APPLICATION NOTE

Advancing and Monitoring EV Stability Using Optimized Blood Collection and High-Sensitivity ELISA Quantification

Everest Biolabs, in collaboration with Streck Research and Development

Altas® Lumi EV ELISA and Nucleic Acid Plus BCT™ are for research use only. Not for use in diagnostic procedures.

Introduction

Extracellular vesicles (EVs) are emerging as powerful biomarkers for a wide range of diseases, including neurodegenerative disorders and cancer, due to their ability to reflect the molecular state of their cells of origin. However, reliable EV analysis is highly dependent on pre-analytical variables, particularly blood collection, handling, and storage conditions.

One of the major challenges in EV-based biomarker research is maintaining EV integrity following blood collection. Cellular stress, activation, or lysis after the draw can introduce artificial changes in EV abundance and composition, confounding downstream analyses. At the same time, clinical samples exhibit substantial biological variability, with EV concentrations spanning several orders of magnitude, presenting an analytical challenge: enabling accurate quantification of both low- and high-abundance EVs within a simple workflow that supports consistent monitoring of EV changes across post-collection time points.

To address these challenges, a robust workflow must combine (1) reliable sample stabilization at the point of collection and (2) a highly sensitive, wide-dynamic-range detection method capable of capturing this variability in a single measurement.

Blood Collection and EV Stabilization

Blood is a complex biological matrix that undergoes substantial *ex vivo* changes after collection. Loss of physiological circulation, temperature shift, and altered gas composition trigger platelet activation, hemolysis, and leukocyte degranulation, processes that drive progressive, time-dependent increases in blood cell–associated EVs. When processing is delayed, these changes accumulate, contaminating EV-associated biomarker signals and compromising reproducibility across studies.

Streck's Nucleic Acid Plus BCT is designed to minimize these pre-analytical variables by inhibiting cellular activation and breakdown following blood draw. Whole blood is collected directly into the tube at the point of care, where a proprietary preservative chemistry acts immediately to stabilize the cellular and molecular composition of the sample. Unlike standard EDTA or ACD-A tubes, Nucleic Acid Plus BCT is formulated to better maintain EV population integrity (and preserve nucleic acid signals) during delayed processing, supporting multi-site and decentralized clinical workflows.

Sensitive and Robust EV Quantification with a Wide Dynamic Range

The Atlas Lumi EV ELISA (Everest Biolabs) is a one-step assay that targets the tetraspanins CD9, CD63, and CD81 on intact EVs, with a chemiluminescence readout. It runs directly on biofluids (plasma, serum, urine, CSF, or cell culture supernatant) or on purified EVs (e.g., SEC fractions), using only 50 µL of sample without the need for purification or concentration steps.

The assay was optimized to provide a broad linear dynamic range spanning approximately 4 orders of magnitude, enabling accurate measurement of both low- and high-EV concentrations within the same run. As a result, the Atlas Lumi EV ELISA is particularly suited for analyzing clinical samples with widely varying EV concentrations, enabling reliable quantification across samples from a single dilution and minimizing the need for repeat measurements or extensive optimization.

Atlas Lumi EV ELISA Reveals Improved EV Stability with Nucleic Acid Plus BCT

EXPERIMENTAL DESIGN

Whole blood from self-declared healthy volunteers was collected into EDTA, ACD-A, or Nucleic Acid Plus BCT tubes. Plasma was isolated at Streck using an in-house double-spin centrifugation protocol (1800 ×g, 15 min, followed by 2800 ×g, 15 min, full brake) at draw time (t = 0) and at 72 h, and 144 hrs post-collection. Plasma aliquots were frozen prior to EV quantification. EV concentrations were measured using the Atlas Lumi EV ELISA; all plasma samples were diluted 1/40 to fall within the linear range of the assay, and EV concentrations were reported as particles per mL plasma by comparing luminescence signals against a serial dilution of the provided EV standard (Figure 1).

The large cohort size in this study (N = 23–53 donors per group) captures the broad biological variability and represents a stringent test of the ELISA assay's dynamic range and the tube's stabilization capacity across a realistic spectrum of baseline EV concentrations.

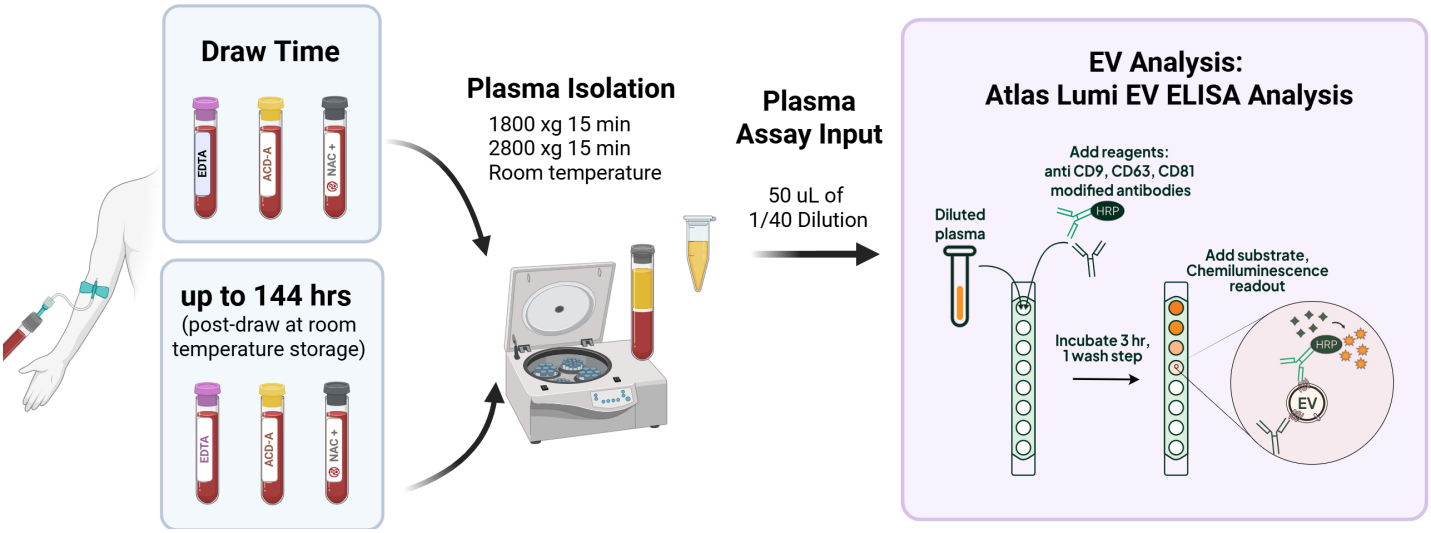

Figure 1. Experimental workflow for evaluating EV stability across delayed processing intervals.

EV Concentration Stability During Delayed Processing

Whole EV concentrations, measured by CD9/CD63/CD81-based ELISA, increased progressively during delayed blood processing in EDTA and ACD-A tubes, while Nucleic Acid Plus BCT maintained near-baseline EV levels across all time points (Figure 2).

At 72 hrs post-collection, EV concentrations rose approximately 10.4× in EDTA and 8.0× in ACD-A relative to draw time. By 144 hrs, these increases reached 12.6× and 41.1×, respectively. In contrast, Nucleic Acid Plus BCT showed minimal increases at both 72 hrs (~1.7×) and 144 hrs (~2.4×), remaining in the low single-digit fold-change range with markedly reduced inter-donor variability.

Importantly, the Atlas Lumi EV ELISA revealed that a donor with unusually **low baseline EV concentrations at t = 0** remained stably low throughout the entire 144-hour collection window in Nucleic Acid Plus BCT. In EDTA and ACD-A tubes, the same donor samples exhibited pronounced, time-dependent increases in EVs, with trajectories indistinguishable from those of donors with higher baseline EVs. These observations support the conclusion that the *ex vivo* EV expansion can be driven by the sample collection and handling environment. Together, these findings demonstrate both the stabilization efficacy of Nucleic Acid Plus BCT and the sensitivity of the Atlas Lumi™ EV ELISA to resolve biologically meaningful differences across a broad EV concentration range within a single assay run.

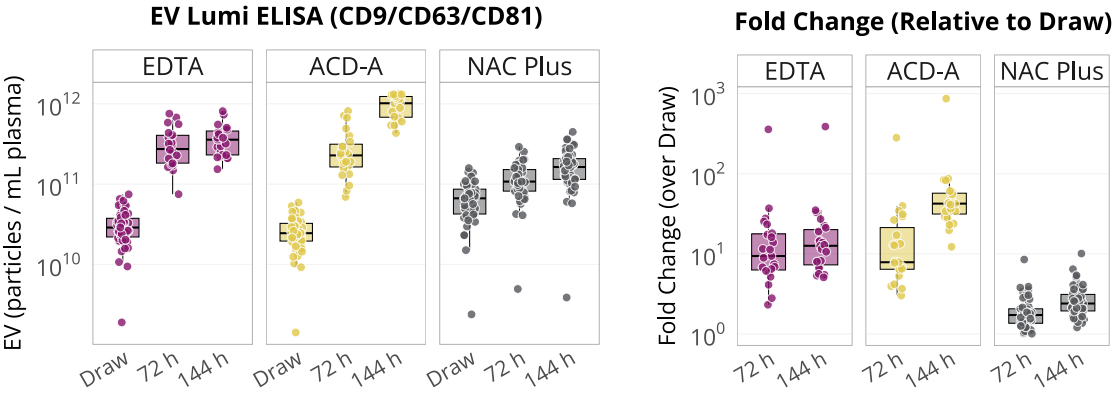

Figure 2. Time-dependent changes in total EV concentrations during delayed blood processing measured by Atlas Lumi EV ELISA. (A) Absolute EV concentrations per mL plasma. (B) Fold-change relative to draw time. N = 23 or 53 donors (EDTA, ACD-A); N = 53 donors (Nucleic Acid Plus BCT).

KEY TAKEAWAYS

- Nucleic Acid Plus BCT limits ex vivo increases in blood cell-derived EVs during delayed blood processing, helping preserve plasma EV populations.
- Bulk EV concentrations measured by the Atlas Lumi EV ELISA (CD9/CD63/CD81) remain near baseline in Nucleic Acid Plus BCT.
- The Atlas Lumi EV ELISA assay supports ~4 orders-of-magnitude dynamic range, enabling accurate quantification of both low- and high-EV concentrations in a single measurement without EV purification.
- Together, Streck's Nucleic Acid Plus BCT and Everest Biolabs' Atlas Lumi EV ELISA provide an integrated pre-analytical and analytical solution for reproducible EV biomarker research.

Conclusion

Reliable EV-based research requires robust sample handling from the point of collection. pre-analytical variability introduced by standard collection tubes can artificially inflate EV concentrations and obscure true biological signals, particularly when sample processing is delayed. Optimizing this critical step demands a quantification tool capable of accurately tracking changes in EV concentration across a wide dynamic range.

The combination of Streck's **Nucleic Acid Plus BCT** and Everest Biolabs' **Atlas Lumi EV ELISA** provides a powerful, integrated solution for overcoming these key pre-analytical and analytical challenges. By preserving sample integrity at the point of collection and enabling accurate quantification across a wide dynamic range, this workflow supports reproducible EV analysis across diverse clinical samples—facilitating biomarker discovery, validation, and translation into clinical application.

PRODUCTS REFERENCED

Product	Description	Supplier
Nucleic Acid Plus BCT	Blood collection tube for EV and nucleic acid stabilization	Streck
Atlas Lumi EV ELISA	Chemiluminescence ELISA for whole EV quantification (CD9/CD63/CD81)	Everest Biolabs

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