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EXTENDED ABSTRACT

# Establishment and characterization of a new ELISA for selenoprotein P ☆

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The accurate quantification of selenoprotein P (SePP) is of growing interest for basic research and clinical studies in a variety of areas. Currently, there is some discrepancy on SePP concentrations in humans. Two major reasons contribute to this inconsistency; the characterization and validation of some (even commercial) SePP assays is marginal or missing and there is no uniform reference material for standardization.

Out of the need to compare clinical results across research groups we decided to develop a monoclonal antibody-based enzyme immunoassay according to highest standards of laboratory-developed molecular assays suitable for 96-well analysis (DeSilva et al., 2003). The assay procedure is optimized to follow a standard enzyme-linked immunoassay protocol and uses a chromogenic detection method available in most laboratories. The assay standard curve is calibrated against NIST SRM 1950 standard reference plasma (Ballihaut et al., 2011, 2012).

Multi-laboratory validation tests were performed according to international guidelines (FDA, 2001). The accuracy was measured with an average deviation from the true concentration of +2.9% RE. Precision was determined at three levels: repeatability (with-in plate variation), intermediate precision (within-laboratories variation), and reproducibility (between-laboratories variation), with coefficients-of-variation (CV) each below 15%. The limit of quantitation was less than 10 μg/l, i.e., around 500-fold below average serum SePP concentrations of well-supplied subjects. The signals were linear on dilution within the working range of the assay, and SePP was stable in serum for 24 h at room temperature.

The analytical performance characteristics of this ELISA indicate that it is suitable to provide comparable results for multi-laboratory studies with clinical samples. We have thus decided to make this assay commercially available in order to support research on Se and SePP status across the different research and clinical disciplines.

In our first measurements using this test more than ten thousand samples have been analyzed. These measurements showed high precision and reproducibility and generated the data needed for risk analysis of colorectal cancer in Western Europeans, which proved to be increased in individuals with relatively low Se status (Hughes et al., 2014), Epub ahead of print.

In summary, the newly developed SePP ELISA based on monoclonal antibodies represents a highly accurate and reproducible quantification of SePP from human body fluids and cell culture experiments. We assume that this novel

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in vitro assay system will prove of high value for future experiments aiming to elucidate the importance of the Se status and selenoproteins in human health and disease.

## Conflict of interest

The authors declare that there is no conflict of interest.

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