Pre-analytical variables: an underestimated challenge for soluble cytokine analysis in blood samples

Jing Li, Ph.D., and Sama Mehta
Streck Research and Development, La Vista, Nebraska

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INTRODUCTION

Blood-based circulating cytokines are pivotal diagnostic, prognostic, and pharmacodynamic biomarkers. However, a lack of understanding of the sample matrix and minimal standardization in blood collection and processing can make precise cytokine measurement in blood samples challenging. Because many soluble cytokines reside in granules of blood cells like platelets, neutrophils, NK cells, and macrophages, they are vulnerable to *ex vivo* changes in a blood sample. These changes can occur immediately after draw (e.g., platelet/leukocyte activation) and continue during storage (e.g., blood cell degradation), complicating the analysis. To address these issues, Streck developed Protein Plus BCT™, a whole blood stabilization tube specialized for plasma protein analysis.

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.

RESULTS

Protein Plus BCT limits *ex vivo* platelet activation at the time of blood draw.

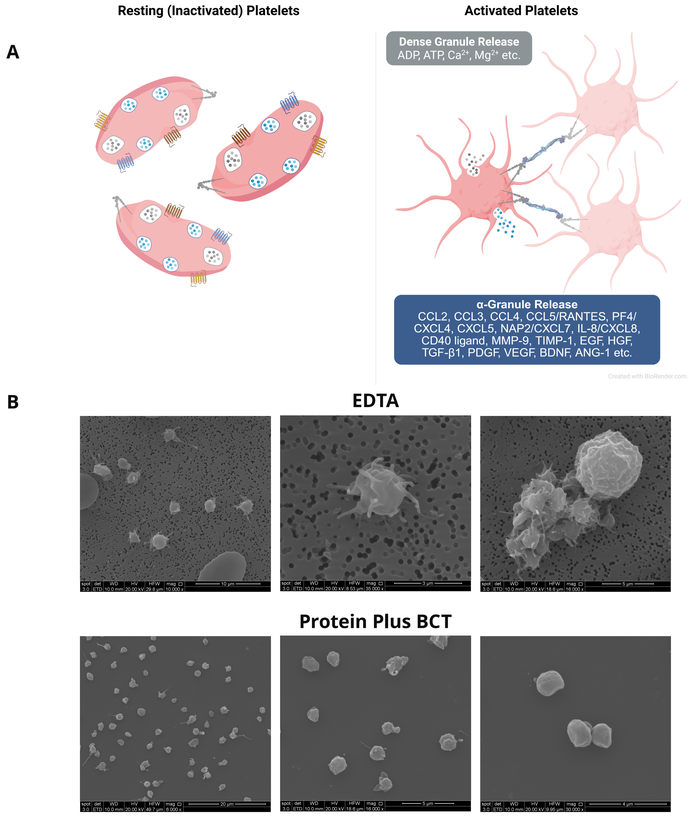
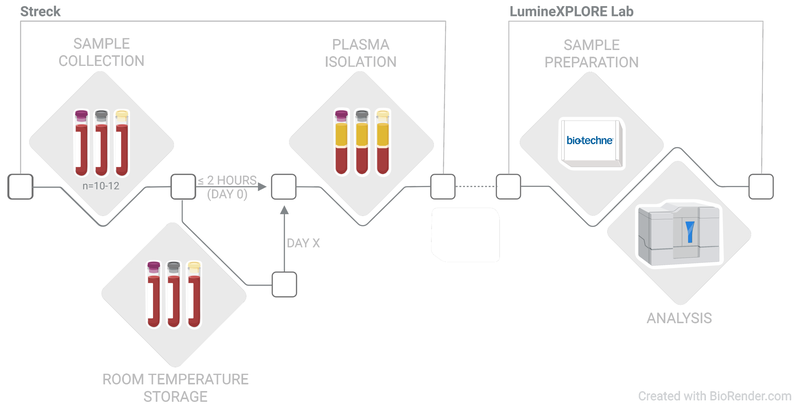


Figure 1. (**A**) Depiction of platelet activation and associated irreversible protein release from α-granules. (**B**) Representative SEM images of platelets in blood samples collected into EDTA and Protein Plus BCT from the same donor at draw time. Scanning Electron Microscope (SEM) images of platelet-rich plasma or buffy coat were taken by the University of Nebraska Medical Center Electron Microscopy Core Facility.

RESULTS (CONTINUED)

To investigate the consequences of blood collection into ACD-A on platelet activation, blood from 12 self-declared healthy donors was collected into EDTA, Protein Plus BCT, and ACD-A. At draw or after up to 5 days of ambient temperature storage, samples were analyzed using the Human XL Cytokine Luminex® Performance Assay 46-plex Fixed Panel (Bio-Techne®, samples tested by LuminexPLORE Lab (Diasorin, S.p.A.)).



Protein Plus BCT limits release of blood cell-associated cytokines during ambient whole-blood storage.

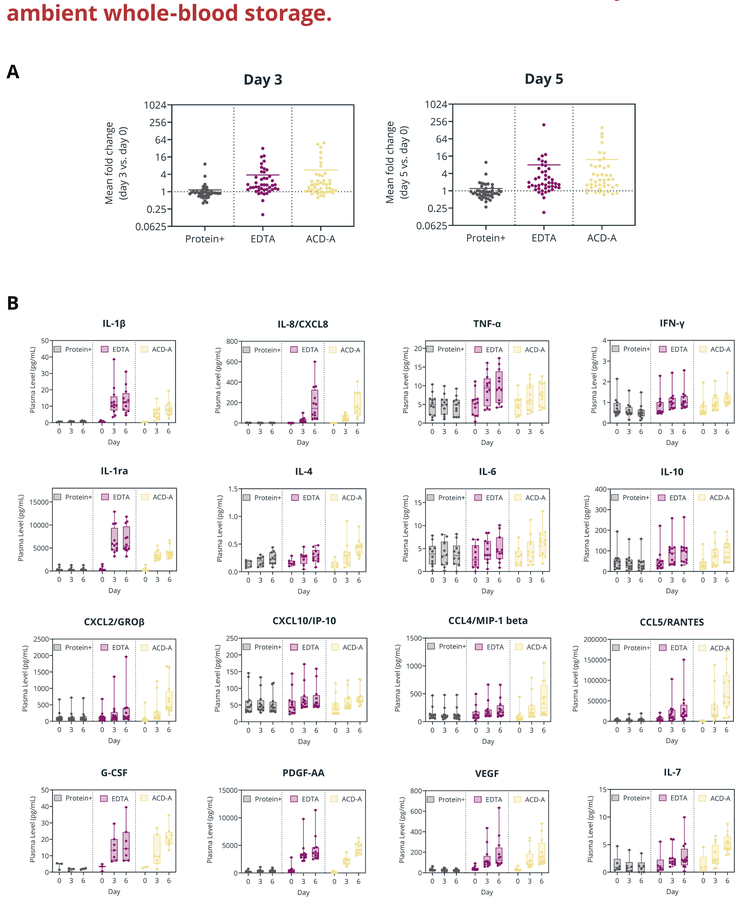
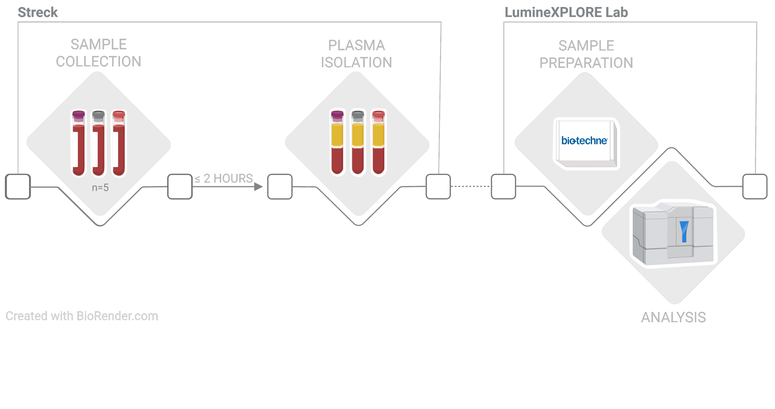


Figure 2. (**A**) Mean protein fold change relative to draw-time abundance of cytokines of interest in samples collected into Protein Plus BCT, EDTA, or ACD-A after 3 and 5 days of ambient temperature storage (all detected cytokines from the Human XL Cytokine Luminex Performance Assay 46-plex Fixed Panel are shown, n=12). (**B**) Plasma levels of proteins of interest in samples collected into Protein Plus BCT, EDTA, or ACD-A at draw time or after 3 and 5 days of ambient temperature storage (representative biomarkers shown, n=12).

RESULTS (CONTINUED)

To identify the consequences of blood collection in serum tubes on platelet activation, blood from five self-declared healthy donors was collected into EDTA, Protein Plus BCT, and a serum tube. Plasma or serum was separated within two hours following draw. samples were analyzed using the Human XL Cytokine Luminex Performance Assay 46-plex Fixed Panel (Bio-Techne, samples tested by LuminexPLORE Lab (Diasorin, S.p.A.)).



Protein Plus BCT minimizes release of cytokines from platelets and leukocytes at draw time.

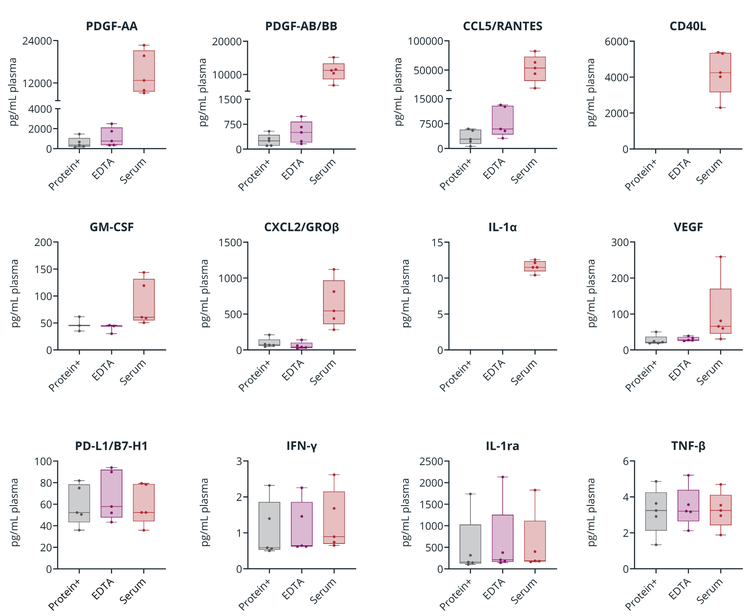


Figure 3. Concentration of various cytokines in plasma isolated from samples collected into Protein Plus BCT, EDTA, or serum tubes at draw.

CONCLUSIONS

Our data show that Protein Plus BCT minimizes *ex vivo* blood cell activation at the time of draw and maintains cytokine levels for up to 5 days, suggesting that incorporating Protein Plus BCT into pre-analytical workflows ensures reliable results in plasma cytokine analysis.

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