Evaluation of blood-based sample types for deep plasma proteomics

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About this application note

New technologies to identify and assess proteins are allowing plasma proteomics biomarker studies to be conducted at greater depth and scale than ever before. Sample collection and processing methods are important for ensuring accurate results from a large cohort study. To evaluate the relative differences and potential advantages of blood-based sample types for large cohort LC-MS based proteomics studies, we compared three sample types: serum, EDTA-plasma, and citrate-plasma. Our results show significantly deeper proteome coverage using Seer's proprietary engineered nanoparticle based Proteograph™ workflow compared to a traditional neat sample digestion workflow. This deeper proteome coverage revealed significant differences between these three blood-based sample types. Therefore, we recommend using a single sample type following the same collection protocol for large cohort studies to minimize the impact of sample collection and processing differences.

Introduction

Serum and plasma are common blood-based sample types used for biomarker discovery studies. While these sample types are similar, the abundance of proteins could be altered depending on the method of collection and processing.[1,2] Proteomics analysis provides valuable insight to explain complex biology, but it remains challenging to collect deep proteome coverage at the scale necessary for large cohort studies. To determine if significant differences or advantages exist between sample types, we undertook this study to characterize the differences between serum and plasma proteomes and assess their suitability for large-scale, unbiased, protein biomarker discovery studies.

We collected matched serum and plasma from 15 individual donors and processed them with the Seer Proteograph workflow (Figure 1). Both peptides and protein group identification performance using LC-MS analysis were evaluated in this study. This application note will explore how deep and unbiased proteomics analysis reveals differences between the serum and plasma proteomes.

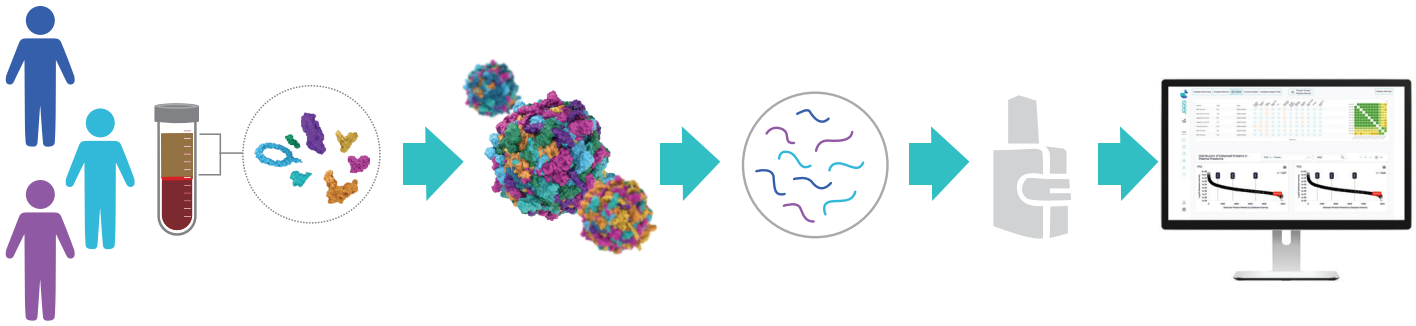

Figure 1. The Proteograph Workflow. Plasma or serum from donor samples were added to Seer's Proprietary engineered nanoparticles to form protein coronas. The protein corona was digested with trypsin and desalted, clean peptides, ready for analysis on most LC-MS instruments, were obtained. Data analysis was performed using the Proteograph™ Analysis Suite (PAS).

Study Design

The study design incorporated three sample types and two workflows. Blood-based samples were collected from 15 donors. Three sample types (citrate-plasma, EDTA-plasma, and serum) were prepared from a single blood draw from each donor (Figure 2). Purified peptides were dried and reconstituted before Data Independent Acquisition (DIA) LC-MS analysis with a 33 min run-to-run method using a Bruker timsTOF Pro2 mass spectrometer. Data were searched with DIA-NN using PAS with library-based and library-free searches.[3]

To compare proteomics workflows for each of the 45 samples (3 sample types x 15 donors), each was processed with a standard neat digestion workflow or with a standard Proteograph™ Assay. Proteograph samples were processed on three multiwell plates with sample types and donors randomized and equally distributed between the plates.

Results

Processing samples with the Proteograph workflow yielded a >3-fold increase in the number of Protein Groups IDs compared with a neat digest (Figure 3). Serum yielded in slightly higher Protein Group IDs compared to either citrate-plasma or EDTA-plasma.

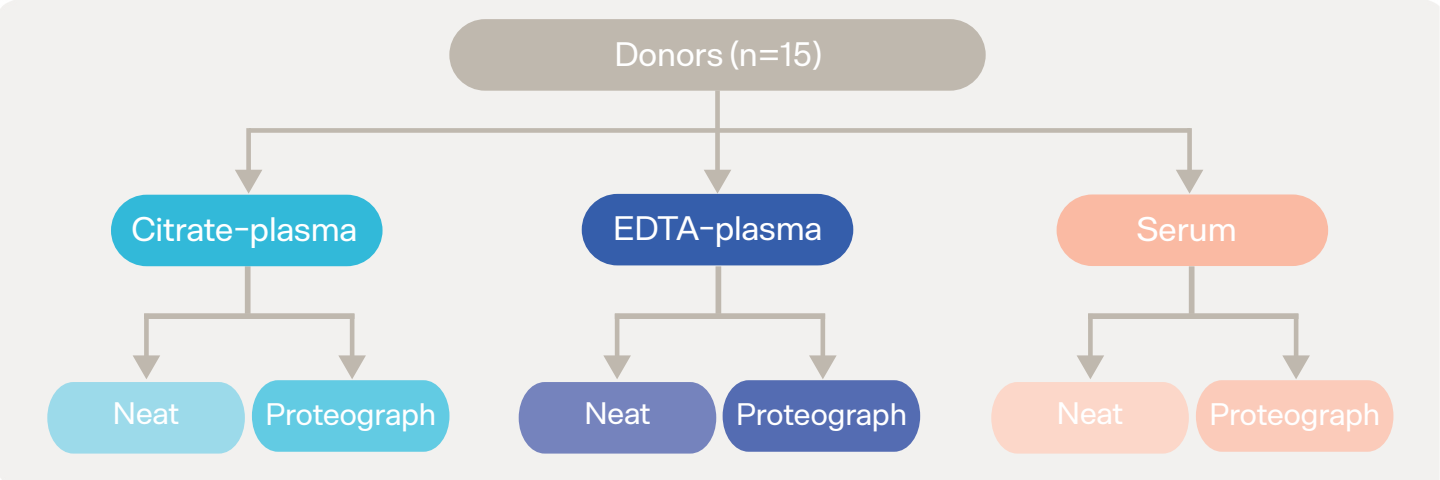

Figure 2. Samples were processed into peptides using the Proteograph Assay[2] with a standard neat digest workflow for comparison.

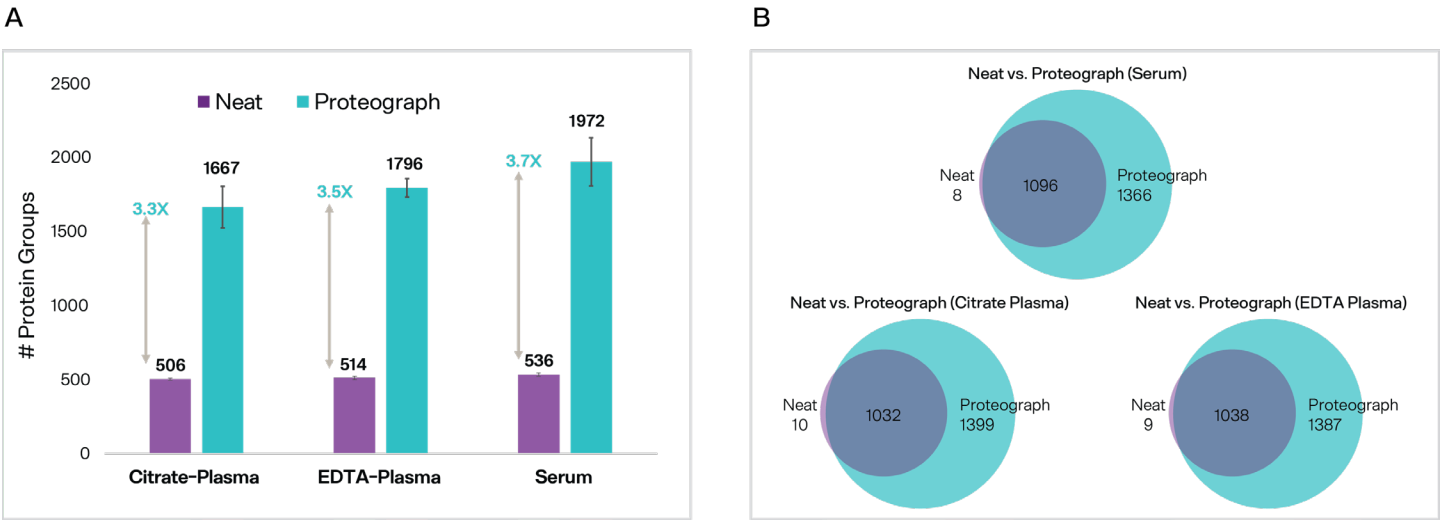

Figure 3. Protein Group IDs show a significant increase in proteome coverage with the Proteograph workflow.
A) Protein Group identifications from a DIA-NN library-based search. Bars represent mean values from 15 donors; error bars represent the standard deviation. **B)** Venn diagram showing the overlapping coverage between Proteograph workflow and neat workflows.

Principal component analysis (PCA) plots reveal significant differences between sample types (Figure 4A-B). The PCA plot of neat digested samples shows almost no difference between citrate and EDTA plasmas, and only a slight difference between serum and the two types of plasma. The PCA plot of samples processed with the Proteograph workflow shows that all three sample types are significantly different and distinguishable, as expected from the greater depth of proteome coverage with Proteograph Product Suite providing higher resolution to see these differences. A Venn diagram showing overlapping protein group IDs across the 15 donors between the three sample types detected by the Proteograph workflow identifies few unique proteins in each sample type (Figure 4C). Differential protein abundances were observed between EDTA-plasma and serum with the Proteograph workflow (Figure 5).

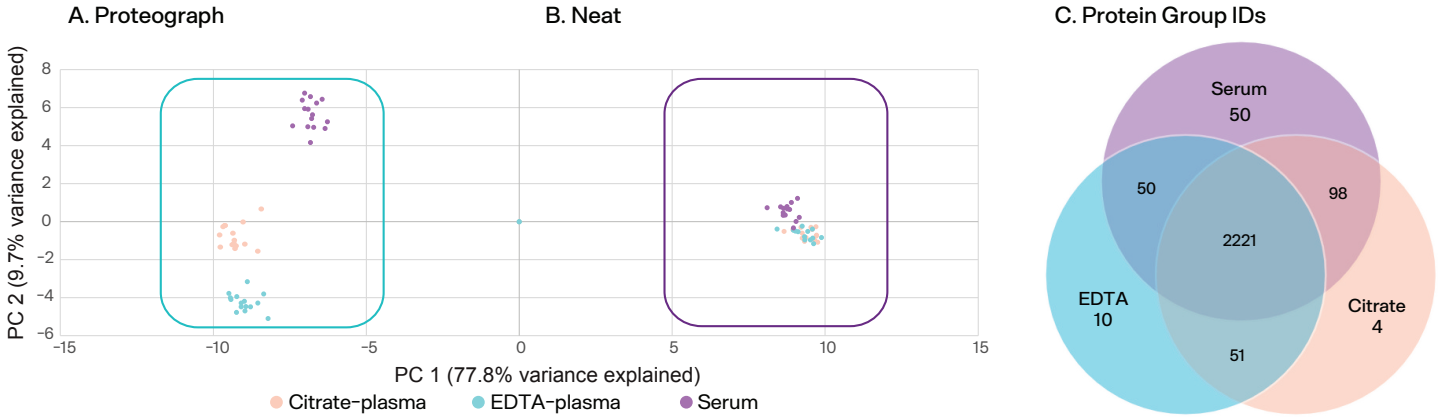

Figure 4. A) Proteograph. PCA plot of samples processed with the Proteograph workflow. **B) Neat**. PCA plot of neat digest samples. **C) Protein Group IDs**. Venn diagram showing overlapping protein group IDs (across 15 donors) between the three sample types detected by the Proteograph Assay.

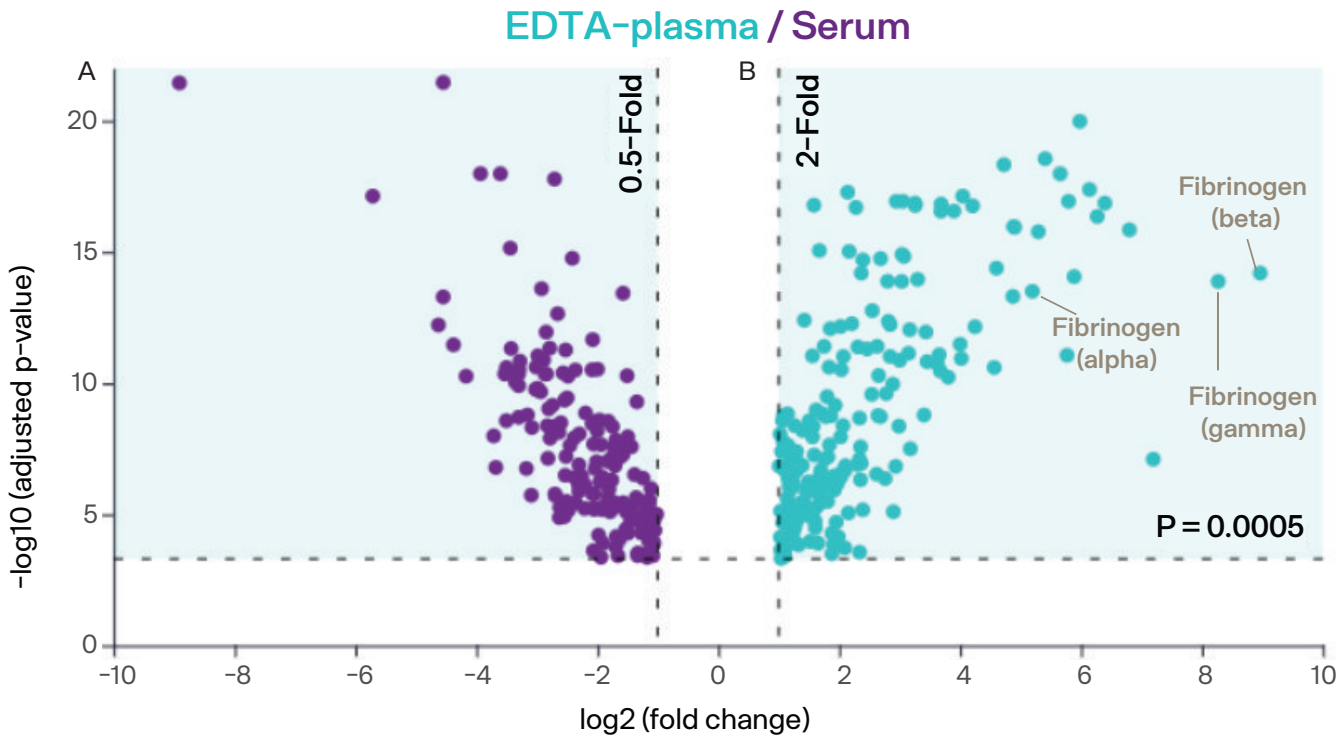

Figure 5. A) Volcano plot showing differential protein abundance between EDTA-plasma and serum with the Proteograph workflow. **B)** Volcano plot demonstrating 355 proteins with differential abundance between EDTA-plasma and serum (p-value < 0.0005). Similar results are seen comparing citrate-plasma with serum.

Conclusions

This study examining deep proteome coverage with the Proteograph Product Suite, providing >3-fold increase in the number of Protein Groups IDs compared with a neat digestion enabling to reveal significant differences between plasma samples and serum samples protein contents. Our data show that serum yields slightly more protein group IDs compared to the two types of plasma, perhaps due to depletion of abundant proteins during clotting. Across all three sample types, EDTA-plasma performed the most consistently, and serum shows depletion of many proteins, such as fibrinogen, as expected.

This work suggests that serum and plasma proteomes are in fact different in their protein contents, and a single sample type is strongly recommended for large-scale biomarker discovery research studies or care must be taken to account for quantitative protein expression differences that could be produced from confounding sample-type differences.

Reference

1. Geyer P.E., et al. Plasma Proteome Profiling to detect and avoid sample-related biases in biomarker studies. EMBO Mol Med 11: e10427 (2019).
2. Thavasu P.W., et al. Measuring cytokine levels in blood. Importance of anticoagulants, processing, and storage conditions. J. Immuno. Methods 153(1-2):115-24 (1992).
3. Bludau, I., et al. Systematic detection of functional proteoform groups from bottom-up proteomic datasets. Nat Commun 12, 3810 (2021).

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