Evaluation of different PNGase F enzymes in immunoglobulin G and total plasma N-glycans analysis

Marija Vilaj¹, Gordan Lauc¹,², Irena Trbojević-Akmačić¹

¹Genos Glycoscience Research Laboratory, 10000 Zagreb, Croatia
²University of Zagreb, Faculty of Pharmacy and Biochemistry, 10000 Zagreb, Croatia

SUPPLEMENTARY MATERIAL
Supplementary Figure 1. Ultra-performance liquid chromatography (UPLC) profiles with major 2-aminobenzamide labeled N-glycan structures identified by LC-MS/MS analysis in each chromatographic peak as previously described (Pućić et al. 2011; Zaytseva et al. 2020).

(a) Typical human immunoglobulin G N-glycome separated into 24 chromatographic peaks.

(b) Typical human total plasma N-glycome separated into 39 chromatographic peaks.
Supplementary Figure 2. Comparison of three different PNGase F protocols/enzymes on different amounts of immunoglobulin G (IgG). IgG N-glycome chromatographic profiles are represented by average %Area values and standard deviations (n = 8) for each N-glycan chromatographic peak. Deglycosylation with different PNGase F enzymes and protocols results in strikingly different IgG N-glycome profiles quantification.
Supplementary Figure 3. Comparison of three different PNGase F protocols/enzymes on different amounts of immunoglobulin G (IgG). Representative IgG N-glycome chromatographic profile is shown for each condition. Chromatograms are scaled according to chromatographic peak 4. Deglycosylation with different PNGase F enzymes and protocols results in strikingly different IgG N-glycome chromatographic profiles.
Supplementary Figure 4. Comparison of three different PNGase F protocols/enzymes on plasma sample. Representative plasma N-glycome chromatographic profile is shown for each condition. Chromatograms are scaled according to chromatographic peak 20. Deglycosylation with different PNGase F enzymes and protocols results in strikingly different plasma N-glycome chromatographic profiles.
**Supplementary Figure 5.** Comparison of three different PNGase F protocols/enzymes. (a) Reproducibility of IgG N-glycome quantification represented by %Area coefficient of variation (CV) values for each of 24 chromatographic N-glycan peaks. (b) Reproducibility of total plasma N-glycome quantification represented by %Area CV values for each of 39 chromatographic N-glycan peaks. Differential reproducibility of early and late eluting plasma N-glycan peaks quantification was observed with different PNGase F enzymes.
Supplementary Figure 6. Comparison of two recombinant PNGase F enzymes (E1 – Promega, E2 – NEB) in combination with two protocols/denaturing conditions (P1 – in house protocol, P2 – recommended NEB protocol modified to be comparable with P1 in terms of sample amounts and reaction volumes) on different amounts of immunoglobulin G (IgG). IgG N-glycome chromatographic profiles are represented by average %Area values and standard deviations (n = 8) for each N-glycan chromatographic peak. Deglycosylation with different recombinant PNGase F enzymes and protocols results in different IgG N-glycome profiles/quantification.
Supplementary Figure 7. Comparison of two recombinant PNGase F enzymes (E1 – Promega, E2 – NEB) in combination with two protocols/denaturing conditions (P1 – in house protocol, P2 – recommended NEB protocol modified to be comparable with P1 in terms of sample amounts and reaction volumes). (a) Reproducibility of IgG N-glycome quantification represented by %Area coefficient of variation (CV) values for each of 24 chromatographic N-glycan peaks. (b) Reproducibility of total plasma N-glycome quantification represented by %Area CV values for each of 39 chromatographic N-glycan peaks. Deglycosylation with different recombinant PNGase F enzymes and protocols results in different reproducibility of IgG and plasma N-glycome quantification.
REFERENCES
