



Rapid Analysis of N-Glycans on the LabChip GXII Microchip-CE Platform

Abstract

A high throughput microchip-CE method on the LabChip GXII has been developed for profiling N-glycans from recombinant IgG antibodies. In this method PNGase F-released N-glycans in a 96-well microtiter plate are fluorescently labeled by hydrazide reaction in the presence of released antibodies. The labeled samples within the plate are then introduced onto the microfluidic chip through a sipper by applied vacuum. Once on the chip, the sample is electrokinetically injected and separated in a 20 mm long channel filled with polymer solution. The method achieves adequate separation of all major glycan peaks in 60 seconds or less. We describe the derivatization protocol and show profiles of released N-glycans illustrating the resolution, the speed, and ease of use for high throughput screening in early stage development.

Introduction

Glycan Profiling: Background

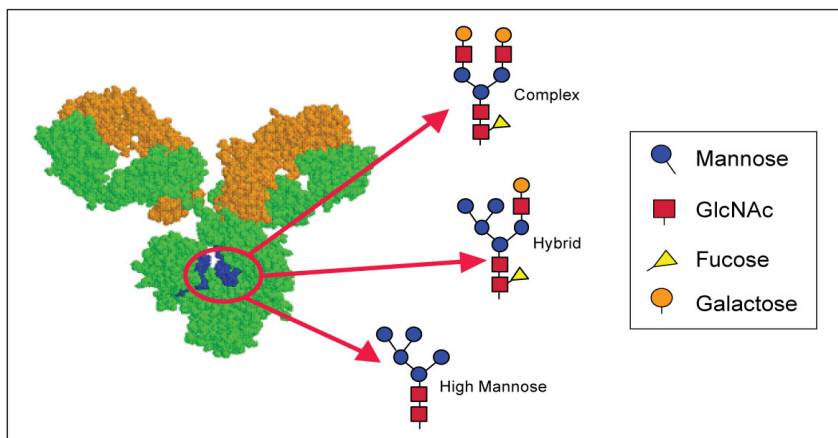


Figure 1. Antibody N-linked Glycans

- Glycans N-linked to the Asn-X site in the Fc region of the antibody have been shown to affect pharmacokinetics, efficacy, and safety of therapeutic rMabs¹
- Operational parameters for antibody manufacturing (media, pH, temperature) can affect the glycosylation pattern (glycosylation site occupancy, degree of branching, linkages, sialylation, etc)

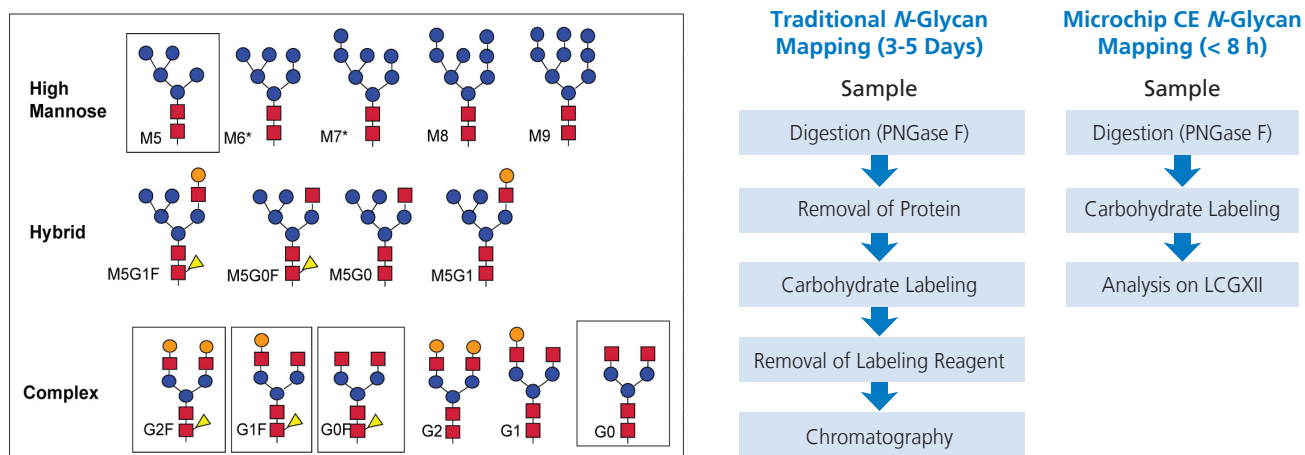
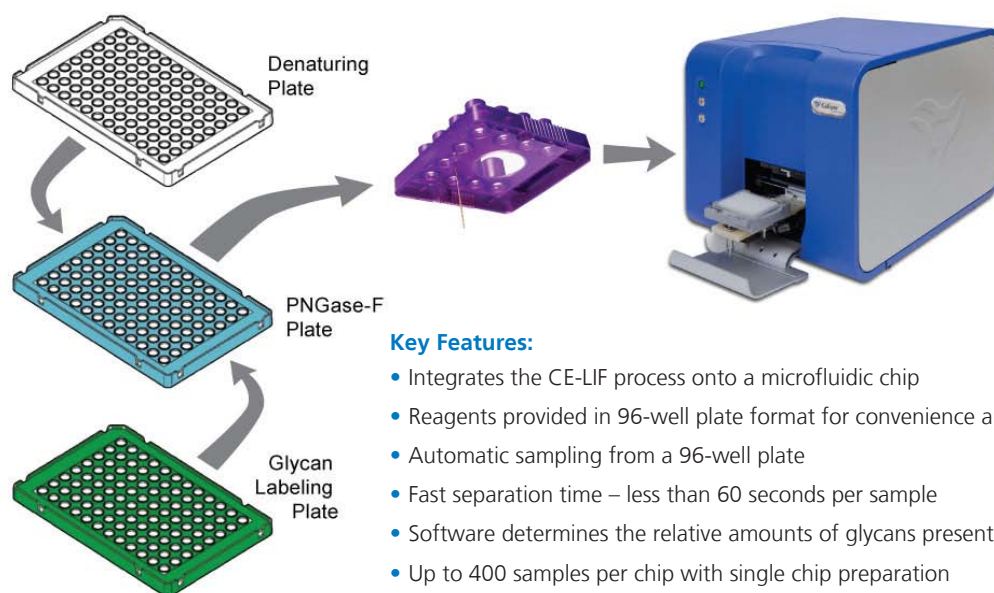


Figure 2. IgG *N*-Glycans. The five main glycans are identified via Microchip

LabChip GX Microchip Electrophoresis System



Materials and Methods

Glycan Profiling Protocol

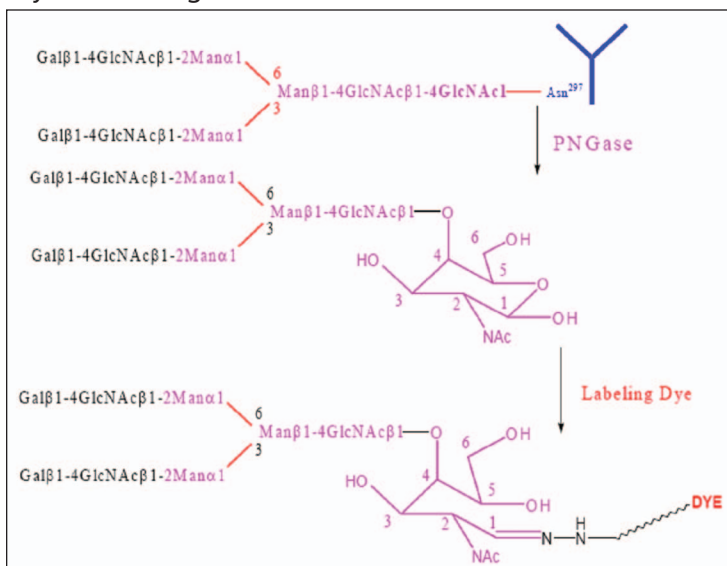
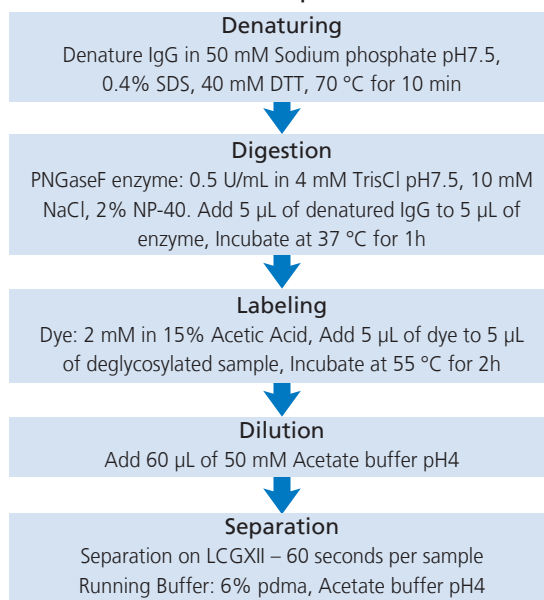


Figure 3. Schematic of glycan digestion and labeling

Workflow of Microchip-CE Method



Results

Glycan Release

- The HT Protein Express 200 assay was run on the LabChip GXII as a control to test for the level of deglycosylation. Under reducing conditions there is a complete shift from the HC to the NGHC.

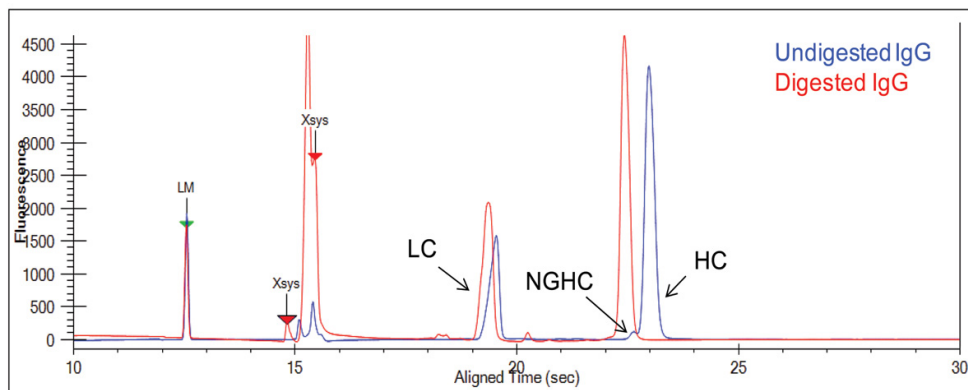


Figure 4. Electropherogram showing complete digestion by PNGaseF after 1h

Labeling of Standards

- Individual glycan standards purchased from ProZyme show good resolution of the five main glycans
- Standards were then used to identify the peaks in the IgG glycan profile

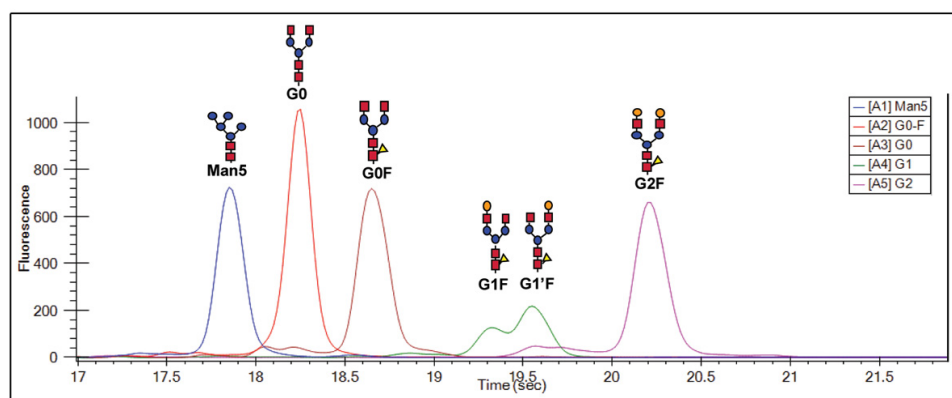


Figure 5. Overlay electropherogram of the five main glycan standards

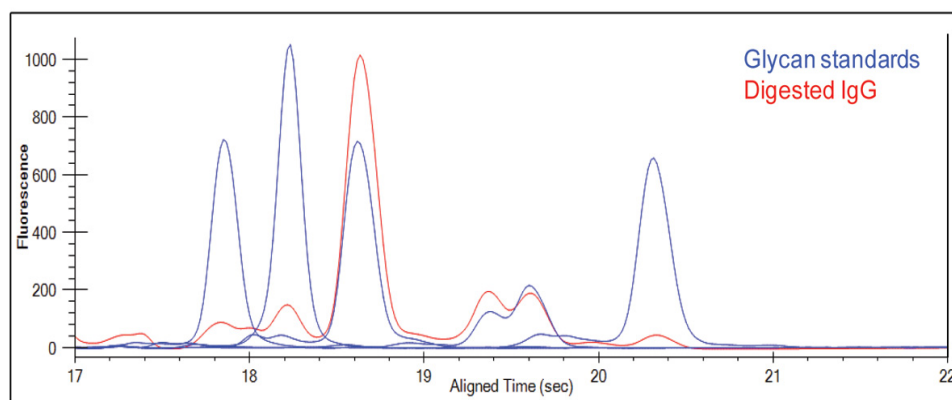


Figure 6. Overlay electropherogram of glycan standards and IgG glycan profile

Reproducibility of IgG Digest and Labeling

- The relative standard deviation (RSD) was calculated for six reactions (complete from deglycosylation to labeling and separation)
- RSD's for the five main glycan peaks were all below 4%

Peak	Sugar	Relative Amount (%)	RSD (%)
1	Man5	3.6	2.7
2	Peak2	1.7	3.4
3	G0	6.5	1.8
4	G0F	54.9	1.0
5	G1F	13.3	2.5
6	G1'F	13.9	1.6
7	Peak7	1.9	6.4
8	G2F	4.3	3.1

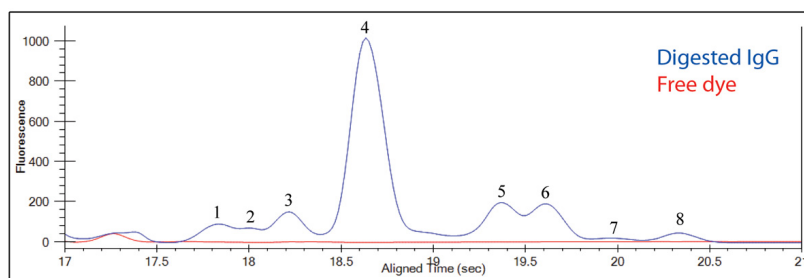


Figure 7. IgG glycan profile with corresponding RSD values

Effect of Digestion Time

- Complete deglycosylation has been shown for a 1h digestion time

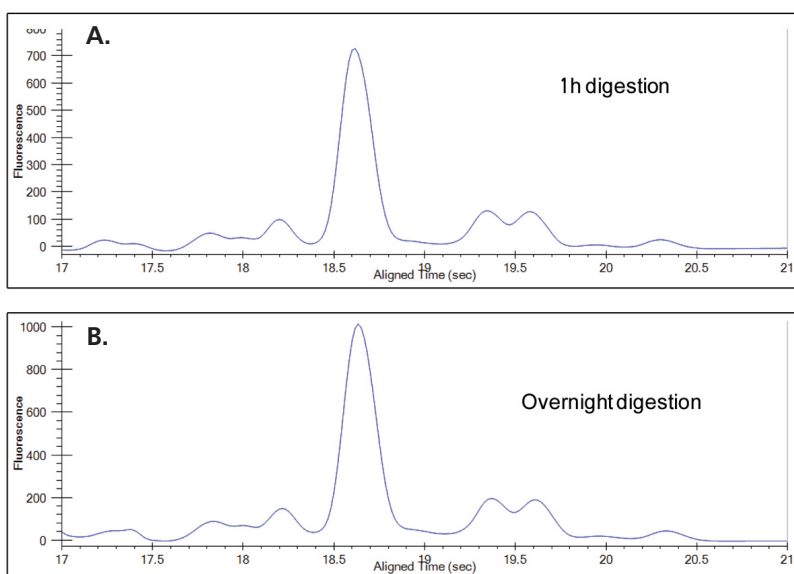


Figure 8. IgG glycan profile for A: 1h digestion, B: overnight digestion

Effect of Digestion pH

- Deglycosylation was tested using buffers over the pH range of 4-8
- All buffers tested were kept at constant ionic strength (100 mM)
- Acetate was used for pH4,5; Citrate for pH6; Phosphate for pH7.5, and TAPS for pH8
- There was no effect to the glycan profile due to pH at the digestion step (Data not shown)

Conclusion

- A microchip-CE method has been developed for profiling N-linked glycans
- The five major glycan peaks are easily resolved in less than 60 seconds per sample
- Assay precision is <4% for the major glycan peaks
- Reagents are provided in a 96-well plate format for ease of use and automation

References: ¹Jefferis R. *Biotechnol. Prog.* 2005, 21, 11-16