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A-308

Residual Platelets: An Underestimated Pre-Analytical Challenge in Circulating Biomarker Research

Jing Li, Ph.D., Eunju Seong, PhD, Rachel Miller, Ph.D., Tyler Morris, MLS(ASCP)CM, Lisa Bartron, MB(ASCP)CM

Streck Research and Development, La Vista, NE, USA

BACKGROUND

Circulating biomarkers, including soluble proteins, extracellular vesicles (EVs) and cell-free nucleic acids, are widely used in biomarker research and clinical diagnostics. However, pre-analytical variables, such as blood collection tube type, plasma processing protocols, and platelet (PLT) contamination can critically impact the accuracy and reliability of biomarker measurements. Unlike red blood cells (RBCs) and white blood cells (WBCs), platelets are small, moderately abundant and densely granulated. They serve as a major reservoir of circulating proteins, EVs, and EV-associated cargos. Residual PLTs in plasma are often overlooked and can artificially elevate biomarker signals through activation or lysis during processing and storage. The commonly used single-spin protocol (1100-1300 x g, 10 min) is sufficient for routine plasma separation in clinical chemistry but is inadequate for assays targeting sensitive or low-abundance biomarkers. Double-spin protocol (e.g.,1800 x g, 10 min, followed by 2800 x g, 15 min) significantly improves platelet and cellular debris removal; however, effectiveness is highly dependent on immediate sample processing after blood draw. In this study, we systematically evaluate the impact of residual platelets in plasma collected using standard EDTA tubes and a specialized stabilization tube (Nucleic Acid Plus BCT™; formerly Protein Plus BCT™). We further assess how different processing methods influence biomarker abundance and stability.

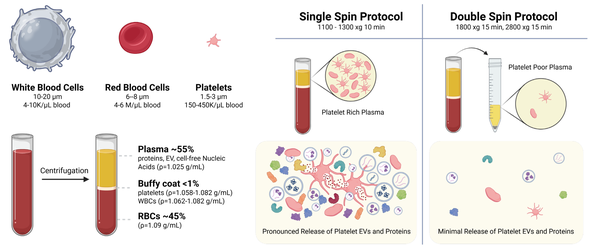

Figure 1. Schematic of composition and characteristics of blood sample matrix and the impact of plasma processing on residual platelets and downstream biomarker release. Following blood collection and centrifugation, incomplete platelet removal leaves residual platelets in plasma, which can release EVs and proteins upon activation or lysis, leading to elevated biomarker signals. In contrast, optimized processing that minimizes platelet carryover reduces platelet-derived EV and protein release, resulting in a cleaner plasma matrix and more accurate biomarker measurements. Created with BioRender.com

RESULTS

Nucleic Acid Plus BCT Combined with Double-Spin Plasma Processing Protocol Enables Superior Platelet Depletion

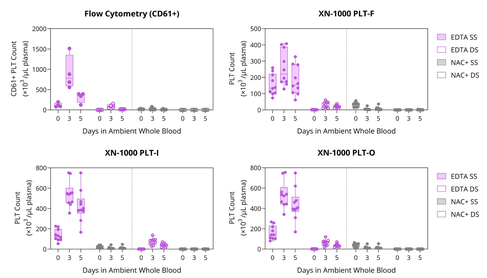

Figure 2. Impact of blood collection tube type and processing conditions on residual platelet abundance in separated plasma. Platelet particle count was assessed by flow cytometry (CytoFLEX, CD61+) and hematology analysis (Sysmex XN-1000; three PLT channels). EDTA samples processed using a single-spin (SS) protocol showed substantial platelet carryover, which increased further with delayed plasma processing. A double-spin (DS) protocol significantly improved platelet removal, particularly under extended storage conditions. In contrast, Nucleic Acid Plus BCT (NAC+) effectively minimized residual platelets under the tested stabilization conditions, even with single-spin processing, with double-spin providing optimal depletion (n= 4–10).

Nucleic Acid Plus BCT Combined with Double-Spin Plasma Processing Enables Reliable Platelet-Associated Protein Analysis

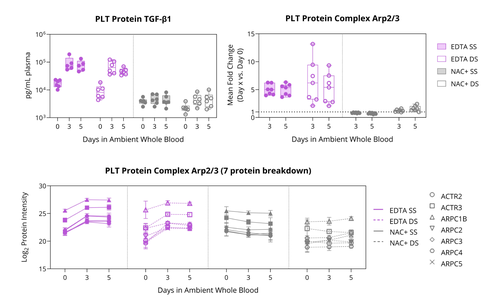

Figure 3. Impact of blood collection tube type and processing conditions on platelet-associated protein measurements. Two representative markers are shown. TGF-β1, a key immunosuppressive cytokine found highly abundant in platelets, is highly susceptible to pre-analytical variability; draw technique, tube type, time-to-spin, freeze-thaw conditions can all inflate measured levels. The Arp2/3 complex, a seven-protein regulator of platelet actin cytoskeleton remodeling, is also presented. EDTA causes *ex vivo* platelet activation immediately upon draw, and even with double-spin processing, EDTA plasma exhibits higher platelet-associated protein abundance than Nucleic Acid Plus BCT (NAC+) BCT as the activation followed by granule release is fast and irreversible. In contrast, the Nucleic Acid Plus BCT (NAC+) formulation effectively minimizes platelet activation and limits platelet-derived protein contamination in plasma. TGF-β1 remains stable from the time of draw with minimal changes over time, and spin protocol has limited impact on measured levels (n=3-6).

Nucleic Acid Plus BCT Combined with Double-Spin Plasma Processing Minimizes Platelet-Derived EV Contamination

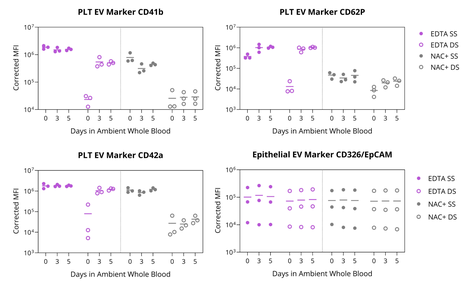

Figure 4. Impact of blood collection tube type and processing conditions on platelet-associated EV measurements. For all three platelet markers, double spin effectively depleted platelet-derived EV signal by 1–2 logs at Day 0 but this depletion was progressively lost at Days 3 and 5, consistent with *ex vivo* platelet activation and EV release during storage. In contrast, Nucleic Acid Plus BCT (NAC+) kept platelet EV markers low across all timepoints in the double-spin fraction. The epithelial marker CD326/EpCAM showed minimal change across tube, spin protocol, and storage day, demonstrating that a genuine tissue-derived EV marker remains stable in both EDTA and Nucleic Acid Plus BCT (NAC+) tubes. Together, these data indicate that platelet contamination and activation are among the dominant pre-analytical variables affecting EV measurements.

Nucleic Acid Plus BCT Combined with Double-Spin Plasma Processing Minimizes Platelet-Derived mRNA Signal

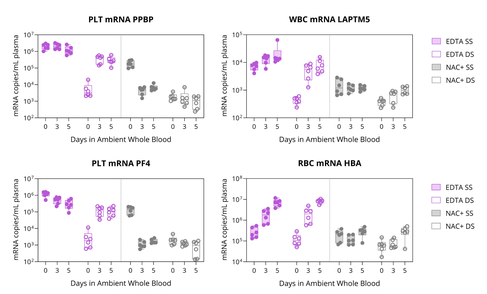

Figure 5. Impact of blood collection tube type and processing conditions on circulating mRNA marker signal contamination. For both platelet markers (PPBP, PF4), leukocyte marker LAPTM5, double spin depleted mRNA contamination signal by 2–3 logs at Day 0 in EDTA, but this depletion was progressively lost over storage, consistent with platelet activation and blood cell breakdown, releasing intracellular mRNA into plasma. For mRNA marker HBA, spin protocol has limited effect in EDTA tubes. In contrast, Nucleic Acid Plus BCT (NAC+) kept all four markers low and stable across all timepoints when processed using double spin (n=6). Together, these data show that double spin alone does not protect against storage-induced release, and that Nucleic Acid Plus BCT (NAC+) preserves a cleaner plasma for reliable downstream mRNA analysis.

CONCLUSIONS

Residual platelets represent a critical yet underestimated pre-analytical variable in circulating biomarker research. Blood collection tube choice, plasma processing strategy, and storage duration significantly influence platelet contamination and biomarker accuracy and stability. Nucleic Acid Plus BCT combined with double-spin plasma processing effectively minimizes residual platelets and preserves platelet-sensitive biomarkers and supports broader plasma biomarker integrity. Standardizing mitigation of residual platelet effects is essential for robust and reproducible plasma-based biomarker analyses.

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

METHODS

Blood Collection and Processing: Blood from matching self-proclaimed healthy donors was collected into K_2EDTA, and Nucleic Acid Plus BCT and stored at ambient temperature for up to 5 days to mimic delayed clinical processing. Plasma was isolated using either single-spin (1100–1300 × g,10 min) or double-spin (1800 × g, 15 min; 2800 × g, 15 min) protocols.

Residual Platelet Count: Residual platelet levels in isolated plasma were quantified using flow cytometry (CytoFLEX, CD61+) and a Sysmex XN-1000 hematology analyzer.

EV analysis: EV profiling was performed using a bead-based MACSPlex EV Immuno-Oncology kit (Miltenyi Biotec) by flow cytometry. All reactions used 10 µL plasma input to enable comparison across all spin conditions within the assay's dynamic range. Background-corrected MFI was reported against isotype control or mIgG1 control.

Plasma Protein Analysis: Platelet-associated protein abundance and stability were assessed over time using Proteograph™ XT workflow coupled with Orbitrap™ Exploris™ 240 mass spectrometry (Seer) or Ella™ Simple Plex™ assay (BioTechne).

Circulating mRNA Analysis: Total circulating RNA in plasma was extracted from 500 µL thawed plasma using the Promega Maxwell® RSC miRNA Plasma and Serum Kit on the Maxwell RSC automated extraction platform. Eluted RNA was then reverse transcribed using ThermoFisher SuperScript™ IV kit and mRNA target quantification was performed on QIAcuity digital PCR.

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