Conventional anticoagulants allow preanalytical variation that confounds the accuracy of plasma proteomics

Rachel M. Miller[1], Sama Mehta[1], Xiaoyuan Zhou[2], Nicholas Mucci[2], Ryan W. Benz[2], Jing Li[1]

[1] Streck LLC., La Vista, NE, United States
[2] Seer Inc., Redwood City, CA, United States

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INTRODUCTION

In proteomics, the accuracy of downstream biological conclusions depends on sample integrity. This is especially true in analysis of biofluids like plasma, where pre-analytical variables such as anticoagulant used and duration of whole blood storage can significantly alter the proteomic landscape of a sample. If the plasma proteome analyzed is no longer representative of a donor's native proteome, inaccurate conclusions could be made. In this study, we investigate the impact of prolonged whole blood storage on the plasma proteome of blood collected into conventional anticoagulants (EDTA, ACD-A) and a specialized stabilization tube (Protein Plus BCT™) using the Seer Proteograph XT™ platform to determine sample handling conditions that minimize proteomic variation.

Protein Plus BCT is For Research Use Only. Not for use in diagnostic procedures.
Protein Plus BCT should only be used for research or the development of new assays.

METHODS

Blood was collected from five healthy donors into EDTA, ACD-A and Protein Plus BCT. Plasma was isolated from whole blood at draw time (within 2 hours), or after 24 hours, 72 hours and 120 hours of ambient temperature storage using a double spin protocol (1800 xg for 15 min, 2800 xg for 15 min) and frozen at –80 °C until analysis. Samples were prepared for bottom-up proteomic analysis using the Seer Proteograph XT workflow and analyzed using data-independent acquisition on an Orbitrap Astral™ mass spectrometer (Thermo Scientific™). Data was analyzed using the Proteograph Analysis Suite (PAS) and the DIA-NN label-free search workflow.

RESULTS

Prolonged whole blood storage in conventional anticoagulants profoundly alters the observable plasma proteome.

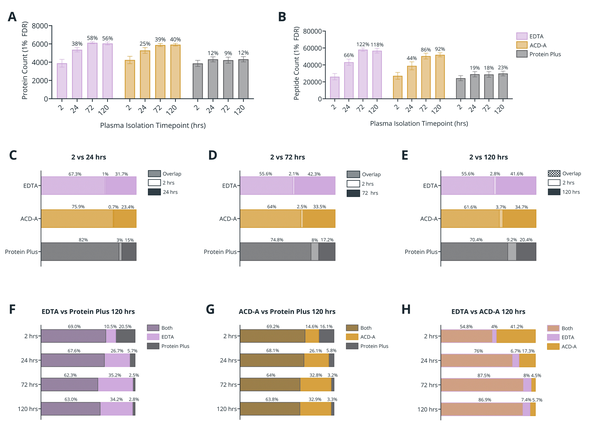

Figure 1. (A-B) Average number of proteins **(A)** and peptides **(B)** identified at 1% FDR in plasma isolated from samples collected into EDTA, ACD-A or Protein Plus BCT at draw time (2 hrs) or after 24, 72 or 120 hrs of whole blood storage at ambient temperature. The percent increase of identifications relative to draw time is displayed above each additional plasma isolation timepoint. **(C-E)** Protein identification overlap between draw time (2 hrs) and 24 **(C)**, 72 **(D)** and 120 **(E)** hrs for EDTA, ACD-A and Protein Plus BCT. **(F-G)** Protein identification overlap between plasma isolated from samples in EDTA **(F)** and ACD-A **(G)** at draw time (2hrs) and after 24, 72 and 120 hrs of storage and plasma isolated from samples in Protein Plus BCT after 120 hrs of storage. **(H)** Protein overlap identification overlap between plasma isolated from samples in EDTA and ACD-A at draw time (2 hrs) and after 24, 72 or 120 hrs of storage. For the purpose of identification overlap, a protein was considered identified if it was present in 60% of samples for any one condition.

RESULTS (cont.)

Protein Plus BCT minimizes proteome alterations during whole blood storage.

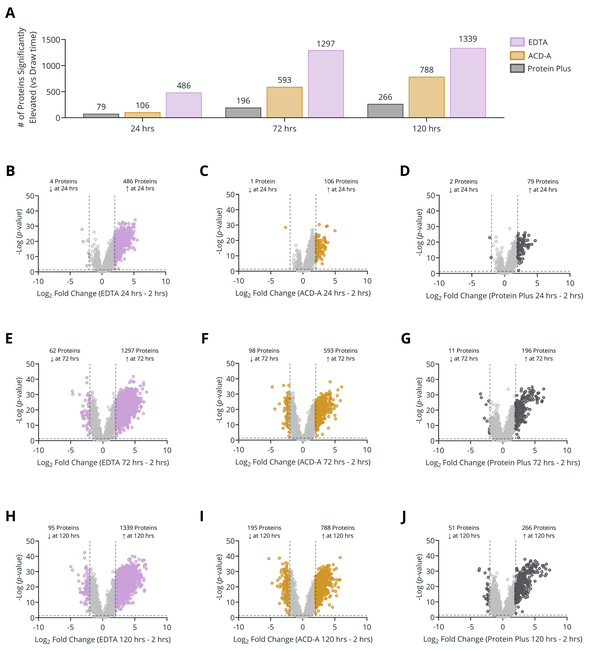

Figure 2. (A) Number of proteins that are significantly elevated in abundance after 24, 72 or 120 hrs of whole blood storage in EDTA, ACD-A and Protein Plus BCT relative to draw time (2 hrs). **(B-J)** Differential analysis of plasma protein abundances between plasma isolated from blood drawn into EDTA, ACD-A or Protein Plus BCT at draw time (2 hrs) or after 24 hrs **(B-D)**, 72 hrs (E-G), or 120 hrs of whole blood storage at ambient temperature. **(H-J)**. Colored markers: Log_2 fold change >2 or < -2, -Log(p-value) > 1.3 and FDR ≤ 1%. All quantitative analysis was performed for proteins which were quantified in at least 60% of samples in each collection tube and plasma isolation timepoint (2730 plasma proteins).

RESULTS (cont.)

Protein Plus BCT maintains draw time abundances of blood cell proteins during prolonged storage.

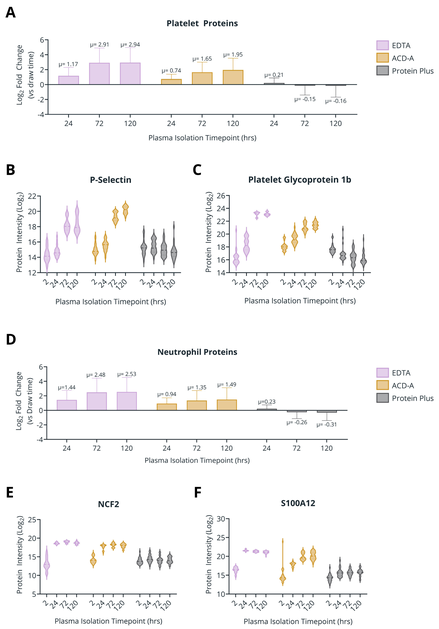

Figure 3. (A) Mean Log_2 fold change of platelet-associated protein abundance in plasma isolated from samples collected into EDTA, ACD-A and Protein Plus BCT after 24, 72 or 120 hrs of whole blood ambient temperature storage relative to draw time (2 hrs) protein abundance (N= 341 proteins). **(B-C)** Plasma levels (Log_2 transformed) of P-Selectin **(B)** and Platelet Glycoprotein 1b **(C)** at draw time (2 hrs) and after 24, 72 and 120 hrs of ambient temperature whole blood storage. **(D)** Mean Log_2 fold change of neutrophil-associated protein abundance in plasma isolated from samples collected into EDTA, ACD-A and Protein Plus BCT at 24, 72 or 120 hrs relative to draw time (2hr) protein abundance (N= 297 proteins). **(E-F)** Plasma levels (Log_2 transformed) of Neutrophil Cytosolic Factor 2- NCF2 **(E)** and S100A12 **(F)** and at draw time (2 hrs) and after 24, 72 and 120 hrs of ambient temperature whole blood storage.

RESULTS (cont.)

Proteins associated with biological pathways of interest are stabilized by Protein Plus BCT.

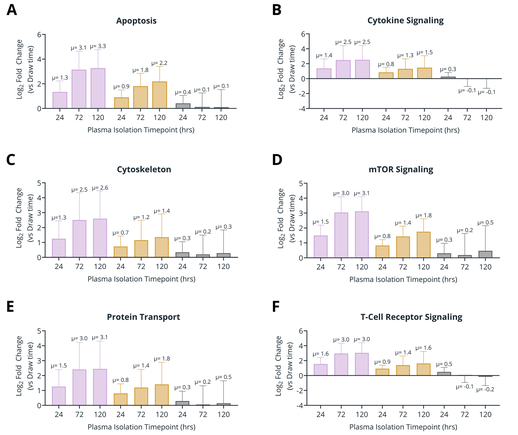

Figure 4. (A) Mean Log_2 fold change in abundance of **(A)** apoptosis related proteins (N= 85, Reactome R-HSA-109581), **(B)** proteins involved in the immune system's cytokine signaling (N= 220, Reactome R-HSA-1280215), **(C)** cytoskeletal proteins (N= 461, Gene Ontology GO:0005856), **(D)** proteins involved in mTOR Signaling Pathway (N= 16, Reactome R-HSA-165159), **(E)** proteins involved in protein transport (N= 258, Gene Ontology GO:0015031), and **(F)** proteins involved in T-cell receptor signaling pathway (N= 63, Reactome R-HSA-202403) in plasma isolated from samples collected into EDTA, ACD-A or Protein Plus BCT after 24, 72 or 120 hrs of whole blood storage at ambient temperature relative to draw time (2 hrs) abundances.

CONCLUSION

The stabilization formulation in Protein Plus BCT greatly reduces the impact of prolonged whole blood storage on the plasma proteome by limiting the ex vivo activation, lysis and degradation of blood cells often seen in sample collected and stored in conventional anticoagulants (EDTA, ACD-A). Samples collected into EDTA and ACD-A show major qualitative and quantitative proteome changes with delayed plasma processing, whereas those collected into Protein Plus BCT display minimal proteome alteration in plasma isolated up to 5 days after draw. Protein Plus BCT ensures sample integrity and minimizes the preanalytical variables, providing researchers greater flexibility in sample handling and improved confidence in downstream biological analysis of plasma proteomic results.

Conflict of Interest Disclosure

At the time of abstract submission, the authors of were full-time employees at Streck, LLC or Seer, Inc. As such, the research described here was funded by Streck, LLC and Seer, Inc.