Providing an analytical platform for state-of-the-art absolute apolipoprotein quantification in human blood plasma using prm-PASEF

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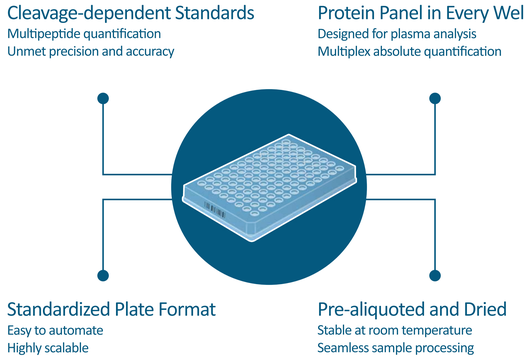
Introduction

With proteomics making a move into precision medicine, there is an increasing pressure on quantitative data quality in studies of blood plasma profiles. Despite the endeavors to develop new data-analysis pipelines, options for improving quantification results early on and prior to the sample preparation are limited. Heavy labeled protein standards such as Quantitative Recombinant Protein Standards (qRePS™) are promising solution to this challenge with the potential to ensure precise quantification that is clinically translatable when combined with targeted mass spectrometry. Apolipoproteins are proteins that have broad diagnostic importance in cardiovascular risk assessment, identification of lipoprotein abnormalities, monitoring treatment efficacy and genetic disorders.

The design of Quantitative Recombinant Protein Standards (qRePS™). qRePS are internal standards co-digested with samples providing high precision and accuracy in absolute quantification.

2. Materials and Methods

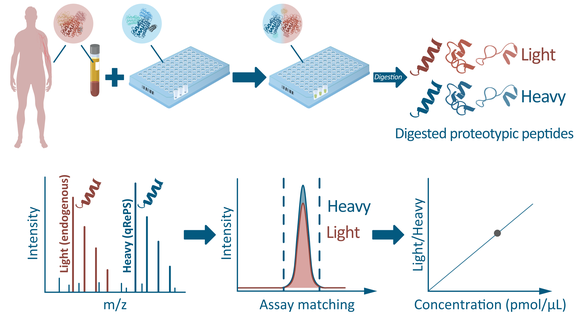
ApoEdge™ (ProteomEdge AB, Sweden), a panel of qRePS targeting all human apolipoproteins, was utilized with prm-PASEF, a powerful targeted mass spectrometry technique, to develop a reference target assay for absolute quantitation of apolipoproteins in human blood plasma.



Each ProtoemEdge product is packed with features in ready to use 96-well plate format. The design allows for the blood plasma and all the reagents to be added into the same plate.

3. Absolute Quantification Workflow

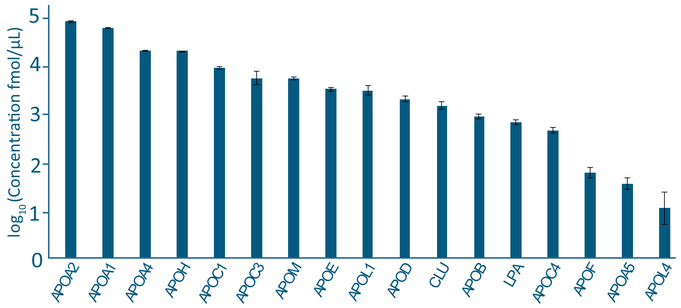
One microliter of blood plasma was diluted and added to dry panel of ApoEdge followed by reduction, alkylation and digestion. Peptide mixture was analyzed using timsTOF HT (Bruker, Germany) operating in prm-PASEF mode. Extracted chromatograms were overlapped and absolute concentrations of target proteins calculated.



ProteomEdge quantitative workflow starts by addition of blood plasma onto a multiplex panel of qRePS followed by digestion and LC-MS/MS analysis providing targeted absolute quantification.

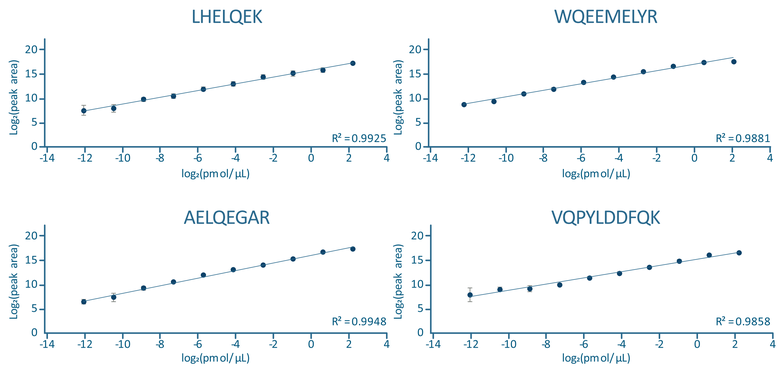
4. Results

The final PRM assay was developed using dia-PASEF workflow within SpectroDive (Biognosys, Switzerland) and contained 57 peptides. Data was acquired on 8 replicates revealing excellent reproducibility of the assay. All ApoEdge target proteins were absolutely quantified in raw blood plasma with a median coefficient of variation of 7%. The absolute quantitative data revealed concentrations of blood plasma apolipoproteins to range from 49.89 pmol/µL down to 0.002 pmol/µL covering 5 orders of magnitude in concentrations.



All human apolipoproteins were absolutely quantified in multiplex using ApoEdge and TIMS-TOF HT operating in prm-PASEF mode targeting 57 peptides.

A dilution curve with 12 calibration points (3-fold dilution series) was generated and measured using prm-PASEF in 6 replicates. Calibration curves were generated for 39 peptides covering all 18 human apolipoproteins. Example calibration curve of labelled peptides from APOA1 are shown.



All human apolipoproteins showed good linear range of response proving great synergy of ProteomEdge internal standards with prm-PASEF workflow.

5. Conclusions and future prospects

The quantitative performance of multiplex panel of internal standards ApoEdge with prm-PASEF was evaluated. All 18 human apolipoproteins were absolutely quantified with great precision and linearity of response. Combining the simplicity and easily automated sample preparation workflow with high precision in absolute quantification, the mutual synergy has a potential in being translated into the clinical practice to replace the current antibody based assays that lack the edge of reproducibility.

Additionally, the high multiplexing capabilities of timsTOF HT and prm-PASEF workflow allowed to initiate a development of a targeted assay for the DiscoveryEdge175, a panel consisting of 177 protein targets including apolipoproteins, complement proteins, coagulation factors, inflamation markers and other clinically interesting markers. The method consists of 346 peptides to be scheduled within a single targeted MS run. Initial experiments showed good coverage of endogenous and labelled peptides with 312 peptides from 173 protein groups being identified and quantified.

The current work outlines a future potential of internal protein standards and targeted mass spectrometry as direction towards clinical application and diagnostics.