Protein Plus BCT™: A Novel Blood Collection Tube for Stabilizing Plasma Proteins of Interest in Ambient Whole Blood Storage

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INTRODUCTION

Circulating soluble plasma proteins (e.g., cytokines) that are crucial for cancer diagnosis, prognosis, and prediction of cancer immunotherapy outcomes are found in blood cells such as platelets and leukocytes. During blood collection and sample storage, *ex vivo* platelet activation, hemolysis, and blood cell degradation can occur, which causes the release of blood cell-secreted proteins into the plasma. The contamination of these proteins may confound true *in vivo* levels of plasma proteins of interest, resulting in inaccurate or inconsistent assay results. Here, we show that the new blood collection tube, Protein Plus BCT™, addresses this issue by maintaining accurate plasma protein levels during ambient whole blood storage.

RESULTS

Conventional serum or plasma blood collection tubes do not meet the sample requirements for protein analysis.

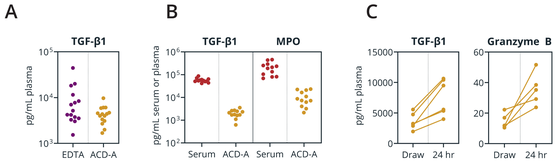

Figure 1. Whole blood was drawn into EDTA, ACD-A or serum tubes and protein levels of TGF-β1, MPO, or Granzyme B in isolated plasma or serum were analyzed at draw time (**A**,**B**) or after 24 hours of whole blood storage (**C**).

Protein Plus BCT limits *ex vivo* hemolysis and platelet activation.

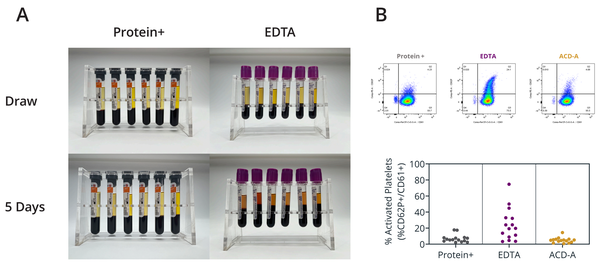

Figure 2. (**A**) *Ex vivo* hemolysis in blood samples drawn into Protein Plus BCT or EDTA at draw or after 5 days of ambient temperature storage. (**B**) Activated platelet populations (CD62P+/CD61+) in matched donor samples immediately following draw into Protein Plus BCT, EDTA or ACD-A (n=15).

RESULTS (cont.)

Analysis with Luminex xMAP® and Ella™ Simple Plex™ immunoassays reveals that Protein Plus BCT maintains draw-time concentrations of plasma proteins of interest.

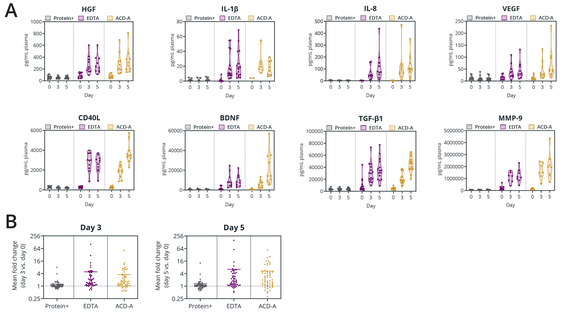

Figure 3. (**A**) Plasma levels of proteins of interest in samples collected into Protein Plus BCT, EDTA, or ACD-A at draw time or after 3 or 5 days of ambient temperature storage (representative biomarkers shown, n≥10). (**B**) Mean protein fold change relative to draw–time abundance for 55 plasma protein markers of interest in samples collected into Protein Plus BCT, EDTA, or ACD-A after 3 or 5 days of ambient temperature storage (n=10).

Protein Plus BCT is compatible with Olink® Proximity Extension Assay (PEA) technology.

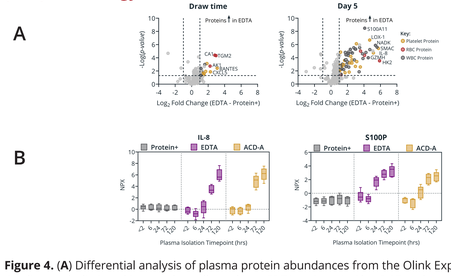

Figure 4. (**A**) Differential analysis of plasma protein abundances from the Olink Explore 384 Cardiometabolic Panel in samples collected into EDTA or Protein Plus BCT at draw time and after 5 days of whole blood storage at ambient temperature (n=5). Markers with fold change < -1 or > 1 and a –Log(p-value) > 1.3 represent proteins that are statistically significantly changing in abundance between the two BCTs. Proteins with a positive Log2 fold change are more abundant in EDTA than Protein Plus BCT. Colored markers denote changing proteins known to come from platelets (gold), RBCs (red), or WBCs (dark grey). (**B**) Abundance of representative proteins (IL-8 and S100P) during storage in Protein Plus BCT, EDTA, or ACD-A.

RESULTS (cont.)

Mass spectrometry analysis indicates that Protein Plus BCT provides increased proteome stability.

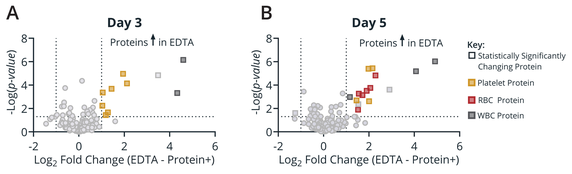

Figure 5. Differential LC-MS/MS analysis of neat plasma protein abundances between EDTA and Protein Plus BCT after three (**A**) or five (**B**) days of whole blood storage at ambient temperature (n=6). Square markers represent proteins statistically significantly changing in abundance between the two BCTs (Log2 fold-change >1 and -Log(p-value) >1.3). Proteins with a positive Log2 fold change are more abundant in EDTA than Protein Plus BCT. Colored markers are for changing proteins known to come from platelets (gold), RBCs (red), or WBCs (dark grey).

Protein Plus BCT maintains proteome stability to the expanded depth of coverage enabled by the Seer Proteograph™ XT platform.

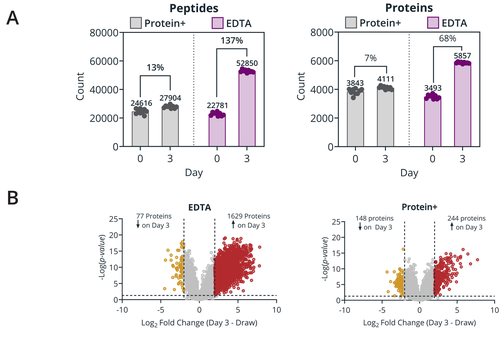

Figure 6. (**A**) Overall protein and peptide counts in plasma isolated from Protein Plus BCT or EDTA at draw time or after three days of ambient temperature storage (n=3). (**B**) Differential analysis of protein abundance between plasma isolated at draw time or after three days of whole blood storage for EDTA (left) and Protein Plus BCT (right). Red markers: Log2 fold change >2, -Log(p-value)> 1.3.

METHODS

Blood Collection: Blood samples from self-proclaimed healthy donors were drawn into Protein Plus BCT, EDTA, and ACD-A tubes. All the samples were stored at ambient temperature for up to 5 days. Plasma was isolated using a double-spin protocol (1800 ×g for 15 min and 2800 ×g for 15 min, both at room temperature) and frozen at -80 °C until use.

Platelet Activation Analysis by Flow Cytometry: Whole blood samples were stained with an antibody cocktail of CD61-PerCP (a platelet surface marker, BD® Biosciences) and CD62P-PE (an activated platelet surface marker, BioLegend) and analyzed on a BD FACSCanto® II clinical flow cytometry system. Acquisition and analysis were performed by scatter gating (forward scatter [FSC] and side scatter [SSC]) followed by fluorescent gating. Activated platelets (%) were quantified by the CD61+CD62P+ platelet population of all gated platelets (FlowJo v10.7.2).

Analysis of Plasma Level of Protein Biomarkers by Immunoassay: Plasma levels of protein biomarkers were measured by i) Ella Simple Plex Assays (Bio-Techne); ii) Luminex xMAP Technology Assays (Bio-Techne, samples tested by LuminexPLORE Lab (Diasorin, S.p.A.); or iii) Olink Proximity Extension Assay (Olink Explore 384 Cardiometabolic panel, samples tested by Vanderbilt University Medical Center High-Throughput Biomarker Core Lab), following manufacturers' dilution and processing recommendations, respectively. Resulting data was analyzed by Streck.

Analysis of Plasma Proteome by Mass Spectrometry: Neat plasma samples were prepared for bottom-up analysis using the S-Trap 96-well plate (Protifi) and analyzed on a Thermo Fisher Exploris 240 Orbitrap mass spectrometer. All raw spectra files were analyzed using MetaMorpheus (v0.0.320) and quantitative statistical analysis was performed using Perseus (v2.0.7.0). To achieve deep proteomic coverage, Streck collaborated with Seer, Inc., who prepared samples using the Seer Proteograph XT workflow and analyzed with a Thermo Fisher Orbitrap Astral mass spectrometer using data-independent acquisition (DIA). Data were analyzed using DIA-NN-1.8.1 and the Proteograph Analysis Suite (PAS).

CONCLUSION

We demonstrate that Protein Plus BCT maintains draw-time plasma protein levels during extended whole blood storage at room temperature by limiting *ex vivo* platelet activation, hemolysis, and blood cell degradation. Plasma isolated from this tube is compatible with both immunoassay platforms and mass spectrometry-based proteomic workflows. Protein Plus BCT ensures sample integrity and minimizes preanalytical variables, providing cancer researchers and clinical assay developers greater flexibility in sample handling and improved confidence in downstream plasma protein analysis.

Protein Plus BCT is for Research Use Only. Not for use in diagnostic procedures.

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