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# Multi-Site Plasma and Serum LC-MS Analysis to Assess Comparability Using Targeted Acquisition and Reference Peptides Covering More Than 500 Proteins

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## Introduction

In modern medicine the analysis of easily accessible blood derived plasma or serum is an important cornerstone in detecting the onset and monitoring the progress of a multitude of diseases. Mass-spectrometry based proteomics is increasingly utilized, due to its inherently high precision in identifying and quantifying analytes. With the number of studies and cohort sizes increasing the comparability between studies is coming more into focus.
We show a state-of-the art LC-MS proteomic workflow for plasma and serum, employing an optimized set of 804 stable isotope standard peptides representative of 582 proteins in combination with the SureQuant quantitation workflow. To assess the comparability, the same set of samples was measured on seven different LC-MS systems, with only partially overlapping instruments set-ups.

## Methods

For the proof-of-concept study a cohort of seven prostate cancer patients and matching healthy controls where plasma and serum was prepared from the same blood draw was used. The cohort consisting of 28 samples plus QC pools was acquired on seven different LC-MS systems, consisting of six Thermo Exploris 480 and a Fusion Lumos in nano-flow. All runs were performed over a two hour non-linear gradient with the same SureQuant method and all systems passed the same QC thresholds. Deviations between the LC-MS systems were columns and column materials, acquisition software versions and queue interruptions. Data analysis was performed fully automated with SpectroDive, based an optimized ion scores composition.

## Preliminary Data

Overall, the acquisition of plasma and serum samples with an optimized set of SIS peptides (PQ500) and SureQuant acquisition showed an excellent measurement depth of more than 480 proteins per sample on average. The absolute amount of the measured peptides calculated from the amount of spiked-in reference peptides correlated very well when the same sample

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was acquired on different LC-MS systems with $R^2$ values of 0.95 and above. When comparing plasma against serum, the proteins found significantly regulated were very stable, basically independent of the LC-MS set-up used. A factor influenced by the instrument was the measurement sensitivity, meaning the number of protein IDs fluctuated by 10 % depending on the LC and MS. The quantitative variation on individual instruments spread between 3 and 6 % with a tendency towards more sensitive instruments leading to lower CVs. Instrument specific effects, like fragmentation patterns leading to interferences in peptide quantifications were observed at a very low rate (less than 0.2 %) and other anomalies, like two distinct peptides leading to different protein quantities could be reproducibly measured across set-ups and therefore determined to be sample specific and not caused by the instrument.

In total we demonstrate the results obtained by the plasma and serum acquisitions can be used as a digital record of the biological samples only marginally influenced by the instrument used to obtain them.

## Novelty aspect

MS acquisition with SIS peptides can create a directly comparable digital record of plasma and serum samples without instrument bias.