BIOGNOSYS
NEXT GENERATION PROTEOMICS

Application Note

REPRODUCIBLE AND CONSISTENT RESULTS IN LARGE-SCALE PLASMA AND SERUM STUDIES WITH **PQ500™ REFERENCE PEPTIDES**

In this technical note you will learn about:

- why plasma and serum are important biospecimens in current clinical practice.
- the challenges large sample cohorts pose in MS-based proteomics applications.
- how SIS peptides and PQ500 help researchers get consistent and reproducible results in multi-site, multi-system setups.

If you have any questions about this technical note or about Biognosys' solutions for targeted proteomics, please contact us at **support@biognosys.com**

INTRODUCTION

Blood plasma and serum are important cornerstones in detecting and monitoring the progression of many diseases. As blood flows through every organ in the body, its protein composition uniquely reflects the body's physiological state. In addition, blood samples are easy to collect, making plasma and serum especially attractive biospecimens when looking for diagnostic biomarkers.

Traditionally, affinity-based assays have been used to detect and quantify biomarkers in plasma and serum. However, these methods lack specificity, and the ability to handle multi-analyte panels. Mass spectrometry (MS)-based proteomics is, therefore, an increasingly popular and superior method as it can simultaneously measure hundreds of proteins and identify and quantify them more precisely than classical approaches. Because of its unbiased nature, MS proteomics is highly suitable for biomarker discovery purposes.

Current biomarker discovery in plasma and serum using MS-based proteomics requires an increasing number of replicates and consequently, sample cohorts are becoming larger. In addition, there is a growing interest in comparing or using data from different facilities. This introduces the need for compiling data generated in different instruments, different sites, and on different dates. However, data compiled in this way reflects the different statuses and types of instrumentation, making it challenging to properly compare them.

One solution to overcome this challenge is the use of stable isotope standard (SIS) peptides. By spiking a known amount of a heavy labeled peptides in the samples, it is possible to normalize for the variability introduced by instrumentation, making data generated directly comparable.

To show the benefits of this approach, we used Biognosys' PQ500™ Reference Peptides kit in a multi-site study where we acquired prostate cancer cohort samples on several independent LC-MS systems. Biognosys' PQ500 Reference Peptides is the most comprehensive and refined peptide kit for plasma proteomics available.

The PQ500™ Reference Peptides kit contains an optimized set of 804 stable isotope standard (SIS) peptides targeting 582 proteins. PQ500 provides a greatly improved comparability across analytical platforms, making it especially helpful in large studies and cohort sizes.

METHODS

Human plasma and serum samples were precipitated using cold acetone, processed to peptides and cleaned up using Sample Preparation Kit Pro (Biognosys AG.). Subsequently, PQ500 Reference Peptides were spiked into the ready-to-inject plasma or serum samples before MS acquisition.

Data was acquired using SureQuant Targeted Quantitation Workflow (Thermo Scientific). The SureQuant PQ500 acquisition method was adjusted to Biognosys' standard acquisition set-up. A SureQuant PQ500 Survey Run was acquired, as pre-programmed on the Thermo Scientific™ Orbitrap Exploris™ 480 MS and processed with SpectroDive (Biognosys AG) to generate analytical methods with different MS intensity thresholds (between 0.1% and 20%).

The method with the best performance (3% MS1 intensity threshold) was used throughout the study and transferred to all seven LC-MS platforms. All acquisitions were performed using two-hour non-linear gradients, phase B (80% ACN) ranging from 1% to 42%. Finally, fully automatic data processing was performed using SpectroDive.

RESULTS

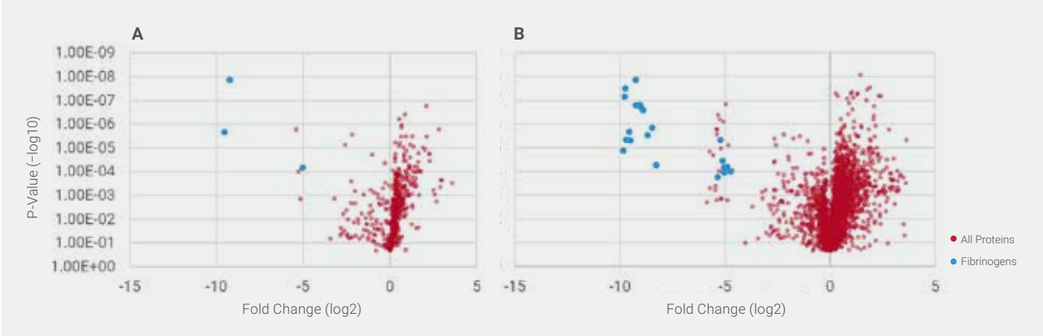
High Repeatability Between Instruments

A comparison of plasma and serum samples from the same blood draw revealed differences between the two sample types. When looking at the results from a single instrument *(Figure 1A)* levels of fibrinogens, clotting factors and certain calcium-binding proteins were significantly different between serum and plasma, as expected. When comparing multiple instruments, a statistically comparable pattern was observed *(Figure 1B)*, indicating high repeatability of the workflow between different analytical platforms.

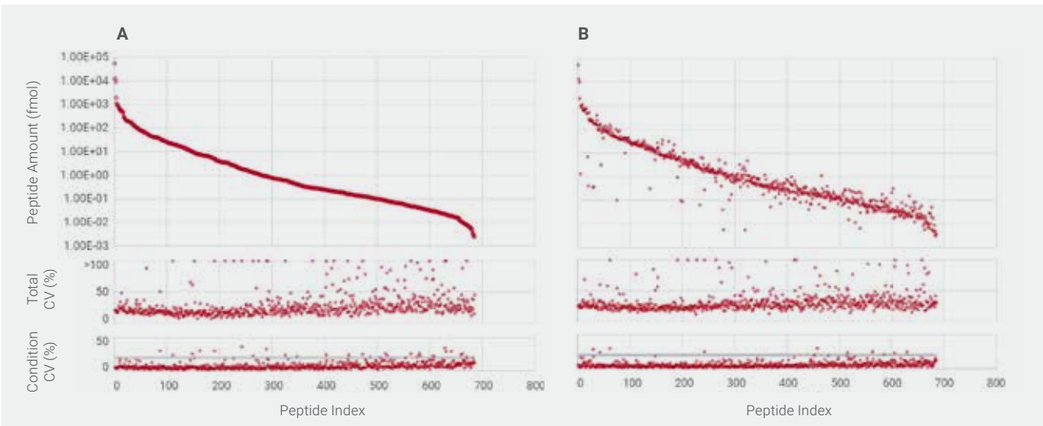
Exceptional Analytical Depth

Analysis of plasma and serum pools showed an excellent analytical depth of an average of 480 proteins (695 peptides) in plasma *(Figure 2A)* and serum *(Figure 2B)* across more than six orders of magnitude in dynamic range. Median coefficients of variation (CVs) ranged from 2.5% to 7.5% per condition and proved excellent quantitative accuracy. Between instruments, variance remained low (average of 30%) with few outliers, indicating instrument-specific effects.

1A — B. **Analysis of Plasma versus Serum from the Same Blood Draw**
Fibrinogens, clotting factors, and certain calcium binding proteins were detected as significantly different, both on a single instrument (A) and multiple instruments (B).



2A — B. **In Plasma (A) and Serum Pools (B),** an Average of 480 Proteins (695 Peptides) were Quantified Across more than Six Orders of Magnitude



Consistent Biological Results Across Acquisition Systems

The Prostate-specific antigen (PSA) is an important biomarker that increases in abundance in prostate cancer patients. PSA was consistently quantified in the plasma of a patient in all LC-MS systems *(Figure 3A)*. Absolute amounts of PSA were measured with high accuracy (CV <20%) in both serum *(Figure 3B)* and plasma samples *(Figure 3C)*, while a higher fluctuation was observed in label-free intensities.

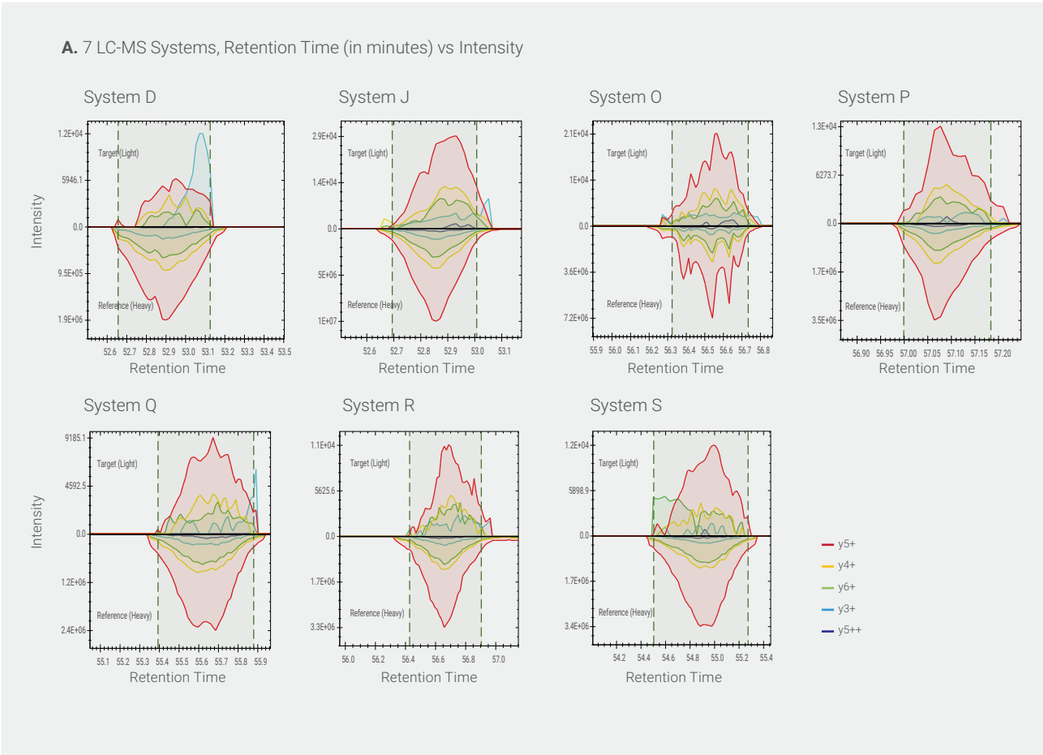
Although PSA increases in prostate cancer patients, overall, it is a very low abundant protein, and its detectability can be influenced by instrument setup *(Figure 3D — E)*. This was observed when the Exploris™ 480 platform and Fusion Lumos platform were compared. SureQuant acquisition on the Exploris™ 480 platform *(Figure 3D)* detected more PSA peptides more consistently than the same method on a Fusion Lumos platform *(Figure 3E)*. However, in both acquisition systems PSA was detected as significantly more abundant in plasma and serum of the patient. The workflow demonstrates that different acquisition systems can effectively detect the difference in abundance of PSA between prostate cancer patients and healthy individuals.

CONCLUSION

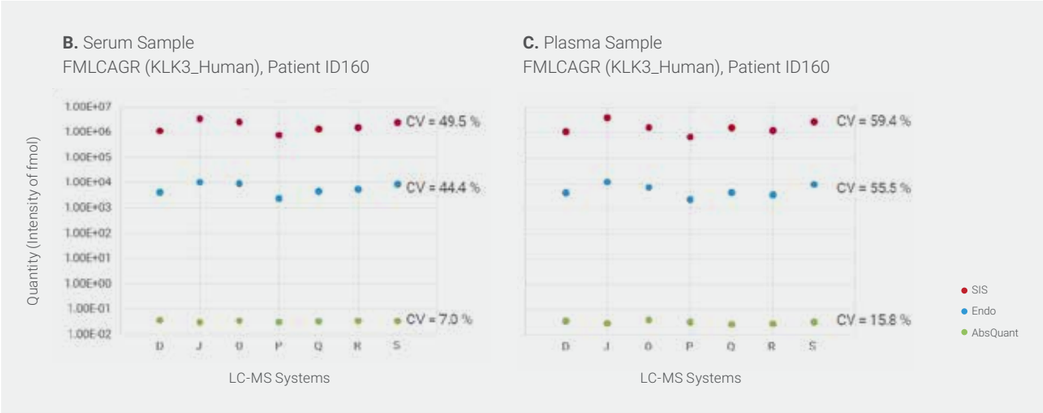
Overall, the consistent results between different LC-MS systems confirm that our PQ500 Reference Peptides kit in combination with targeted proteomics is a powerful tool for large-scale plasma and serum analyses that are spread across multiple analytical platforms.

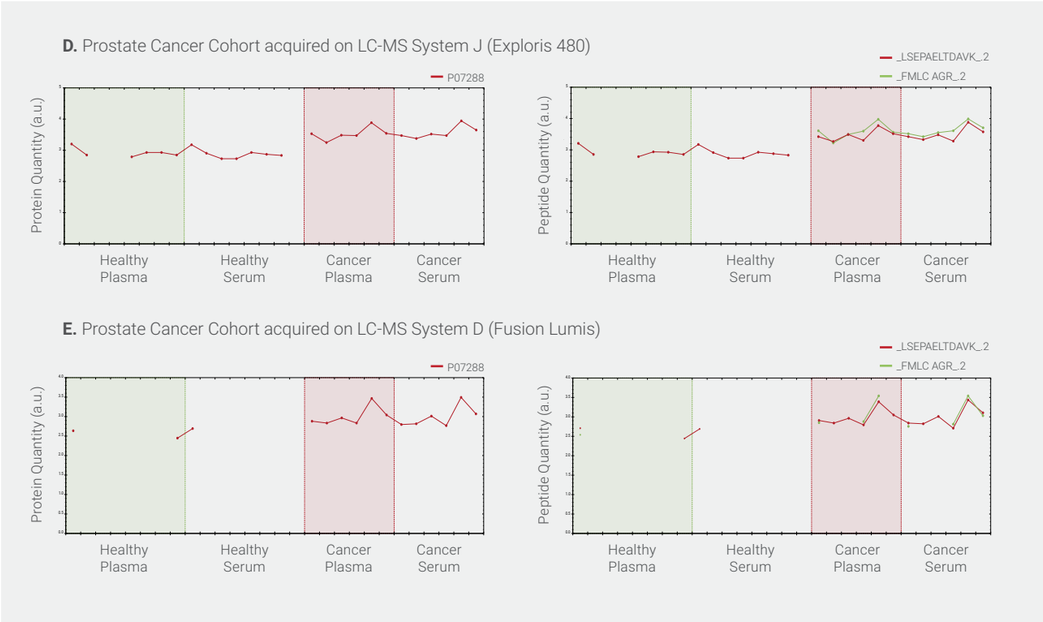
We found that instrumentation only marginally influenced quantitative results and the statistical outcome, while the sensitivity of the analysis was mostly dependent on the MS set-up and performance state.

3A. **Prostate-specific Antigen (PSA)** was Quantified Consistently in a Cancer Patient's Plasma across Seven LC-MS Systems



3B — E. **Absolute amounts of PSA** were measured with a CV <20% in serum (B) and plasma (C). **Detectability can be influenced by instrument setup. Targeted acquisition** on the Exploris480 platform (D) detected more PSA peptides more consistently than the same method on the Fusion Lumos platform (E).





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