High-throughput and reproducible workflows to prepare human plasma samples for proteomic analysis

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Recent advances and improvements in mass spectrometry technology have allowed quantification of proteins (potential biomarkers) with desirable sensitivity and selectivity in a complex bio-fluid matrices, such as plasma and urine. This mass spectrometry based absolute quantification has advantages in assay development time, specificity and multiplexing capability for protein analysis. In particular, two LC-MS/MS platforms: Selective Reaction Monitoring (SRM) and SWATH acquisition (Data independent acquisition) workflows provide avenues to simultaneously quantify multiple protein analytes without the need to generate and characterize antibody pairs for immunoassays while allowing for fold change and absolute quantification. Both highly selective/targeted known proteins for absolute quantitative SRM and highly comprehensive quantitative SWATH platforms can potentially be applied in biomarkers discovery and validation, drug response monitoring, drug development, and assessment of disease state progression. In all situations, sample preparation represents a major bottleneck for these protein / peptide-based mass spectrometry techniques, as there are numerous processing steps that are susceptible to technical variation, especially when large sample numbers are to be processed.

Reproducibility of data across large sample numbers is essential for potential biomarkers to be verified for a particular disease or disease state. Developing suitable automation workflows for mass spectrometry based absolute quantification is key. To increase throughput and reduce technical variation, we have implemented an automated peptide preparation protocol on a liquid handling workstation (Biomek NX^P) coupled with an SRM workflow using triple quadruple mass spectrometer (QTRAP® 6500 system). Human samples arrayed in a 96-well format were denatured, reduced, alkylated, and digested with trypsin in a total time of less than two and half hours. In order to obtain highly reproducible data, our workflow incorporates an internal standard to monitor the sample preparation and LC MS analysis: each sample was spiked with β-galactosidase. Lastly, to monitor the instrument day-to-day performance and minimize the instrument drift, we adapted the strategy of blocking samples and running QC samples throughout the analysis. For quality control, samples were desalted online using post-column diversion of the flow-through to waste prior to peptide elution. For

low-abundant proteins, a workflow extension is under development that adds antibody enrichment either prior to or after trypsin digestion.

The complete analysis had a coefficient of variation of less than 10% based on assessment of the internal β-galactosidase standard. Online desalting as a substitute for solid-phase extraction provided the largest contribution to reduced technical variation.

We present automated high-throughput workflows with sample processing and enrichment robotics for accurate and reproducible large-scale analysis of biological/clinical samples. This workflow can be applied to biomarker validation, drug response monitoring, disease state and progress monitoring.