Establishment and Characterization of a new ELISA for Selenoprotein P

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Introduction

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.

Method

- The Selenotest ELISA is a chromogenic sandwich enzyme immunoassay using two high-affinity monoclonal antibodies.
- The Antibodies were prepared in mice by immunization against a recombinant full-length selenoprotein P.
- The selenocysteine of the recombinant selenoprotein P - produced in baculovirus expression system - were substituted for cysteine.
- The Selenotest ELISA is calibrated against NIST SRM 1950 Standard Reference Plasma [1,2].
- The Selenotest ELISA uses a TMB-based detection system which can be measured at 450nm after stopping the enzymatic reaction.
- The Selenotest ELISA is validated by measuring more than 10,000 healthy and pathological human samples.
- The Selenotest ELISA is validated for use of diluted human serum as sample.
- The working range goes from 10-400 µg SePP/L (depending on actual calibration).
- Limitations: The ELISA is not yet validated for cell culture, tissue extracts, blood plasma or other fluids as sample matrices.

Validation

Selenoprotein P was isolated with 113% recovery from 37ml human plasma by affinity chromatography with the detection antibody of the Selenotest ELISA. 19µg of the eluate were separated by 10% SDS-PAGE and transferred to a PVDF-membran. The blot were stained in Ponceaull. The bands were cut out and human Selenoprotein P was confirmed by N-terminal Edman amino acid sequencing (Proteome Factory AG).

Figure 1 shows 3ng SePP of the plasma load, the flow-through and the eluate of the purification analyzed by Western Blot.

Accuracy (Trueness)

The closeness of the measured concentration to the nominal reference concentration expressed as average %RE: specification: ±15-20% RE (depending on reference) determined by 3 operators (2 plates per operator) on 3 days: ±2.9% RE (operator 1 = 0.4%, operator 2 = 9.6%, operator 3 = 0.4%).

Precision

The closeness of individual measurements of the same homogeneous sample expressed as relative standard deviation (RSD) or coefficient of variation (CV).

There are 3 levels determined by 3 operators (2 plates per operator) on 3 days:

- Reproducibility (intra-assay, intrabatch or within-run precision; here: with-in plate precision)
  - Precision under identical procedure conditions (w/ in one plate):
    - specification: ±15-20% CV (depending on reference)
    - determined: 6.2% (operator 1 = 6.2%, operator 2 = 6.6%, operator 3 = 5.7%)

- Intermediate Precision (inter-assay, interbatch or between-run precision)
  - Variation in one laboratory: 3 different days, 3 different operators:
    - specification: ±15-20% CV (depending on reference)
    - determined: 10.5% (laboratory 1 = 11.1%, laboratory 2 = 9.9%)

Reproducibility

Variation between different laboratories:

- specification: ±15-20% CV (depending on reference)
- determined: ≤11.3%

Linearity

Serial dilutions in sample buffer within the working range of the standard curve of 6 serum samples were tested. The back calculated concentrations were all within 20% of the nominal concentration.

Stability of the analyte in serum

- Stability of the analyte in RT
  - serum at RT
- Stability of selenoprotein P in DMEM/F12 supernatant
  - DMEM/F12
- Stability of selenoprotein P in DMEM/F12 + 10% FCS supernatant
  - DMEM/F12 + 10% FCS

Application of the method for in vitro studies

SePP can be determined from cell culture media. However, dilution linearity is not yet given with cell culture samples.

References


Keywords

450nm

serum

NIST SRM 1950 reference material

selenium

ELISA

selenoprotein P

Summary and Conclusion

The analytical performance characteristics of this ELISA indicate that it is suitable to provide comparable results for multi-laboratory studies with clinical samples. We 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.