COMPREHENSIVE CHARACTERIZATION OF ANTIBODY QUANTITIES, ISOTYPES, SUBCLASS AND GLYCOSYALTION

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Antibodies are a key element of adaptive immunity, critical to its role in protection against pathogens. As glycoproteins, antibodies are a prime example of the diversity of attributes that are regulated by glycosylation, such as effector functions, immune tolerance, structural integrity and half-life. Antibody glycosylation is also a critical element in the efficacy and safety of various therapies, including vaccination. Antibody responses have multiple structural and functional layers interacting in a complex way to achieve an efficient, but appropriate immune response. Levels, class, subclass, and glycosylation of antibodies are structural features that interact extensively to determine antibody effector functions, such as cellular cytotoxicity, phagocytosis, and complement activation.
Over more than a decade, we have developed and refined an LC-MS platform for subclass-specific glycosylation analysis of antigen-specific antibodies which we recently termed GlycoLISA [1]. It combines the efficiency and wide availability of ELISA-type immunosorbent assays with the superior structural resolution of LC-MS. We will showcase the latest continuous development of GlycoLISA for clinical and (bio)pharmaceutical bioanalysis. We can now simultaneously analyze IgG, IgA and IgM glycosylation in a protein- and site-specific manner. Through the implementation of stable isotope labeled protein standards, quantity, subclass, and glycosylation of an immunoglobulin G (IgG) response can now be determined in a single measurement [2]. This allowed us to comprehensively compare vaccination responses, for example between healthy controls and patients with inborn errors of immunity in the context SARS-CoV-2.
In conclusion, a comprehensive coverage of levels, class, subclass, and glycosylation has great potential to further our understanding of dynamics, regulation, and impact of antibody-mediated immune responses. This knowledge can in turn guide therapeutic interventions and help to assess their efficacy.

References:
1. D. Falck and M. Wuhrer, *Nat. Protoc.* **2024**, *19(6)*, 1887-1909.
2. S. Gijze, A. Wasynczuk, L. van Leeuwen, M. Grobben, M.J. van Gils, J. Nouta, W. Wang, V.A.S.H. Dalm, H. Jolink, M. Wuhrer, and D. Falck, *J. Proteome Res.* **2024**, *23(12)*, 5600–5605.