Providing an analytical platform for absolute apolipoprotein quantification in human blood plasma

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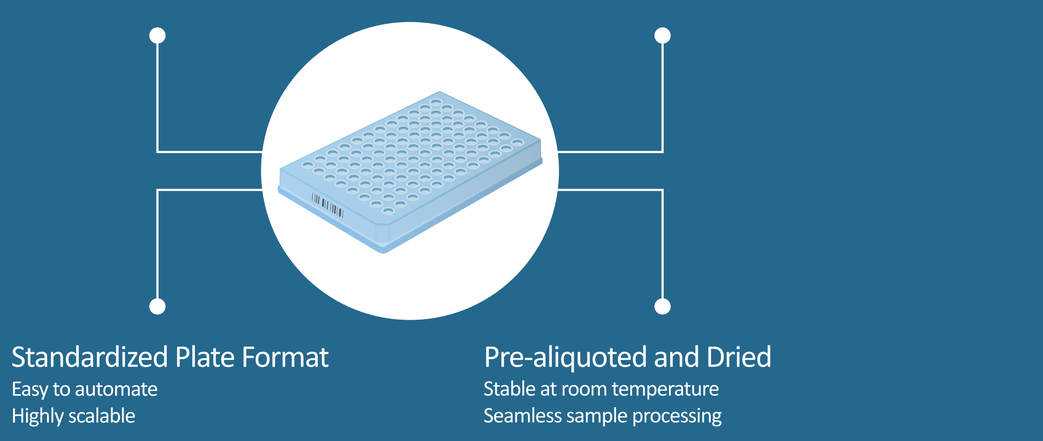
Introduction

With mass spectrometry-based proteomics making a move into personalized medicine, there is an increasing pressure on data quality in studies of blood plasma profiles. The accessibility of AI and its integration will shift the field even further. Nonetheless, a model that is specific and sensitive requires high quality data to be generated, and importantly to be applied with the same quality data at different places and different times for prediction of patients' clinical outcomes. ProteomEdge is a company from Stockholm, providing a solution for this challenge with a proprietary analysis platform for multiplexed absolute quantification. This platform offers prealiquoted and dried multiplex panels of ^{13}C and ^{15}N Lysine and Arginine heavy-isotope labelled Quantitative Recombinant Protein Standards – qRePS™. qRePS are enzymatically cleavable protein standards designed for targets of clinical interest and cover a longer amino acid stretch of single endogenous protein.

The design of Quantitative Recombinant Protein Standards (qRePS) covering multiple proteotypic peptides. qRePS are internal standards that are co-digested with the samples, providing high precision and accuracy in absolute quantification.

Multiplex qRePS panels are distributed vacuum dried and prealiquoted in 96-well plates allowing for direct addition of plasma samples and other reagents on top of the heavy labelled standards. This setup outlines a new strategy for confident results using a variety of experimentally verified peptides, resulting in a previously unmet quantitative precision.

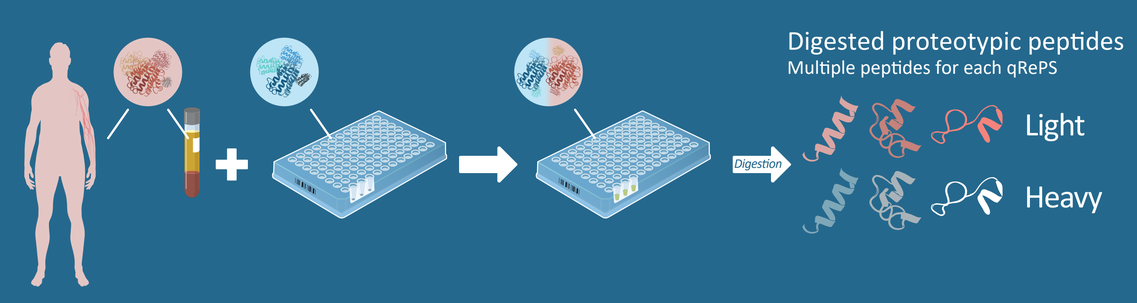


Each ProteomEdge product is packed with features in ready to use 96-well plate format. The design allows for the blood plasma and all the other reagents to be added in the same plate and processed together with qRePS.

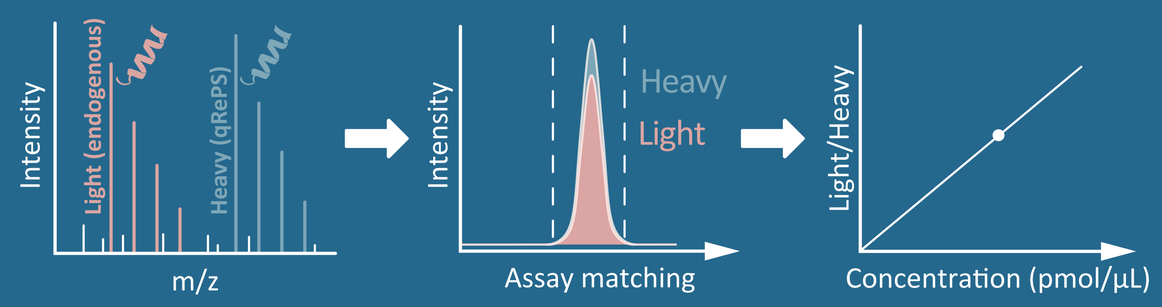
Materials and Methods

Single reaction monitoring (SRM) method targeting all human apolipoproteins was developed using ApoEdge™ (ProteomEdge AB, Sweden), a multiplex panel of 19 qRePS covering all human apolipoproteins. TSQ Altis (Thermo, USA), a powerful targeted mass spectrometry workhorse, and the developed reference targeted proteomics assay was applied to perform absolute quantification in human blood plasma at high sample throughput of 50 samples per day. The ProteomEdge quantitative workflow initiates with addition of diluted equivalent of 1 µL blood plasma into the provided ApoEdge plate followed by consequent addition of all other reagents to perform reduction, alkylation (TCEP + CAA at 90°C, 10 min) and trypsin digestion using automated liquid handling system.

Digested qRePS and plasma mixture was subjected to LC-MS/MS analysis to perform absolute quantification. A single multiplex MS method was used containing a transition list with preselected transitions of Heavy (qRePS) and Light (endogenous) peptides as well as iRT tag peptides which is a natural part of all qRePS. Chromatograms were extracted from the resulting raw files using Skyline (Brendan MacLean et al., 2010) and peptide areas of Heavy and Light signals overlapped to calculate the ratio between them. The Light/Heavy ratios were multiplied by absolute quantities of each qRePS (provided by ProteomEdge) resulting in absolute concentrations of each peptide in pmol/µL of blood plasma.



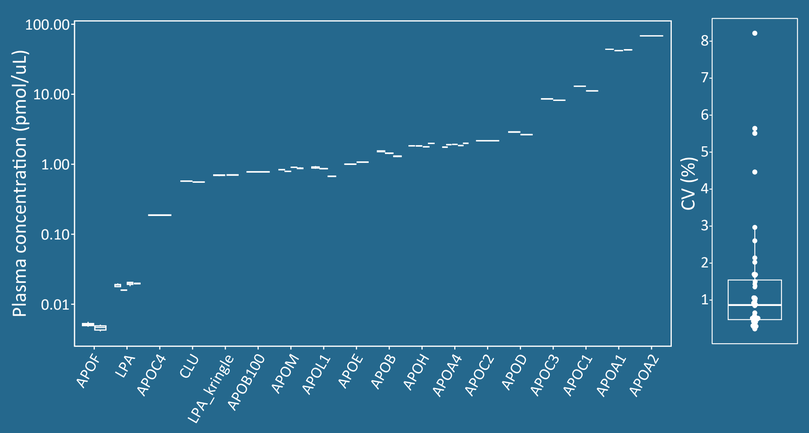
The sample preparation workflow starts by adding blood plasma samples onto a dried panel of qRePS, assuring that the ratio with endogenous proteins is established as the very first step and prior to enzymatic digestion.



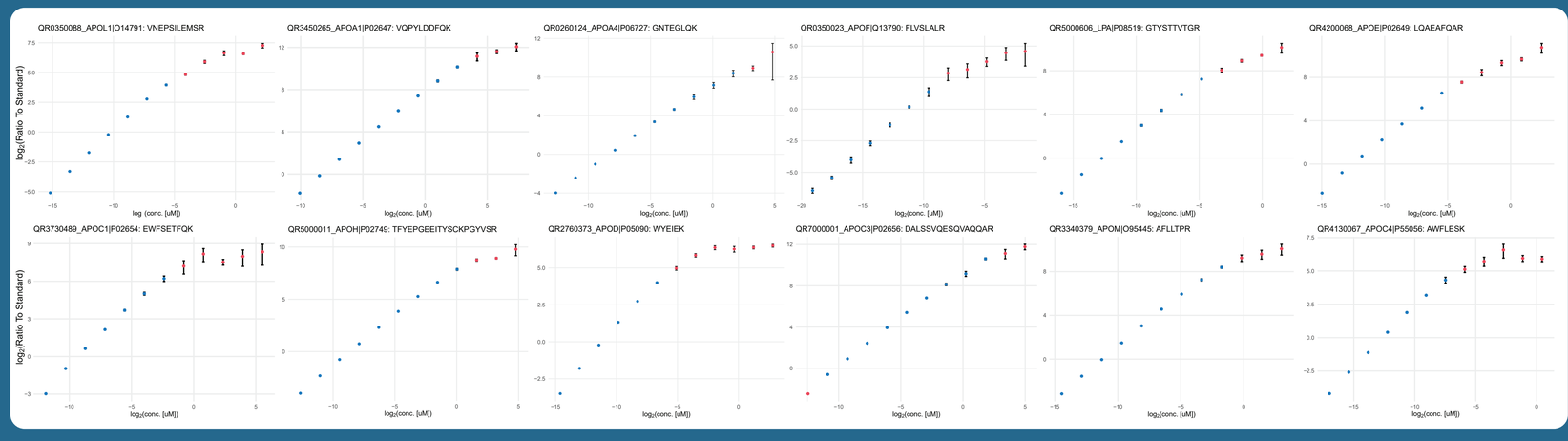
Light and Heavy peptides are analysed using LC-MS/MS and chromatograms extracted to calculate ratios of Light to Heavy peak areas. Resulting ratio is multiplied by provided qRePS quantities to calculate the absolute endogenous concentrations.

Results and Conclusions

All human apolipoproteins except for APOA5 and APOL4 (below LOD) were absolutely quantified with 44 peptides and technical reproducibility with median coefficient of variation CV < 1% (0.22% - 8.2%) over 5 orders of magnitude in concentrations. Additionally, for proteins quantified with multiple peptides, the same results were obtained validating the accuracy of absolute quantification using qRePS. Also, both total APOB and APOB100 and LPA kringle repeats and total LPA were absolutely quantified expanding on the biological and clinical insight into the apolipoproteins.



Absolute quantities of 16 apolipoproteins were measured in multiplex with median coefficient of variation below 1%.



Quantitative linearity was assessed with dilution series of ApoEdge in blood plasma background. Absolute quantification showed high linearity and precision across over 5 orders of magnitude with $R^2 > 0.99$ for all peptides.

- ApoEdge was successfully utilized within an automated sample preparation workflow
- Multiplex reference targeted assay was set up for all human apolipoproteins
- All human apolipoproteins were absolutely quantified in blood plasma except the lowest abundant APOA5 and APOL4
- High precision and accuracy of quantification was achieved over 5 orders of magnitude in concentrations
- This unique and customizable toolbox outlines possibilities for clinical applications, precision medicine and drug development

Find out more at www.proteomedge.com