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# Advanced IgG subunit characterization employing nanoRPLC-MS

IgGs mediate diverse immunological responses by binding antigens via their Fab and interacting with downstream receptors (e.g., Fcγ) via their Fc region, activating different pathways. Their remarkable variability originates from the B cell differentiation and activation in response to antigens, resulting in an immense repertoire of different antigen-specific clones. In addition, the immune system can fine-tune the immune response by using an armamentarium of different Fc characteristics.
This highlights the necessity to characterize IgGs. The large heterogeneity of endogenous antibodies requires highly sensitive methods to tackle critical clinical samples. Therefore, we developed two miniaturized methods focusing on the intact subunit level with enhanced sensitivity: one for Fc/2 and one for IgG1 Fab analysis. Polyclonal IgGs are purified from the serum of single donors using Fc-specific beads. Subsequently, depending on the focus of the analysis, IgGs are subjected to either below-hinge cleavage or above-hinge cleavage for intact Fc/2 or Fab characterization, respectively. For the analysis of IgG subunits - Fc/2 and Fab - separation is conducted by employing a miniaturized RPLC-MS-based method, with a flow rate of 1 µL/min. Furthermore, a CaptiveSpray source with dopant gas is used to enhance the sensitivity and the ionization efficiency. This strategy enables us to achieve high sensitivity while we can analyze intact Fc/2 from only 1 µL of serum and intact Fabs from 10 µL.
When applied to clinical samples, this method provides a comprehensive analysis of the Fc/2 at the proteoform level, providing information on subclasses, allotypes, and glycoforms. On the other hand, Fab profiling offers insights into clonality and potential post-translational modifications (PTMs).

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## User consent

yes

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