# TN0004 Applicability of CIM® Protein G 96-monolithic plate (Pores 2 um) for glycosylation analysis of human IgG isolated from blood

Chromatographic applications in diagnostics call for automated systems and high-throughput analyses in order to cope with the large numbers of samples. BIA Separations offers differently modified monoliths (ion exchanging, affinity, hydrophobic…) in 96-well plate format to follow the increasing needs of DIAGNOSTIC laboratories. The example described below is an affinity-based CIM® Protein G 96-monolithic plate (Pores 2 um), which enables efficient and robust capture of different antibodies from complex samples. The application describes the capture of immunoglobulin G (IgG) from human plasma with subsequent IgG glycosylation studies. The stability of the column for at least 70 isolation steps and proof of no cross-contamination are shown.

Immunoglobulin G is one of the most abundant glycoproteins in human plasma. Its role is to defend the organism by recognizing foreign antigens. Glycosylation is a co-translational and post-translational modification of proteins, essential to their structure and function. It has been shown that glycosylation of IgG significantly affects its function depending on the structure of bound glycans[1,2]. Glycomics studies on large numbers of samples (High-throughput studies) can provide an insight into interindividual glycosylation variability (population studies) as well as glycosylation changes in disease (case/control studies).

## METHOD

| | |
|---|---|
| Plate: | CIM® Protein G 96-monolithic plate (Pores 2 um) |
| Plate conditioning: | 10 CV ultra-pure water (18 MΩ cm at 25 °C)<br>10 CV phosphate buffer saline (1xPBS), pH 7.4<br>5 CV 0.1 M formic acid, pH 2.4<br>10 CV 10x PBS<br>20 CV 1x PBS pH, 7.4 |
| Sample load: | 100 µL of plasma/serum sample, 7x diluted with 1x PBS, pH 7.4 |
| Washing: | 3x 10 CV 1x PBS, pH 7.4 |
| IgG elution: | 5 CV 0.1 M formic acid, pH 2.4 |
| IgG neutralisation: | 1 M ammonium hydrogen carbonate to pH 7.0 |
| Plate regeneration: | 5 CV 0.1 M formic acid, pH 2.4<br>10 CV 10x PBS<br>20 CV 1x PBS, pH 7.4<br>10 CV storage buffer (ethanol $\varphi$ = 20 %, 20 mM Tris, 0.1 M NaCl, titrated with HCl to pH 7.4) |
| Storage | 4 °C |

Around 150 µg of IgG from neutralised elution fraction was dried and deglycosylated. Free glycans were labelled with 2-aminobenzamide and analysed after clean-up by hydrophilic interaction liquid chromatography-ultra performance liquid chromatography (HILIC-UPLC) using Waters ACQUITY UPLC glycan BEH amide column (Scheme 1, Figure 1 ).


*Scheme 1: Schematic presentation of sample processing and analysis*

Protein G 96-well monolithic plates enable fast, reproducible and specific isolation of human (and murine) IgG from blood plasma or serum. This technology represents an essential tool for high-throughput IgG glycosylation analysis workflow.

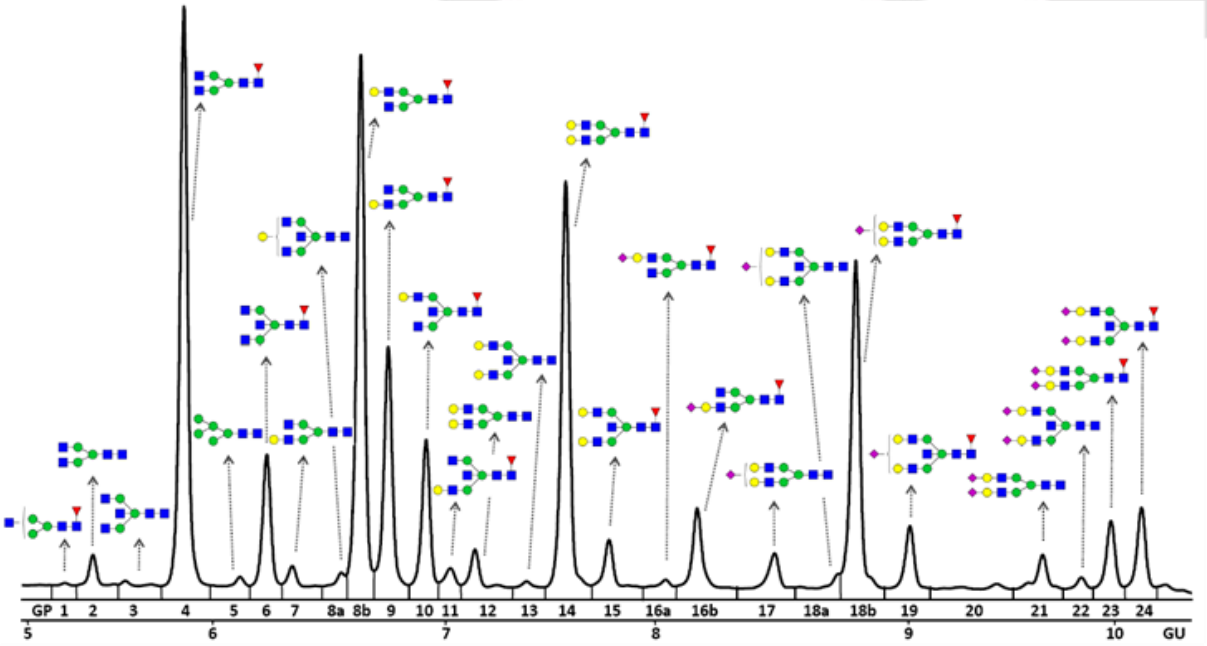


***Figure 1. Chromatographic profile of IgG N-glycans separated into 24 chromatographic peaks (GP1-GP24) by HILIC-UPLC. Major glycan structures in each peak have been shown. N-acetylglucosamine - blue square, mannose - green circle, galactose - yellow circle, sialic acid - purple diamond, core fucose - red triangle[3].***

Reusability and life-time studies have shown that Protein G monolithic plates are stable for several years and can be re-used up to 60-70 times for IgG isolation from human plasma without significant loss of binding capacity. Figure 2 shows the chromatographic profile of IgG N-glycans from Diagene standard during 8$^{th}$ and 47$^{th}$ use of protein G plate. The matching glycan elution profiles indicate high stability of the column.

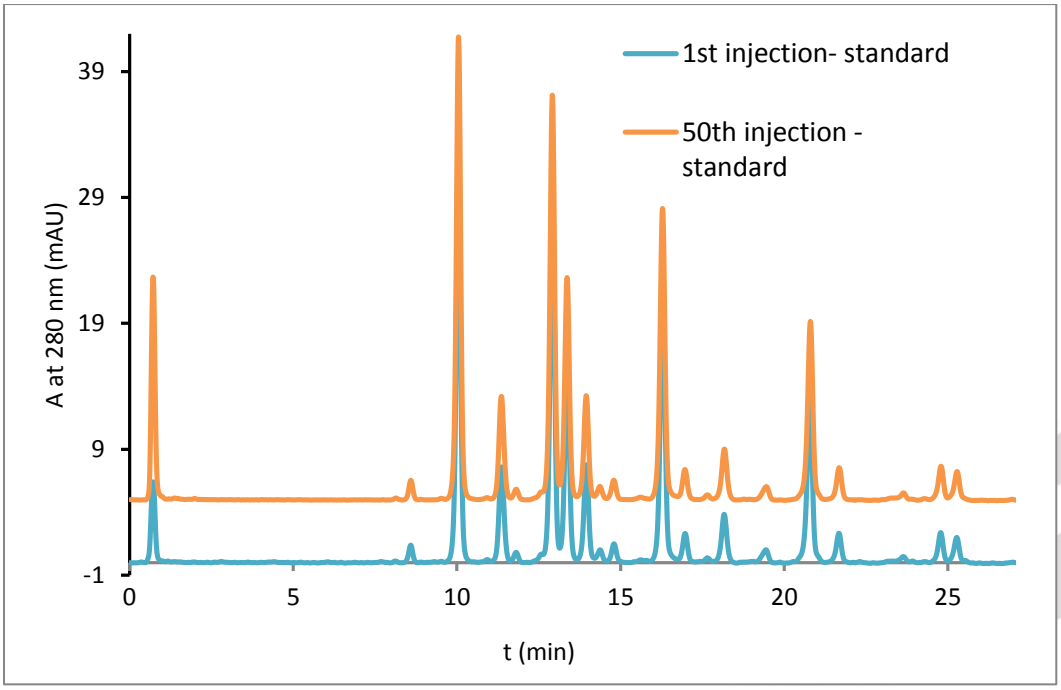


*Figure 2: Chromatographic profile of IgG N-glycans from Diagene standard in 8th and 47th use of protein G plate.*

Additionally, random blank runs (injection of buffer) were performed throughout the lifetime of the CIM protein G 96-well plate on random well positions in order to evaluate the cross-contamination level. The elution fractions from blank runs were treated in the same way as with other samples and a glycan profile from UPLC anaylsis was recorded. As it is obseved from Figure 3, no glycan structures are present in the blank samples, neither after first isolation cycles nor after 70 cycles. This is an indication of no leaching of IgG or other contaminating glycoprotein.

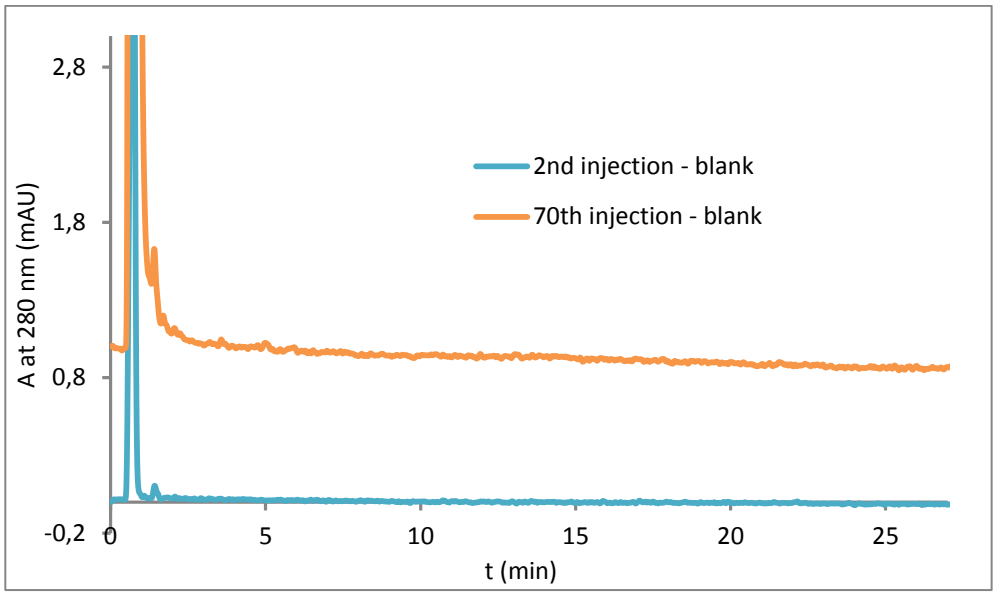


*Figure 3: Chromatographic profile of IgG N-glycans from blank samples in 2nd and 70th use of protein G plate.*

## CONCLUSIONS

An example of high throughput analysis using CIM 96-well plates was demonstrated.

CIM protein G 96-well plate enables fast and efficient capture of IgG from different complex samples.

No cross-contamination between the subsequent isolation steps was observed.

The affinity-based CIM protein G plate could be reused more than 50 times without losing the chromatographic properties.

## REFERENCES


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3. M. Pucic, A. Knezevic, J. Vidic, B. Adamczyk, M. Novokmet, O. Polasek, O. Gornik, S. Supraha-Goreta, M. R. Wormald, I. Redzic, H. Campbell, A. Wright, N. D. Hastie, J. F. Wilson, I. Rudan, M. Wuhrer, P. M. Rudd, D. Josic i G. Lauc, Mol. Cell. Proteomics 10 (2011) M111.010090–M111.010090.


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