	0.7%	11.3%	21	11.7%	0.7%
5	0.8%	2.3%	14	2.4%	1.5%	22	5.0%	1.0%
6	5.8%	7.4%	15	0.3%	4.8%	23	3.6%	0.8%
7	9.1%	2.1%	16	0.7%	1.4%	24	10.6%	0.9%
8	2.3%	9.8%	17	8.2%	0.9%	25	1.7%	1.1%
9	2.7%	0.8%	18	4.2%	1.0%			

Fig. 4. Overlay of glycan peaks from multiple injections Mixture of APTS-labeled glycan standards was distributed in a 96-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. Same results were observed for complicated set of more than 35 APTS-labeled glycans prepared from human plasma (data not shown).

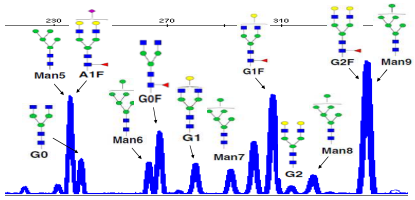


Fig. 5. TEAL dye provides better glycan separation than APTS 15 glycans were labeled with TEAL dye or APTS and were run under the same conditions. TEAL dye separated A1F, Man5, G2F, and Man9; these cannot be separated by APTS. Turquoise dye could separate A1F and Man5, but not G2F and Man9 (data not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

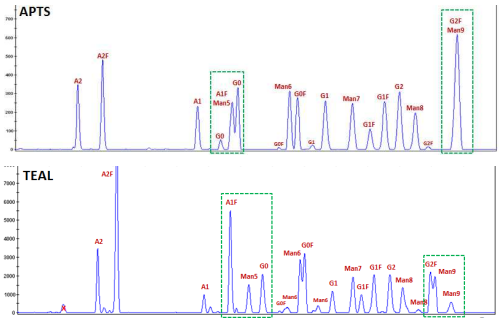


Fig. 6. Responsive of CE detection to increased glycan concentrations 3 mixtures (M1, M2, and M3) of 15 APTS-labeled glycans were created. Concentrations of A2F, Man7, and G2 were increased by 2x in M2, and by 4x in M4. Concentrations of the rest 12 glycans were unchanged. Signals of A2F, Man7, and G2 were increased by 2x in M2 and by 4x in M3 from 3500xL CE analysis.

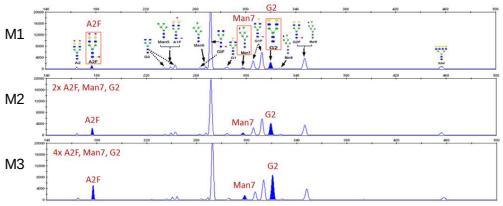
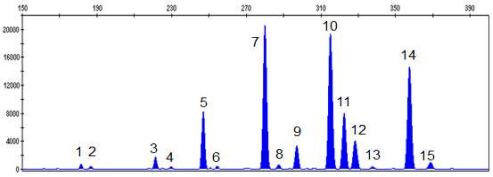


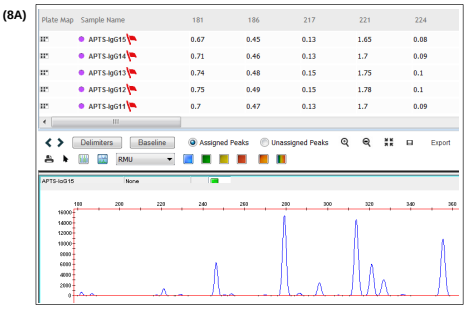
Fig. 7. Robust process produced consistent relative quantities of glycans from wide inputs of 10-100 µg human IgG 8 independent sample preps were performed for each input quantity. Consistent average relative quantities and low %CV were obtained from 10, 50, and 100 µg.



	10µg		50µg		100µg	
Peak #	%Area	%CV	%Area	%CV	%Area	%CV
1	0.90%	4.05%	0.72%	3.06%	0.64%	5.07%
2	0.61%	6.31%	0.45%	3.31%	0.39%	4.77%
3	1.64%	2.22%	1.69%	2.46%	1.66%	2.68%
4	0.42%	7.09%	0.42%	4.67%	0.38%	4.26%
5	8.54%	2.47%	8.31%	2.19%	8.18%	1.48%
6	0.76%	5.89%	0.55%	8.97%	0.42%	6.47%
7	22.51%	2.04%	23.07%	4.25%	23.71%	1.60%
8	0.63%	4.50%	0.71%	9.54%	0.71%	3.36%
9	3.73%	0.83%	3.82%	2.60%	3.85%	1.28%
10	23.52%	0.43%	23.33%	2.52%	23.84%	0.65%
11	9.98%	0.52%	10.16%	3.37%	9.91%	0.88%
12	5.37%	1.00%	5.46%	3.50%	5.42%	1.00%
13	0.43%	4.27%	0.44%	4.53%	0.43%	2.12%
14	19.58%	1.16%	19.53%	2.91%	19.20%	0.85%
15	1.38%	2.25%	1.33%	4.83%	1.27%	1.84%

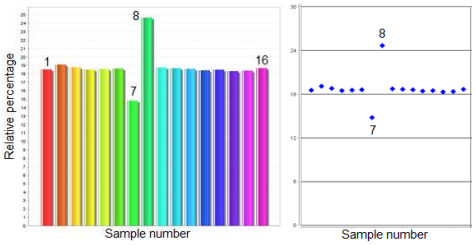
Fig. 8. Data analysis of 96 CE sample results within 1 hour Data analysis program automatically creates a set of bins which can rapidly map and calculate relative quantities of same glycans from 96 samples and calculate %CV among selected samples (8A; an analysis example of 16 samples are shown in the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

(8A)



(8B) Bar chart of relative quantity of a particular glycan from 16 samples.

(8C) Scattering plot of relative quantity.



SUMMARY

- **Easy and rapid high throughput workflow** Analysis of 96 samples can be finished in 7-9 hours (Fig. 1 and Fig.2).
- **High precision in glycan analysis and reproducible alignment of glycan peaks** Very low variation in relative quantities of glycan among 24 capillaries (Fig. 3) and nice overlay of glycan peaks from multiple injections (Fig. 4).
- **Higher resolution by new fluorescent dyes** Availability of three dyes allows selection of a dye for the best resolution of glycans from any particular sample (Fig. 5).
- **CE condition detects the proportional concentrations of glycans in the sample** Selectively increased concentrations of charged fucosylated and sialylated glycan (A2F), high mannose (Man7), and uncharged complex G0 were detected in a mixture with fixed concentration of another 12 glycans (Fig. 6).
- **Consistent glycan profile and relative quantities from wide input sample amounts** Repeated 8 analyses of 10x difference in input sample produce results with low CV% in relative quantities from ~24% down to <1% (Fig. 7).
- **Rapid data analysis of capillary electrophoresis results** The new software allows automatic creation and easy modification of bins to identify glycan peaks from multiple samples (Fig. 8). Relative quantities and %CV can be easily calculated. The overlay and trending feature of the program allows quick identification of samples that produce different glycan profiles for further analysis 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.

ThermoFisher SCIENTIFIC