Deep and unbiased proteomics analysis reveals differences between serum and plasma proteome in matched donors

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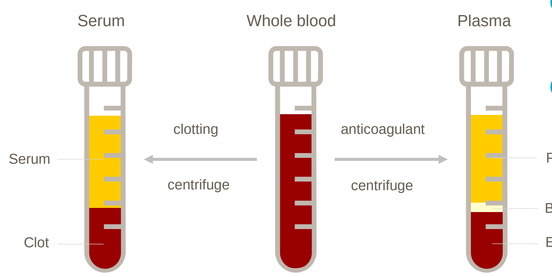
Evaluation of different blood-based sample types for deep plasma proteomics

Serum and plasma are common blood-based sample types used for biomarker discovery studies. While these sample types are similar, the abundance of many proteins could be altered from the original source material (i.e. blood) during their collection and processing[1,2]. To better characterize the differences between serum and plasma proteomes and 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™ Product Suite, and peptides and protein groups identification performance using LC-MS analysis were evaluated in this study.

Sample Preparation Strategies



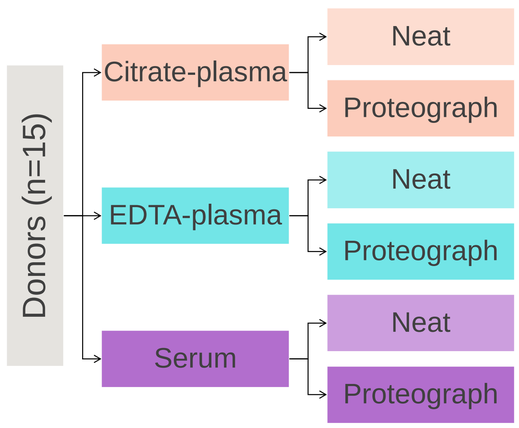
A Are there significant **differences** between sample types?

B Are there distinct **advantages** between sample types?.

Study design: comparing three sample types and two workflows

Samples were collected from 15 donors

1. Three sample types (citrate-plasma, EDTA-plasma, and serum) were prepared from a single blood draw from each donor.
2. To compare proteomics workflows, for each of the 45 samples (3 sample types x 15 donors) we processed each sample with a standard neat digest workflow or with the Proteograph Assay.
3. Proteograph samples were processed on three plates with sample types and donors randomized and balanced between the plates.



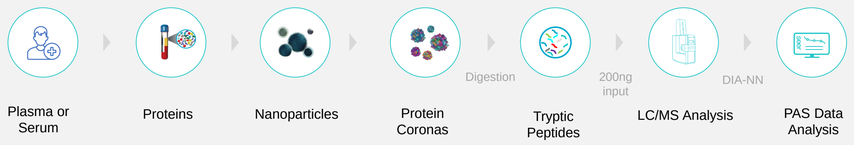
Proteograph Product Suite

Proteograph Product Suite provides unbiased, deep, and rapid proteomics at scale

From sample to peptides, ready for analysis on most LC-MS instruments with a variety of proteomics methods

Data Generation

1. Samples were processed into peptides using the Proteograph Platform[3] and with a standard neat digest workflow for comparison.
2. Peptides were dried and reconstituted before DIA LC-MS analysis with a 33 min run-to-run method using a Bruker timsTOF Pro2.
3. Data were searched with DIA-NN using the Proteograph Analysis Suite (PAS) with library-based and library-free searches. Data shown is from library-based searches.



Highlighting differences between plasma and serum proteomics

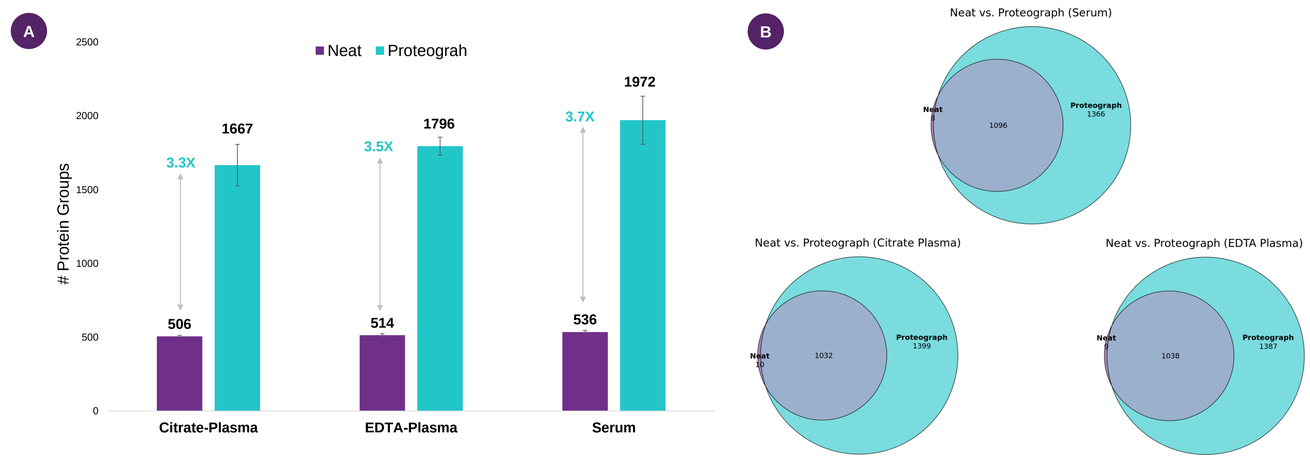


Figure 1. Protein Group IDs show a significant increase in proteome coverage with the Proteograph workflow.

A) Protein Group identifications (IDs) from a DIA-NN library-based search. Processing samples with the Proteograph™ workflow yielded a >3-fold increase in the number of Protein Groups IDs compared with a neat digest. Serum yielded slightly higher Protein Group IDs compared to either type of plasma. 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.

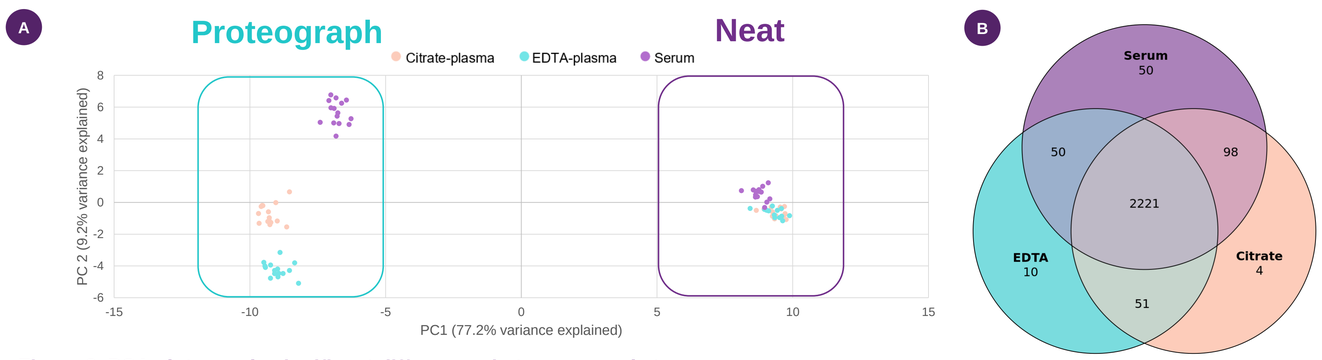


Figure 2. PCA plot reveals significant differences between sample types.

A) PCA plot of neat digest samples shows almost no difference between citrate and EDTA plasmas, and only a slight difference between serum and the two types of plasma. PCA plot of samples processed with the Proteograph workflow reveals that all three sample types are significantly different and distinguishable, as expected from the greater depth of proteome coverage with Proteograph Product Suite. **B)** Venn diagram showing overlapping protein group IDs (across 15 donors) between the three sample types detected by the Proteograph Assay, identifying few unique proteins in each sample type.

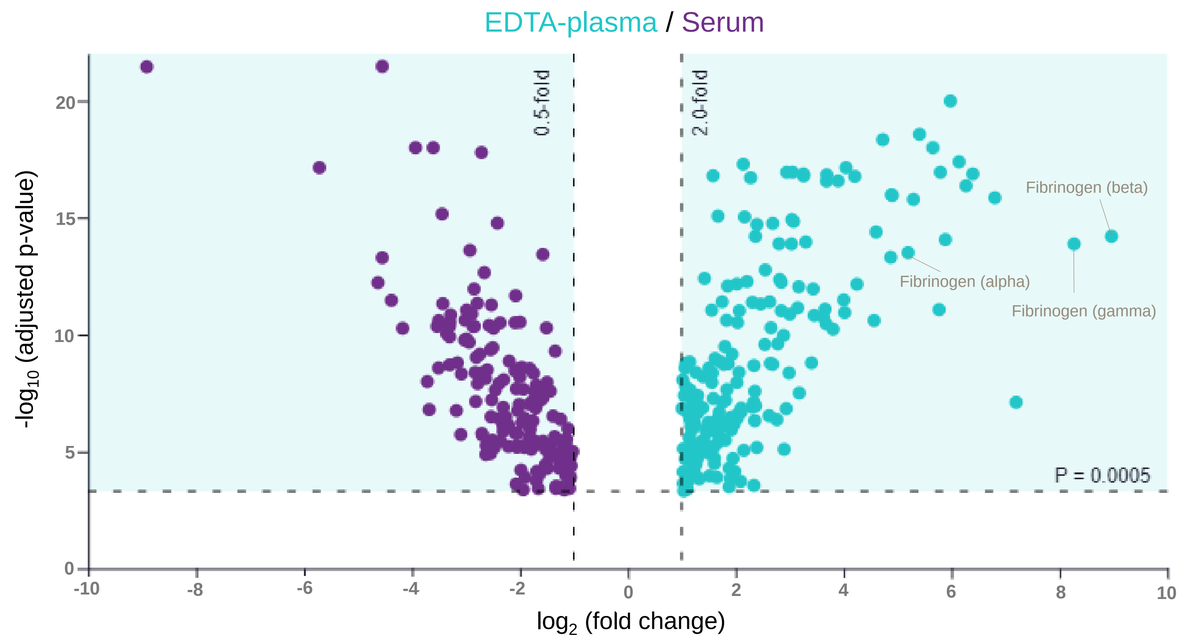


Figure 3. Volcano plot visualizing differential protein abundance between EDTA-plasma and serum with the Proteograph workflow.

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 (not shown). Comparing EDTA-plasma and serum prepared with the neat digest workflow showed 7 proteins, including 3 fibrinogen chains, differentially expressed with a p-value < 0.0005 (not shown).

Conclusion

- Deeper proteome coverage with the Proteograph Product Suite, reveals significant differences between the three sample types tested.
- Serum yields slightly more protein group IDs compared to the two types of plasma, perhaps due to depletion of abundant proteins during clotting.
- EDTA-plasma performed the most consistently across all three sample types.
- Serum shows depletion of many proteins, such as fibrinogen, as expected.
- A single sample type should be used in large-scale biomarker discovery studies, or care must be taken to account for sample-type differences.

References

1. *Geyer et al. EMBO Mol Med (2019).*
2. *Thavasu et al. J. Immuno. Methods (1992)*
3. *Bludau et al. Nat. Comm. (2021)*

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