**Introduction**

Plasma proteome continues to be a good source of biological information to diagnose health and monitor health. Recently, it has been indicated to provide a source and means of providing therapy for age related diseases as well. One of the best tool to quantify the plasma proteome is mass spectrometry based proteomics as it enables discovery of new proteins in the plasma as well as characterization of post-translationally modified plasma proteome. Additionally, it enables protein-protein interaction analysis within the relevant matrix. However, a major challenge in plasma proteomics is the protein dynamic range where close to 60% of the plasma consists of albumin. This challenge exists all the way from plasma preparation, MS analysis, and data analysis (Table 1). Here, we highlight a label-free quantization high-resolution data-independent acquisition (LFQ-HR-DIA) workflow for plasma sample processing using the new Thermo Scientific AcceleOme™ automated sample preparation platform comprising of standardized and optimized software, liquid handler, and reagent kit in combination with Thermo Scientific Vanquish Neo UHPLC system coupled to Thermo Scientific Orbitrap Exploris™ 480 MS system.

**Materials and methods**

Control human plasma samples were aliquoted in an input plate by four different individuals with five aliquots per individual (Table 2) and were processed for mass spectrometry analysis using the AcceleOme platform (Figure 1). Briefly, proteins were lysed, reduced, alkylated, and digested to peptides using AccelerOme LFQ kit. All 20 sample digests were then analyzed by LC-MS/MS. Briefly, ~1μg of peptides were separated using a Thermo Scientific™ EASY-Spray™ 50 cm column on a Vanquish Neo UHPLC system coupled to an Orbitrap Exploris 480 MS (Figure 2).

**Methods**

**MS method**

The samples were analyzed using high resolution data-independent acquisition method. Briefly, the survey scan was acquired using m/z 400-9000 at a resolution of 60K @200mz. The DIA scans were performed at a resolution of 10K @2000mz. DIA scans were acquired using isolation window of 12 m/z and isolated precursor ions were fragmented using higher-collision induced dissociation (HCD) with 30% normalized collision energy (NCE). Figure 4 shows details about the MS parameters.

**Data processing**

DIA raw data files were processed using Spectronaut™ 17 (v17.3.20224.55965) with DirectDIA approach against Human UniProt protein database (20,707 sequences). Quantification was based only on unique peptides with a false-discovery rate (FDR) of 1%. Protein groups were filtered 1% FDR on experiment level. Protein quantities are reported from such filtered protein groups exclusively.

**Results**

HR-LFQ-DIA workflow for plasma sample processing using AcceleOme platform resulted in consistent sample to sample peptide recovery, protein identifications and quantitation with minimal person to person variation.

**Conclusions**

- AcceleOme platform provides consistently high protein alkylation, digestion efficiency and low in vitro artefacts.
- AcceleOme system provides a hands-free solution to overcome this challenge by combining standardized, optimized, and verified hardware, software, and reagents to produce quality samples for plasma proteome quantification.
- AcceleOme platform also enables future development into additional applications for samples preparation for mass spectrometry analysis including high abundant proteins depletion.

**References**

1. Enroth et.al. Protein profiling reveals consequences of lifestyle choices on predicted biological aging. Scientific Reports. 2015 Dec 1;5:17782. PMID: 26719799

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