The TAG FLEX-LB™ Blood Collection Tube (TFLB) is a 10 mL tube that stabilizes cell-free DNA, cell-free RNA, and total RNA from a single draw. This note also reports a pilot plasma protein comparison.

## Abstract

Blood tubes are often shipped and stored for days before plasma is separated, and in that time lysing white cells add genomic DNA and red cells add RNA that bury the cell-free signal. This technical note compares TFLB with Streck cfDNA, Streck Nucleic Acid and Streck RNA tubes, PAXgene DNA, Norgen and CellSave tubes, and $K_2$EDTA, in paired feasibility-scale studies of one or two healthy donors: each donor's blood was drawn into every tube compared. Over 7 days at ambient temperature (20 to 21 °C), the cfDNA fraction of plasma DNA in TFLB followed the same course as PAXgene DNA and both Streck tubes, while in $K_2$EDTA it fell to 22% by Day 3. The miRNA share of small RNA in TFLB stayed within a 12-point band over 7 days, the narrowest of the three tubes tested, with the tightest replicate spread. After 12 hours at 37 °C, TFLB total RNA fell from 88 to 31 ng/uL and remained sufficient for RT-qPCR; Streck RNA tubes extracted with the same silica-column kit did not. In a two-donor spike-in study, all three tubes tested detected EGFR G719S and PIK3CA H1047R through Day 7, and coverage over the PIK3CA amplicon declined the least in TFLB. In a pilot protein comparison in two donors, the rise in plasma TGF-β1 from Day 0 to Day 4 in TFLB was about one seventh of the rise in $K_2$ EDTA. In exploratory flow cytometry of mononuclear cells from two donors, CD45+ leukocytes stayed detectable through 48 hours in TFLB and $K_2$ EDTA and fell to about 1% of events in the Streck Nucleic Acid Plus blood collection tube (BCT). With the MagMAX Cell-Free DNA Isolation Kit, TFLB plasma needs no Proteinase K digestion.

## Why TAG FLEX-LB? *Protect Your Science. Perfect Your Signal.*

- One tube, three nucleic acid analytes: stabilizes cell-free DNA, cell-free RNA and total RNA, with a pilot plasma protein comparison included.
- **Ambient stability:** cell-free DNA and cell-free RNA recoverable after 7 days at ambient temperature (20 to 21 °C), and total RNA still recoverable for RT-qPCR after 12 hours at 37 °C
- **Shorter cfDNA workflow:** with the MagMAX Cell-Free DNA Isolation Kit, TFLB plasma needs no Proteinase K digestion, which the kit specifies for Streck cfDNA tubes (20 minutes at 60 °C per run, plus 15 uL of Proteinase K per mL of plasma).
- **Cost-Effective for High-Quality Results at Scale**: Reliable performance at a lower price than other stabilizing BCTs.

Table 1: Summary Table of Results

| Measure | TFLB | Streck tubes | $K_2$EDTA | Figure, n |
|---|---|---|---|---|
| **cfDNA fraction, ambient, 7 days** | about 90% on draw day, 74% on Day 7 | 70 to 80% on Day 7 (cfDNA BCT, Nucleic Acid BCT) | 22% by Day 3 | Fig 2B, paired n = 2 |
| **miRNA and small RNA, ambient, 7 days** | 34 to 46% | 40 to 62% (RNA Complete BCT); 32 to 48% (Nucleic Acid BCT) | Not tested | Fig 3B, paired n = 2 |

| | | | | |
|---|---|---|---|---|
| **Plasma appearance, 4 °C, Day 6** | Slight color change | Red tint; held below the 6 °C label minimum | Not tested | Fig 4B, paired n = 2 |
| **Total RNA, 37 °C, 1 to 12 h** | 88 to 31 ng/uL; enough for RT-qPCR | 31 to 6.5 and 27 to 7.2 ng/uL with a silica column kit, not enough for RT-qPCR | Not tested | Fig 8B, paired n = 2 |
| **Spiked EGFR G719S and PIK3CA H1047R, 7 days** | Detected through Day 7 | Detected through Day 7 | Not tested | Fig 11, paired n = 2 |
| **PIK3CA amplicon coverage, Day 7** | 13,000 to 18,000 reads | 7,000 to 12,000 reads | Not tested | Fig 12, paired n = 2 |
| **Plasma TGF-β1, Day 4 (rise from Day 0)** | About 5,000 pg/mL (+3,500) | About 7,600 pg/mL (+700), Nucleic Acid Plus BCT | About 28,600 pg/mL (+26,000) | Fig 13, paired n = 2 |
| **Proteinase K step, MagMAX cfDNA kit** | Not required | Required for cfDNA BCT and Nucleic Acid BCT (20 min, 60 °C) | Not tested | Methods; ThermoFisher MAN0014327 |

*Values are means of the replicates shown in each figure.

## TFLB Performance Test Results

Thermal stress and time cause blood samples to degrade from the moment of collection, through shipping, and during lab storage. Sample degradation causes scarce biomarker signals to be destroyed or lost in the noise of genomic DNA released by apoptosis of white blood cells (WBCs), and RNA from hemolysis of red blood cells (RBCs). TFLB is designed to stabilize whole blood so that cell-free DNA and cell-free RNA can be recovered after up to 7 days at ambient temperature.

### Plasma Stability Over 7 Days

Plasma volume stability over 7 days was compared from TFLB and other stabilizing BCTs (i.e., Streck cfDNA, Streck NA, Norgen, and CellSave). In blood from one healthy donor, TFLB, Streck cfDNA, Streck NA and Norgen tubes each returned 1.0 mL of plasma from 2 mL of whole blood at every timepoint through Day 7. CellSave, a fixative tube designed for circulating tumor cells, returned 0.88 to 0.95 mL from Day 3.

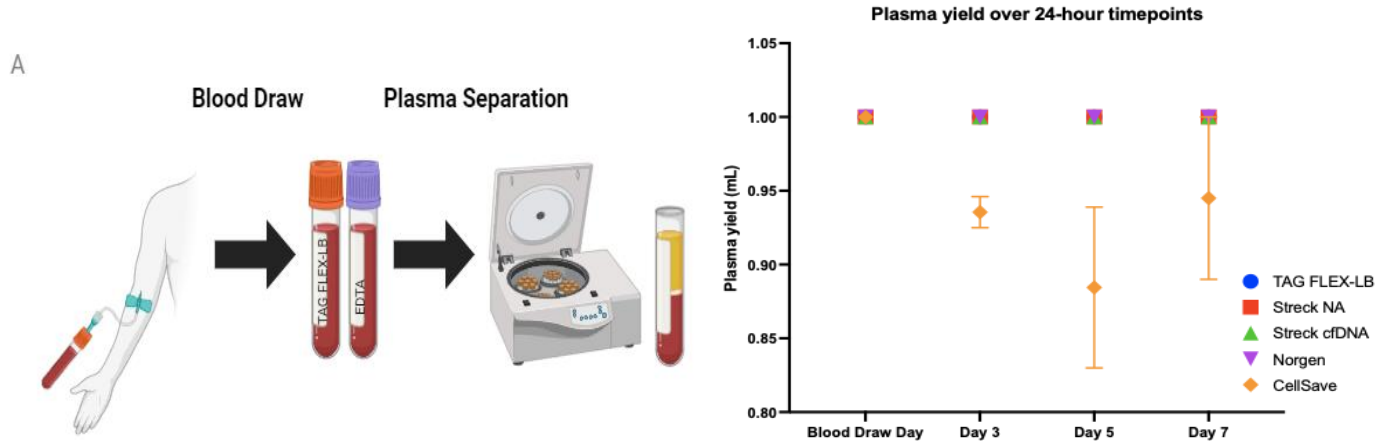



**Figure 1. Plasma volume from 2 mL of whole blood over 7 days at ambient temperature.** A) Blood was collected from a healthy donor into TFLB, Streck cfDNA, Streck NA, Norgen, and CellSave BCTs. Plasma was separated on draw day and on days 3, 5, and 7 at ambient temperature, with a double spin protocol. Plasma volume was measured after centrifuging 2 mL of whole blood.

**Cell-free DNA over 7 days at ambient temperature**

Cell-free DNA (cfDNA) was isolated from 1 mL plasma using MagMAX™ Cell-Free DNA Isolation Kit (ThermoFisher). Per the manufacturer's protocol, TFLB, PAXgene DNA, CellSave, and Norgen BCTs did not use Proteinase K, while Streck cfDNA and Streck NA BCTs did use Proteinase K (per the extraction kit manufacturer's validated protocol for those specific BCTs), which specified a 20-minute, 60°C incubation. cfDNA concentration was measured by QUBIT dsDNA HS Assay, and the cfDNA was measured on the Agilent Cell-free DNA ScreenTape (Agilent 4150 TapeStation). Plasma DNA in the 50 to 700 bp cell-free range measured on the Agilent Cell-free DNA ScreenTape, is a direct readout of genomic DNA contamination from lysing white cells. In TFLB it was about 90% on draw day, 87% on Days 3 and 5, and 74% on Day 7, the same course as PAXgene DNA and both Streck tubes (70 to 80% on Day 7). In EDTA it fell to 22% by Day 3 (paired, n = 2). Norgen BCTs show up to 3 days of stabilization (Figure 2b). EDTA and CellSave show stabilization after blood draw, however, cfDNA recovery decreases by Day 3 for both BCTs. CellSave plasma held under 1 ng/mL of DNA from Day 3 on. Plasma DNA in TFLB stayed between 4.2 and 4.6 ng/mL through Day 7, while EDTA plasma DNA rose from 4.8 to 180 ng/mL, the expected signature of white-cell lysis. (Figure 2C).

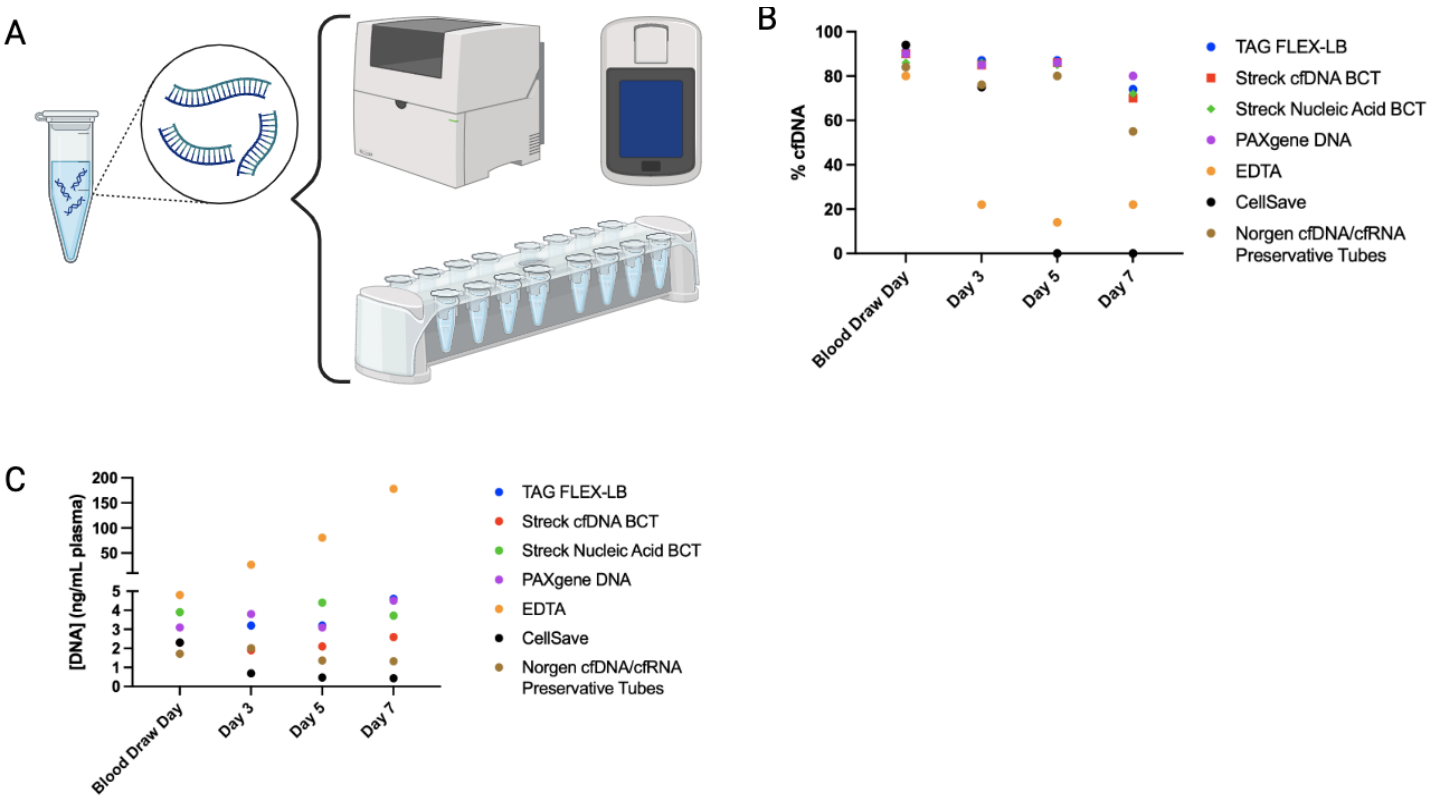



**Figure 2. Plasma DNA in TFLB shows no rise in genomic DNA over 7 days at ambient temperature.** A) cfDNA quality check workflow on TapeStation and Qubit. B) **c**fDNA fraction (% of total DNA in the 50 to 700 bp region), Agilent Cell-free DNA ScreenTape. C) cfDNA concentration (ng/mL) was analyzed by QUBIT dsDNA HS assay.

**TFLB maintains micro- and circulating cell-free RNA for 7 days at ambient temperature**.

Micro-RNA (miRNA) was isolated from 1 mL plasma using Qiagen's QIAamp Circulating Nucleic Acid Kit (55114). miRNA concentration was measured by QUBIT micro-RNA Assay and High Sensitivity RNA ScreenTape (Agilent 4150 TapeStation). Plasma miRNA yield in TFLB rose from about 3.3 ng/mL on draw day to 8.7 ng/mL on Day 7. Both Streck tubes started higher, at about 6.2 ng/mL, and ended at a similar level 8.1 to 8.7 ng/mL (Figure 3 A). Across seven daily time points, the miRNA share of small RNA (Agilent Small RNA assay) stayed between 34% and 46% in TFLB. Streck RNA Complete ranged from 40% to 62%, and Streck NA from 32% to 48%, and the Streck replicates varied more widely at Days 4 and 6 (Figure 3B). Additionally, the electropherogram from Day 0 to Day 7 has no differences in integrity for TFLB (Figure 3D). The Streck RNA Complete and Streck Nucleic Acid profiles were unchanged. To evaluate hemolysis in our samples, we used RT-qPCR and biomarker hsa-miR-16, a miRNA present in RBCs and found in abundance in hemolytic samples (Cabus, et al). TFLB shows a slight downward trend of degradation over time, where TFLB miR-16 Ct falls from about 29.5 on draw day to 27.4 on Day 5, a 2.1-cycle shift (Figure 3C). Measured against each tube's own draw-day value, miR-16 Ct fell by 1 to 2 cycles in all three tubes by Day 5 to 7: in TFLB from about 29.5 to 27.4 at Day 5 and 28.4 at Day 7, in Streck RNA Complete from 27.7 to 26.0, and in Streck Nucleic Acid from 26.9 to 24.8 (paired, n = 2). TFLB had the highest Ct, meaning the least miR-16, at every timepoint. The draw-day differences between tubes probably reflect extraction rather than hemolysis, so we compare each tube with itself. (Figure 3C).

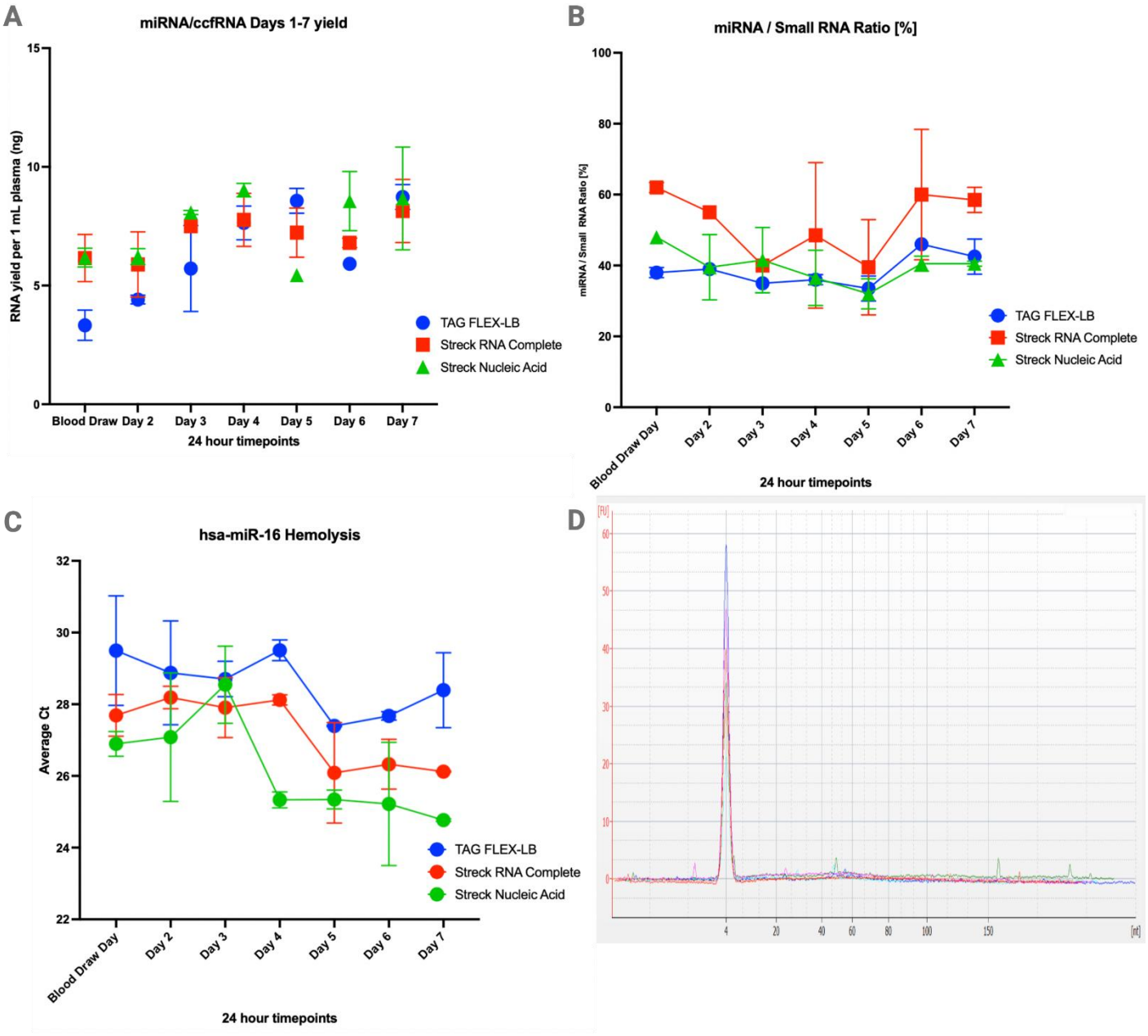



**Figure 3. miRNA ratio and size profile in TFLB and Streck tubes over 7 days at ambient temperature.** A) micro and circulating cell-free RNA yield**.** B) miRNA / Small RNA Ratio [%] utilizing Small RNA Bioanalyzer to evaluate noise. C) hsa-miR-16 Ct by day in each tube; a lower Ct means more miR-16. D) Electropherograms for miRNA for TFLB, Streck RNA, and Streck NA (overlaid traces for all three tubes on draw day to Day 7).

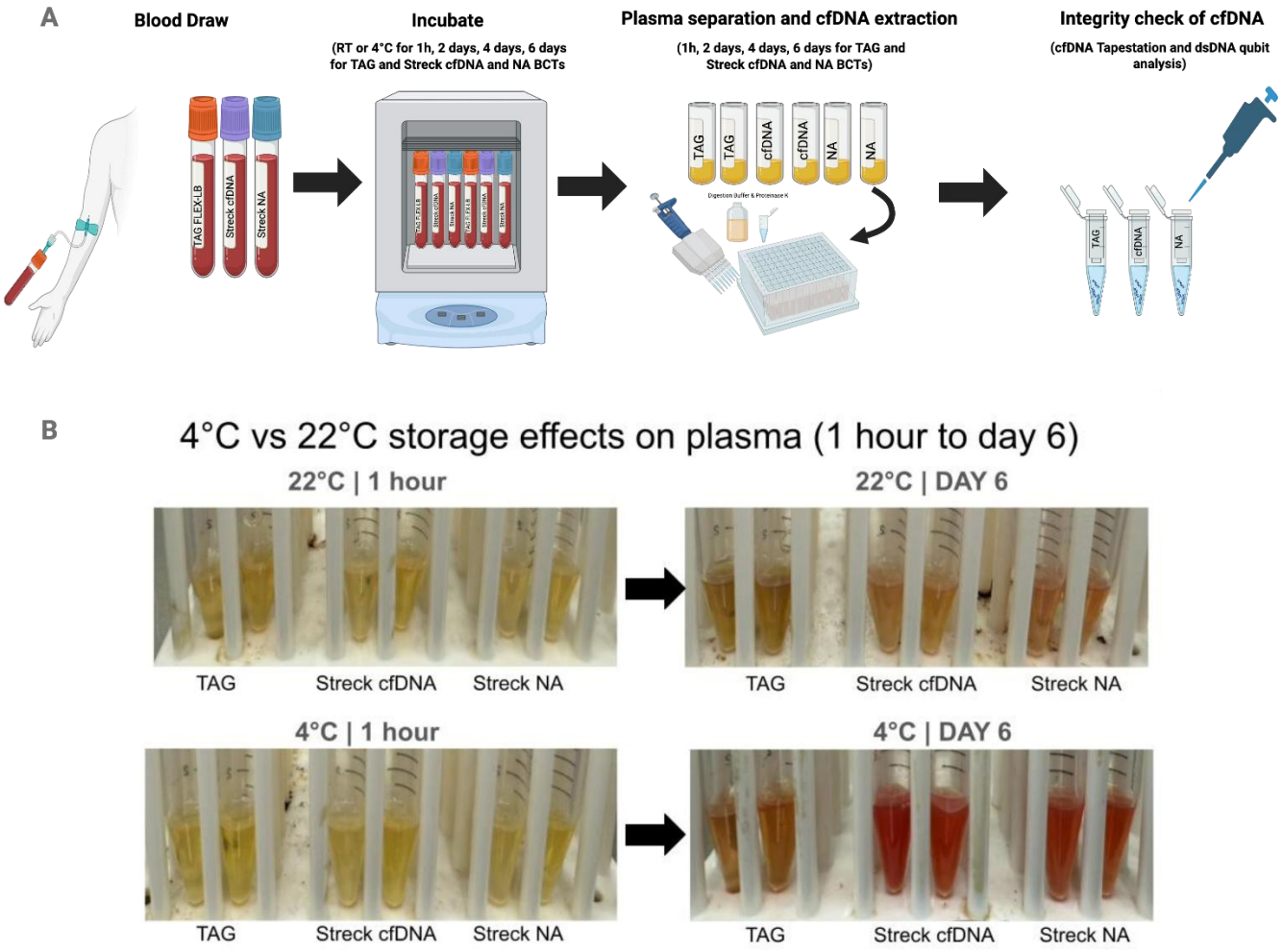



**Figure 4**. **Storage at ambient vs 4°C in TFLB, Streck cfDNA, and Streck NA BCTs over 6 days.** A) Workflow for whole blood storage and extraction of cfDNA from TFLB, Streck cfDNA, and Streck NA BCTs**.** B) Storage temperature at 4°C vs 21°C affects plasma over a 6-day time point in TFLB and Streck BCTs. Changes in plasma appearance at 21°C from 1 hour to 6 days post blood draw in TFLB, Streck cfDNA, and Streck NA BCTs. Changes in plasma appearance at 4°C from 1 hour to 6 days post blood draw in TFLB, Streck cfDNA, and Streck NA BCTs.

**Plasma appearance in TFLB is more stable than competitor BCTs at ambient and 4°C**

Shipments with ice packs can cool tubes to about 4°C, below the 6 °C minimum on Streck's cfDNA tube label. We held tubes at 4°C to test that excursion. Blood was collected into TFLB, Streck cfDNA, and Streck NA BCTs and stored at ambient (21°C) or 4°C for 1-hour, 2-, 4-, 6 days post blood draw. Plasma was separated following cfDNA isolation kit specifications. Photos were taken for all time points to depict changes in plasma appearance for all time points and temperature conditions. Cell-free DNA (cfDNA) was extracted using Applied Biosystems MagMax

cell-free DNA Isolation kit (A29319) from whole blood stored at 21°C and 4°C for 1 hour, 2-, 4-, 6 days post blood draw (Figure 4A). Plasma color is similar for all BCTs at 21°C and 4°C storage 1-hour post-blood draw. A slight color change is seen in TFLB and Streck BCTs at ambient for 6 days post-blood draw (Figure 4B). Streck BCTs have prominent color changes that occur at 4°C storage conditions at 6 days post-blood draw, compared to TFLB. Streck plasma samples have a strong red tint, suggesting increased hemolysis in whole blood stored at 4°C, while TFLB has a slight red tint, indicating less hemolysis (Figure 4B). Blood drawn in TFLB can be stored at 21°C and 4°C, with minimal hemolysis occurring compared to other stabilizing BCTs.

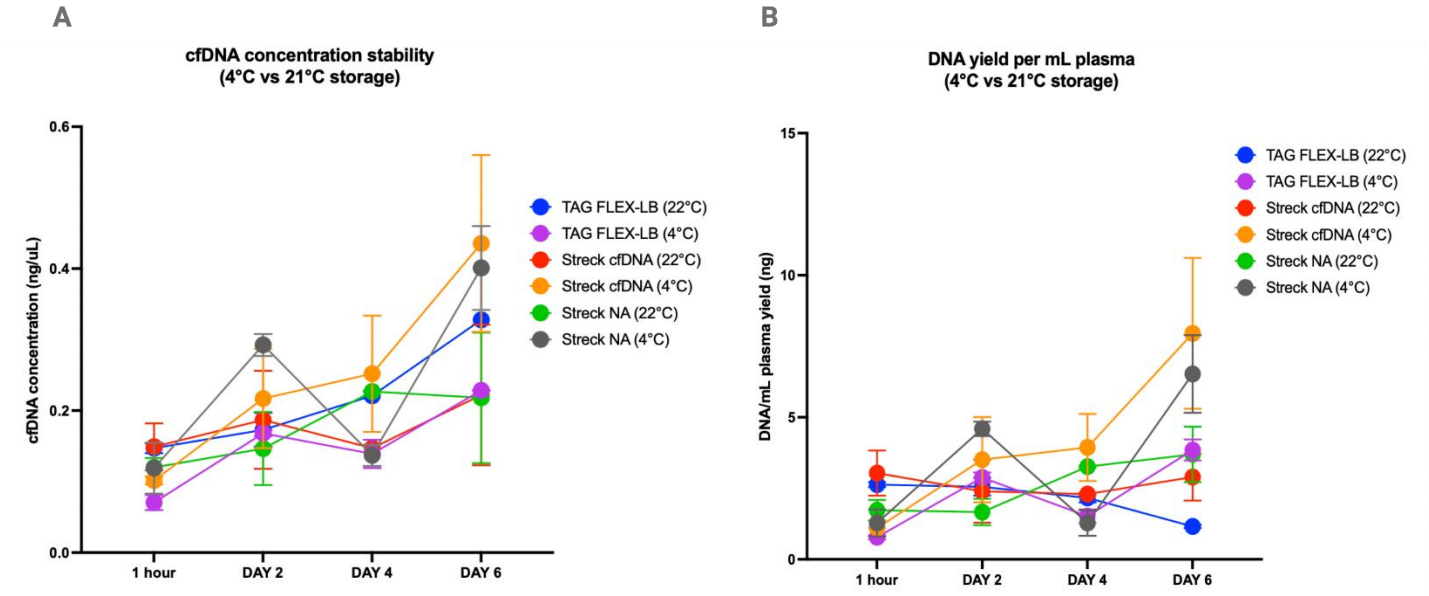



**Figure 5. Plasma DNA concentration and yield at 22 °C and 4°C over 6 days in TFLB and two Streck tubes.** A) Comparison of cfDNA concentration from plasma in TFLB, Streck cfDNA, and Streck NA BCTs at 22°C and 4°C. B) Comparison of cfDNA yield per 1 mL Plasma of TFLB, Streck cfDNA, and Streck NA BCTs at 22°C and 4°C**.** DNA yield for TFLB at 22°C (blue line), TFLB at 4°C (purple line) for 1-hour, 2-, 4-, and 6-day timepoints. Streck cfDNA at 22°C (red line) and Streck cfDNA at 4°C (orange line) for 1-hour, 2-, 4-, and 6-day timepoints. Streck NA at 22°C (green line) and Streck NA at 4°C (black line) for 1-hour, 2-, 4-, and 6-day timepoints.

**Plasma DNA after 6 days at 4°C**

At 4°C, plasma DNA yield rose by Day 6 in all three tubes (Figure 5B): from about 0.8 to 3.8 ng per mL of plasma in TFLB, 1.0 to 8.0 ng in Streck Cell-Free DNA BCT, and 1.3 to 6.5 ng in Streck Nucleic Acid BCT (paired, n = 2 donors). Day 6 value in TFLB was the lowest of the three. At ambient temperature, yield stayed between about 1.2 and 3.7 ng per mL in all three tubes. A rise in plasma DNA in the cold is consistent with white-cell lysis, and the Streck tubes were held below their labeled minimum to model an ice-pack excursion. Fragment-size data are needed to confirm that the added DNA is genomic.

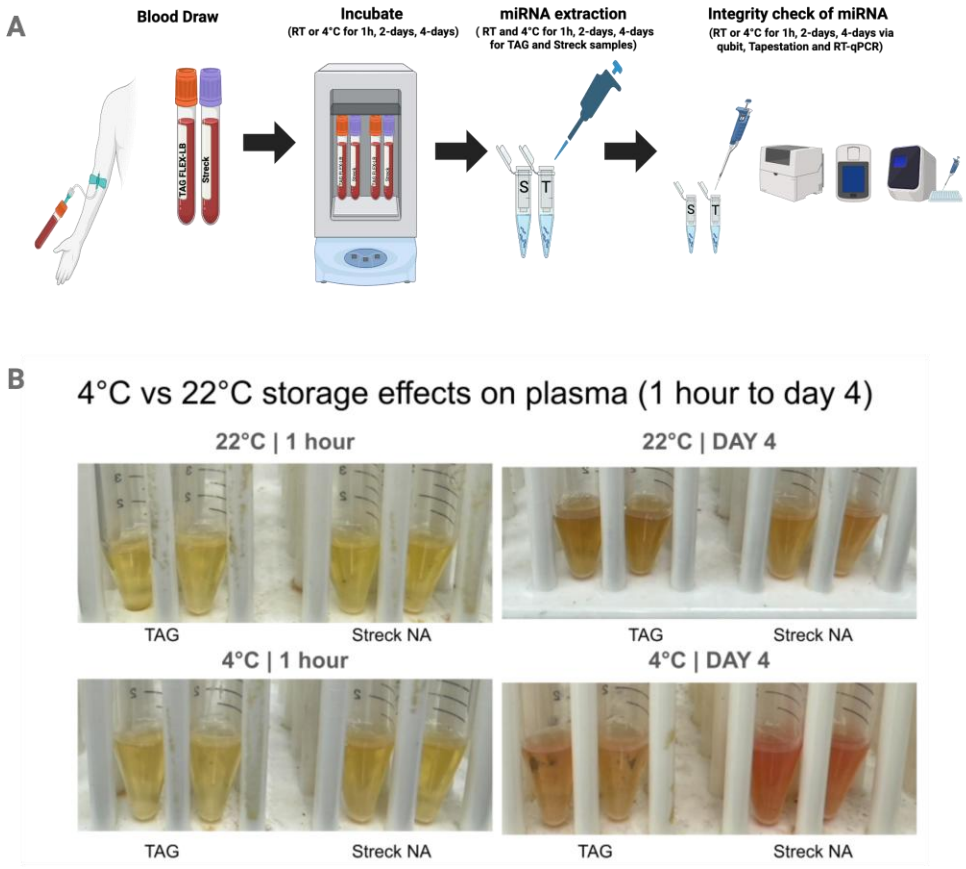



**Figure 6. miRNA stability at 22°C and 4°C in TFLB and Streck NA BCTs over 4 days**. A) Workflow for whole blood storage and extraction of miRNA from TFLB and Streck NA BCTs. B) Effects of storage temperature at 4°C vs 22°C on plasma over a 4-day time point in TFLB and Streck BCTs.

**TFLB stabilizes plasma at 21°C and 4°C compared to Streck BCTs.**

Whole blood was collected into TFLB and Streck NA BCTs and stored at 21°C or 4°C for 1 hour, 2-, 4 days post-blood draw. Plasma was separated following QIAamp Circulating Nucleic Acid Kit (55114) specifications. Photos were taken to demonstrate changes that occur in plasma appearance over all time points and temperature conditions. miRNA was extracted using QIAamp Circulating Nucleic Acid Kit from 2 mL whole blood stored at 21°C and 4°C for 1 hour, 2-, 4 days post-blood draw (Figure 6A). Plasma color is similar for all BCTs under 21°C and 4°C storage at 1 hour. A slight color change is seen in TFLB and Streck BCTs at 21°C for 4 days post-blood draw (Figure 6B). Streck NA BCTs have a red tint color change that occurs under 4°C storage conditions on day 4 post-blood draw, compared to TFLB. The color change in Streck NA BCTs may be indicative of RBC-induced hemolysis (Figure 6B). Blood drawn in TFLB at 21°C and 4°C had minimal changes in plasma appearance compared to Streck NA BCTs over 4 days. In TFLB, plasma appearance at 4°C stayed close to that at 21°C over 4 days. The Streck Nucleic Acid BCTs were held below their labeled storage range for this comparison.

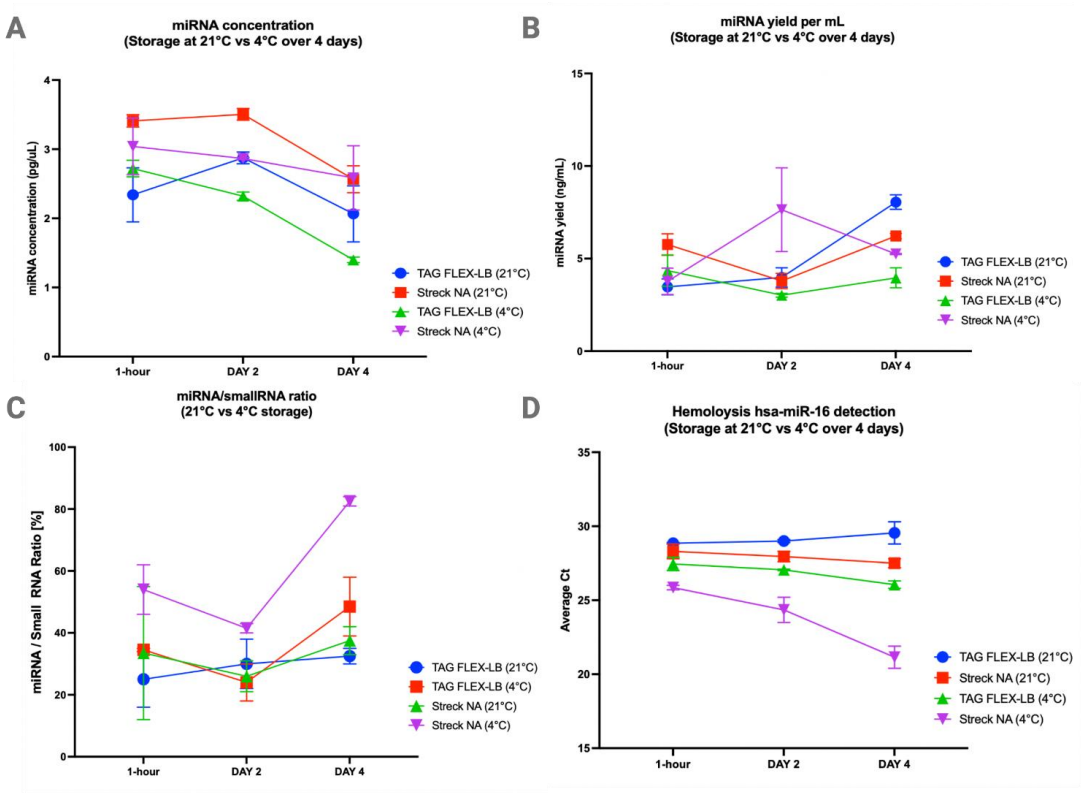



**Figure 7. miRNA concentration, yield, ratio and miR-16 in TFLB and Streck Nucleic Acid BCT at 21°C and 4°C over 4 days.** A) RNA concentration from plasma in TFLB and Streck NA BCTs at 21°C and 4°C**.** miRNA concentration (ng/uL). B) Comparison of miRNA concentration from plasma in TFLB and Streck NA BCTs at 21°C and 4°C. C) miRNA/small RNA ratio percentage in TFLB and Streck NA at 21°C and 4°C. D) hsa-miR-16 hemolysis detection in TFLB and Streck NA BCTs at 21°C and 4°C. TFLB at 21°C (blue line), TFLB at 4°C (green line) for 1 hour, 2-, and 4-day time points. miRNA concentration from Streck NA at 21°C (red line) and Streck NA at 4°C (purple line) for 1-hour, 2-, and 4-day time points.

**Effects of temperature storage conditions on miRNA concentration and yield in TFLB and Streck BCTs**

miRNA concentration in blood drawn in TFLB at 21°C increased from 3.5 to 8.0 ng/mL. At 4°C, plasma miRNA concentration fell over 4 days by about half in TFLB (about 2.7 to 1.4 pg/uL) and by about 15% in Streck Nucleic Acid BCT (3.05 to 2.6 pg/uL) (Figure 7A). The percentage of miRNA/small RNA in TFLB is maintained at 21°C and 4°C, while Streck NA BCTs has a 40% increase in miRNA/small RNA ratio after four days at 4°C compared to samples held at 21°C (Figure 7C). miR-16 activity was analyzed in all TFLB and Streck NA samples to identify any hemolysis occurring under various storage conditions. In TFLB samples, miR-16 Ct values moves from about 27.4 to 26.0 cycles under 4°C, suggesting slight hemolysis over time. In Streck NA samples, miR-16 Ct fell by about 4 cycles at 4°C (about 25.6 to 21.8), against about 1.4 cycles in TFLB (27.4 to 26.0) (paired, n = 2). (Figure 7D). The

findings from this experiment demonstrated that samples drawn into TFLB can be stored at 4°C or 21°C, maintaining miRNA integrity, concentration, and reduced hemolysis over 4 days compared to Streck NA BCTs.

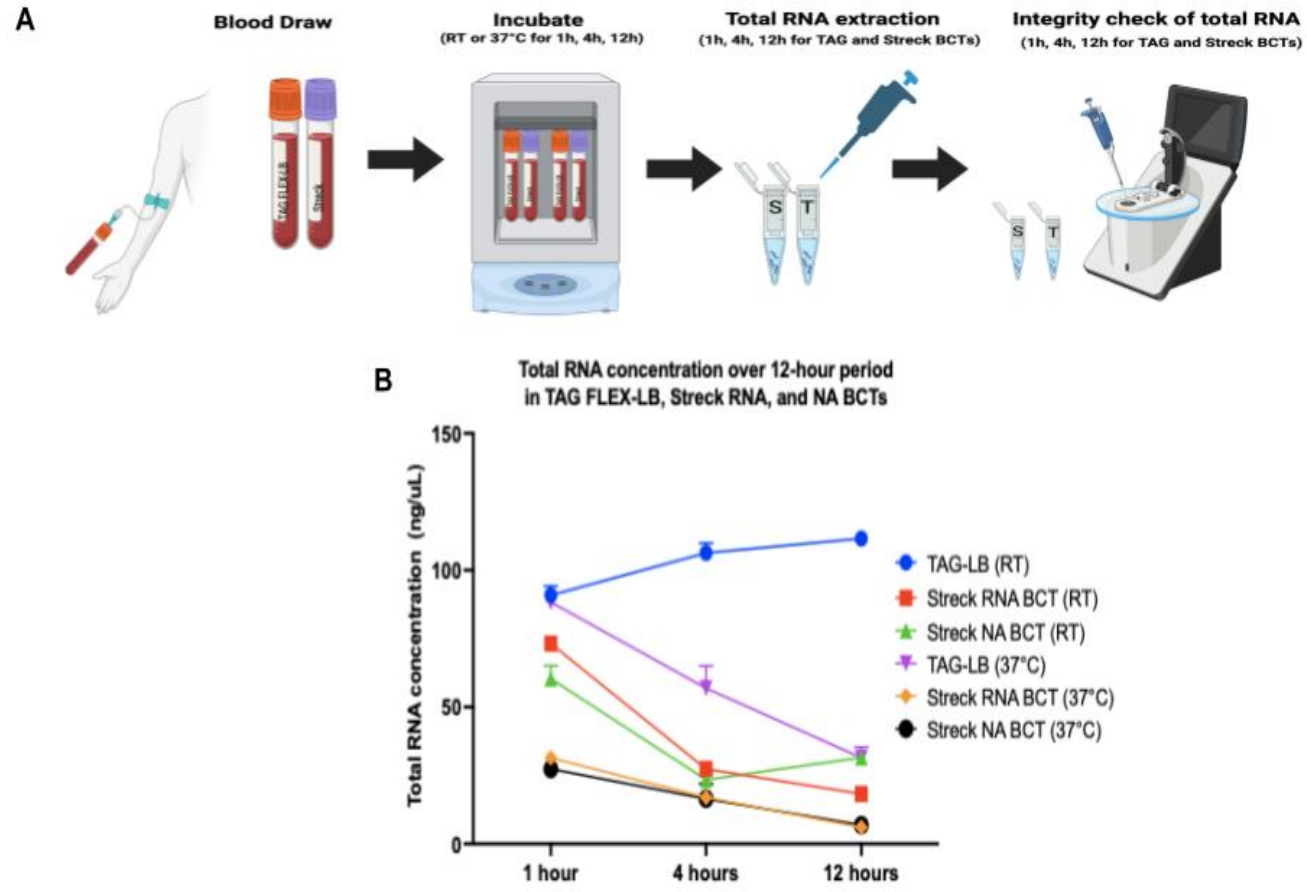



**Figure 8. Total RNA from TFLB, Streck RNA Complete, and Streck Nucleic Acid BCT over 12 hours at ambient temperature and 37 °C.** A) Schematic of experimental design for blood draw, incubation, and total RNA extraction of TFLB, Streck RNA, and Streck NA BCTs. B) RNA concentration (ng/uL) changes for over 12 hours TFLB at 21°C (blue line), Streck RNA at 21°C temp (red line), Streck NA BCT at 21°C temp (green line), TFLB at 37°C (Purple line), Streck RNA at 37°C (Orange line), Streck NA BCT at 37°C (black line).

**Total RNA after 12 hours at 37 °C**

Blood was drawn into TFLB, Streck RNA, and Streck NA BCTs and incubated at ambient temperature (21°C) or 37°C. Total RNA for both temperatures was extracted using QIAamp RNA Blood Mini Kit (52304) for 1-, 4-, and 12-hours. Concentration and purity was measured for all samples using NanoDrop recommended by the manufacturer of the extraction kit. Total RNA in TFLB held at 91 to 111 ng/uL over 12 hours at ambient temperature. At 37 °C it fell from 88 to 31 ng/uL, which was still enough for RT-qPCR (Figure 8B). With the same silica-column kit (QIAamp RNA Blood Mini Kit, 52304), and without the Proteinase K digestion Streck specifies for its RNA tubes, Streck RNA and Streck NA tubes returned 31 and 27 ng/uL after one hour at 37 °C and 6.5 and 7.2

ng/uL at 12 hours, too little to proceed to RT-qPCR (Figure 8B). Since Streck RNA yields at 37 °C were too low for RT-qPCR, the gene expression step was run on TFLB only (n = 2 donors).

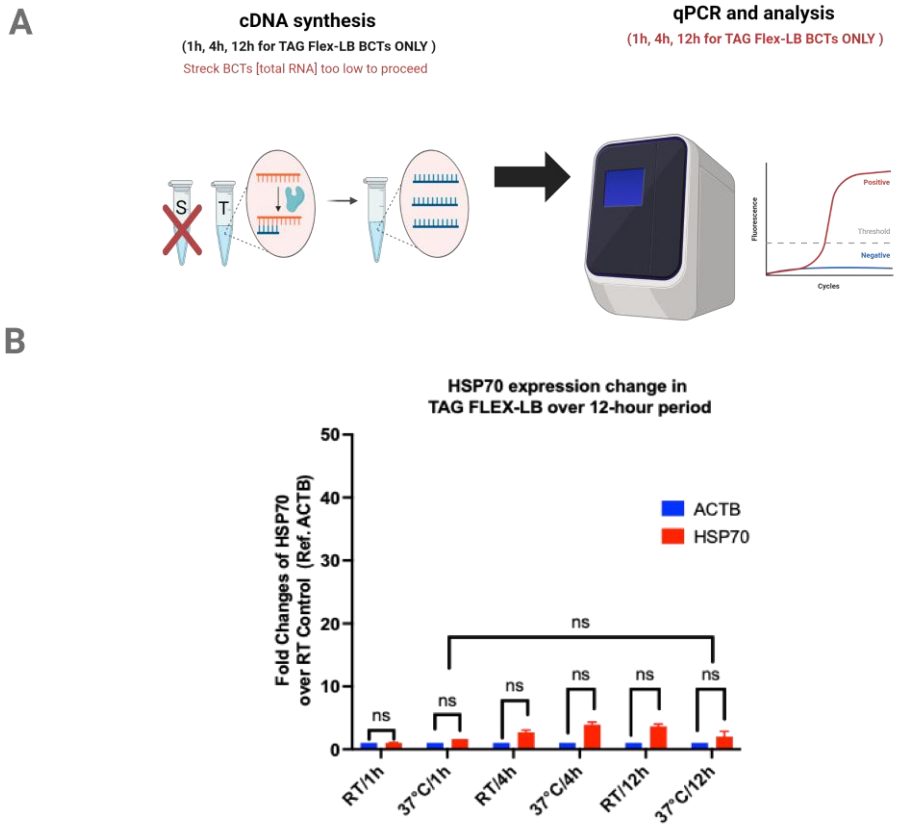



**Figure 9. Gene expression changes for HSP70 in TFLB under heat stress at 37°C over 12 hours. A)** Schematic of experimental design for cDNA synthesis, RT-qPCR, TFLB, Streck RNA, and NA BCTs analysis. **B)** Fold-expression changes of HSP70 (red) normalized over the control, ACTB, at ambient temperature (reference gene; blue).

**HSP70 gene expression change in TFLB under heat stress at 37°C up to 12 hours.**

Since total RNA concentration and purity from Streck BCTs were not optimal for RT-qPCR, analysis was only carried out for TFLB (Figure 9A). Gene expression changes for HSP70 are not different at 21°C or under heat stress at 37°C for 12 hours compared to our reference gene (ACTB), and to HSP70 for 12 hours (Figure 9c). HSP70 gene expression changes over 12 hours under 21°C, and 37°C was unchanged and is likely not altered by minor environmental temperature changes. The cumulative RT-qPCR results further indicate that TFLB can offer thermal protection of whole blood over 12 hours, which was not attainable for Streck RNA and Streck NA BCTs.

**Stabilization of synthetic cancer biomarkers in TFLB**

Variability in storage and transport of samples can impact the stability and preservation of nucleic acids in BCTs, which are needed for the sensitivity and reproducibility of downstream analyses, particularly in next-generation

sequencing (NGS) applications used for diagnosis and targeted gene therapies. The de-identified samples were provided by Your Main Lab, in Reno, Nevada (YML). YML phlebotomists are properly licensed, follow standard blood draw protocols and adhere to the de-identification protocols. Both donors gave written consent. The purpose was to assess the performance of TFLB in stabilizing spike-in nucleic acids under realistic transport and processing conditions compared to other stabilizing BCTs (i.e., Streck cfDNA and Streck NA BCTs). The study did not involve the collection of identifiable human specimens and is limited to in vitro evaluation of biospecimen stabilization technologies for NGS. By validating TFLB in this context, the proposed study sought to improve standardization and reliability of liquid biopsy workflows, which are critical for advancing the clinical translation of NGS-based research.

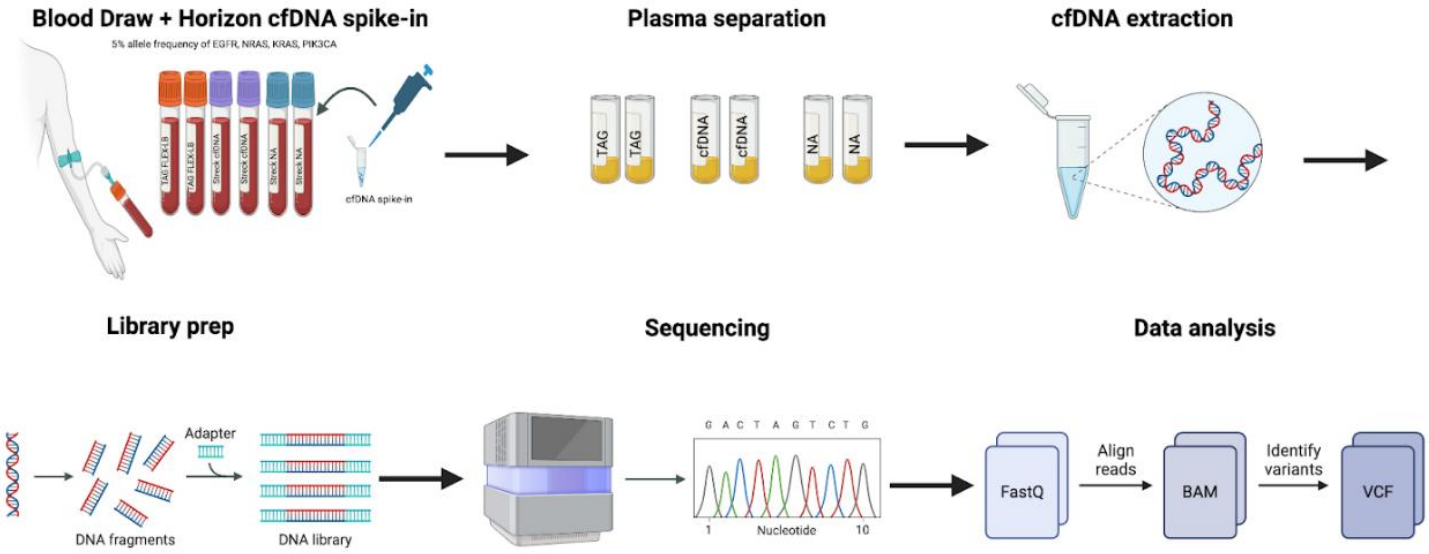



**Figure 10. Workflow for Cancer Spike-in project in TFLB and Streck BCTs.** Blood samples were taken from two study participants and randomly drawn into TFLB, Streck cfDNA, and Streck NA BCTs. Synthetic cfDNA carrying EGFR and PIK3CA variants at 5% allele frequency were added to the blood samples to a final concentration of 11.25 ng/mL (Mimix™ Multiplex I, cfDNA Reference Standard Set; HD780). Plasma was prepared by two-step centrifugation on the first day (day of the blood draw) and after 3, 5, and 7 days of storage at ambient (21°C) temperature. cfDNA was extracted from the plasma samples and sequenced using the Illumina TruSight Tumor 15 panel.

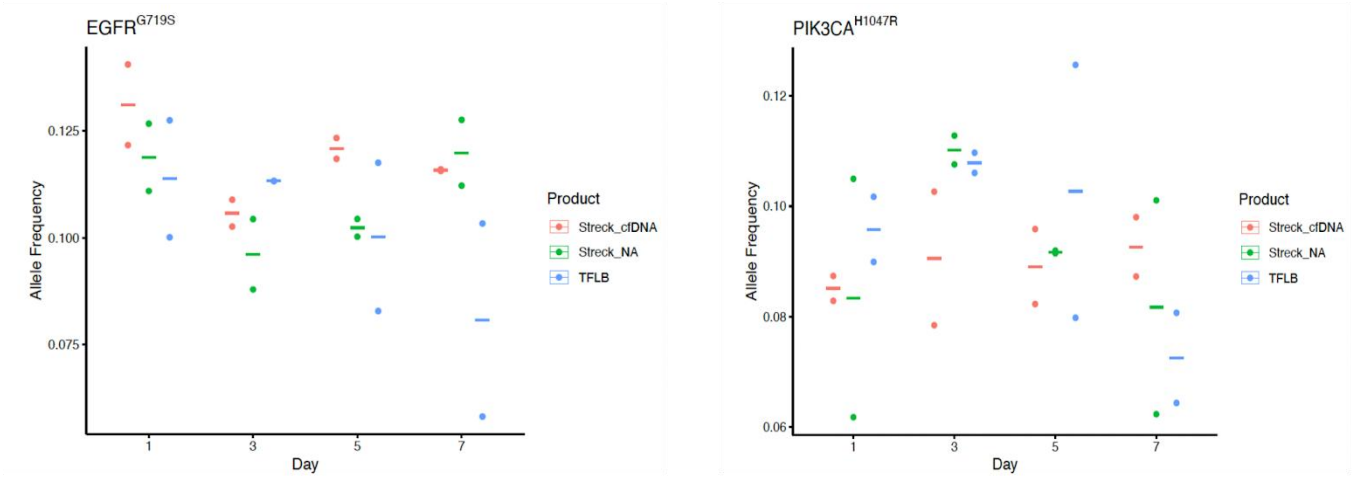



**Figure 11. EGFR and PIK3CA mutations are identified in TFLB up to 7 days.** TFLB (blue dots) identified EGFR and PIK3CA mutations from day 1 (blood draw day) to day 7. Streck cfDNA (red dots) and Streck NA (green dots) BCTs identified mutant alleles from day 1 to day 7.

**Identification of EGFR and PIK3CA in TFLB and Streck BCTs comparison**

Variants were called from the raw sequencing data using the Illumina BaseSpace workflow and filtered for coverage and sequencing quality using BaseSpace defaults. Allele frequencies were calculated from the Allele Depth (AD) field included in the VCF outputs and plotted over time. Horizontal bars show the mean of the two biological replicates for each storage time. In both donors, all three tubes detected EGFR G719S and PIK3CA H1047R at every time point through Day 7. On Day 7, the TFLB mean allele frequency was 0.081 for EGFR and 0.072 for PIK3CA, compared to 0.116 to 0.120 and 0.082 to 0.093 in the Streck tubes (paired, n = 2 donors). KRAS and NRAS variants in the same reference were not analyzed here. Our NGS spike-in project detected cancer markers over 7 days using TFLB.

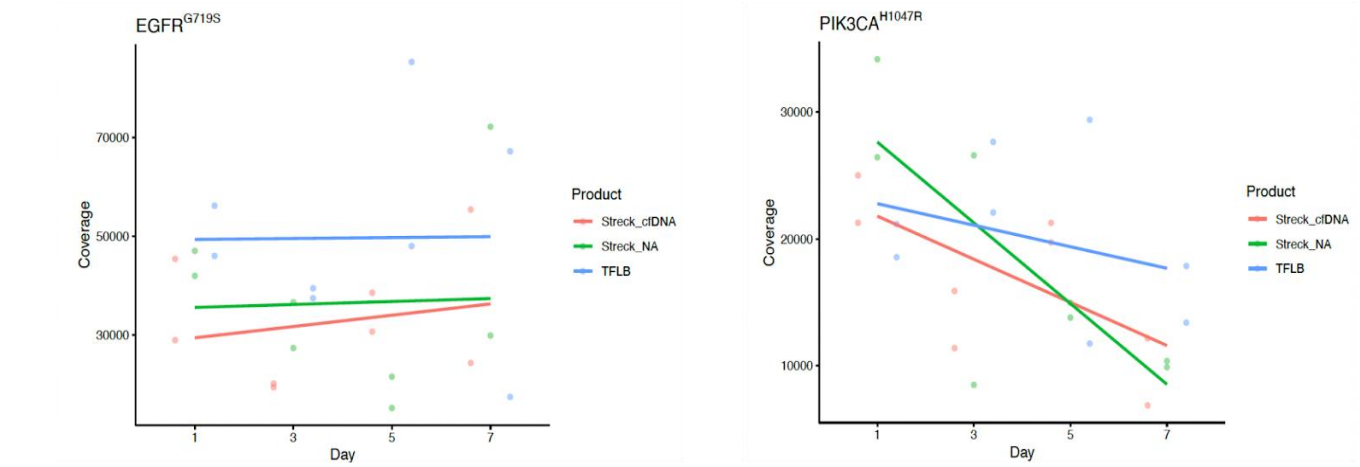



**Figure 12. TFLB has higher PIK3CA amplicon coverage than Streck BCTs at Day 7.** Sequencing depth over the EGFR G719S and PIK3CA H1047R amplicons (TruSight Tumor 15). Points are individual donors; lines are least-squares fits across both donors, shown for trend only.

**Amplicon coverage of EGFR and PIK3CA mutations in TFLB and Streck BCTs comparison**

The trends in amplicon coverage over time for specific amplicons provided by the TruSight Tumor 15 panel, including the variants of interest shown in the Allele Frequency Comparison, were assessed. If cfDNA degradation occurred during storage, a decrease in sequencing coverage of the target amplicons would be expected. Coverage over the PIK3CA H1047R amplicon declined in all three tubes, least in TFLB, which held 13,000 to 18,000 reads at Day 7 against 7,000 to 12,000 in the Streck tubes. Coverage over the EGFR G719S amplicon did not decline in any tube (paired, n = 2 donors). These results from two healthy donors are promising; larger cohort studies are planned to further validate these findings.

**Plasma protein pilot: TGF-β1 and bradykinin over a 6-day ambient hold**

Pre-analytical variability remains a significant challenge in liquid biopsy and multi-omics workflows. During ambient sample holds, metabolically active leukocytes and platelets can undergo *ex vivo* stress or degranulation, artificially inflating circulating protein concentrations and masking true physiological baselines. The study evaluated the stabilization performance of TFLB against standard unpreserved tubes ($K_2$EDTA) and commercial preserved alternatives (Streck NA Plus). This analysis targets platelet-derived factors (TGF-β1) and a kinin peptide (Bradykinin) detection over a multi-day ambient hold to characterize protein stability.

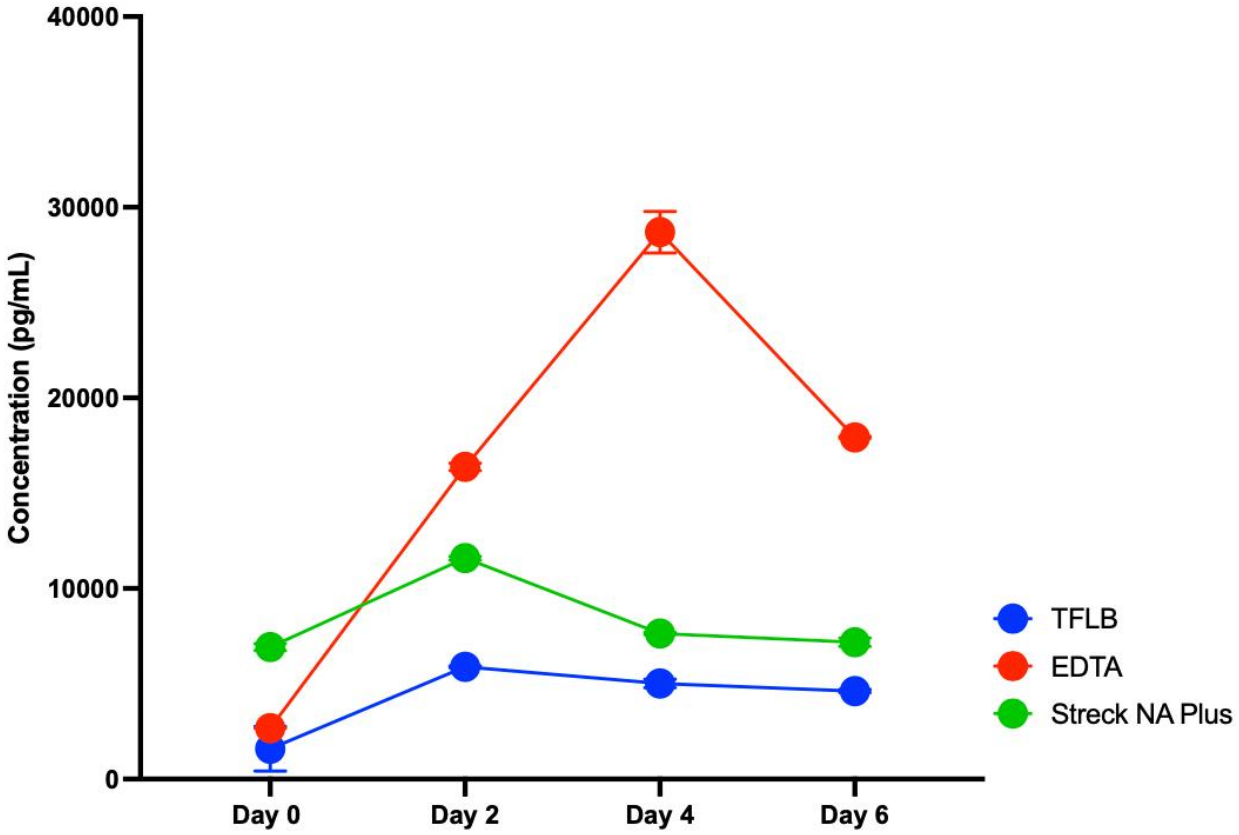



**Figure 13. Plasma TGF-β1 during a 6-day ambient hold in TFLB and other BCTs.** Human TGF-β1 concentration was measured in TFLB (blue line), $K_2$EDTA (red line), and Streck NA Plus (green line) in healthy donor plasma for 6 days at ambient temperature.

**Plasma TGF-β1 over 6 days.** In a pilot comparison at 21 to 22 °C (paired design, 2 donors, 6 tubes per donor, two per tube type), plasma TGF-β1 in $K_2$ EDTA rose from about 2,700 pg/mL on Day 0 to 28,600 pg/mL on Day 4, toward the range R&D Systems reports for healthy serum (32,091 to 95,147 pg/mL), consistent with ex vivo release from platelets. In TFLB, it rose from about 1,500 to 5,900 pg/mL by Day 2 and held between 4,600 and 5,900 pg/mL through Day 6, the lowest of the three tubes at every time point. Streck NA Plus started higher, at about 6,900 pg/mL, and changed least relative to its own Day 0.

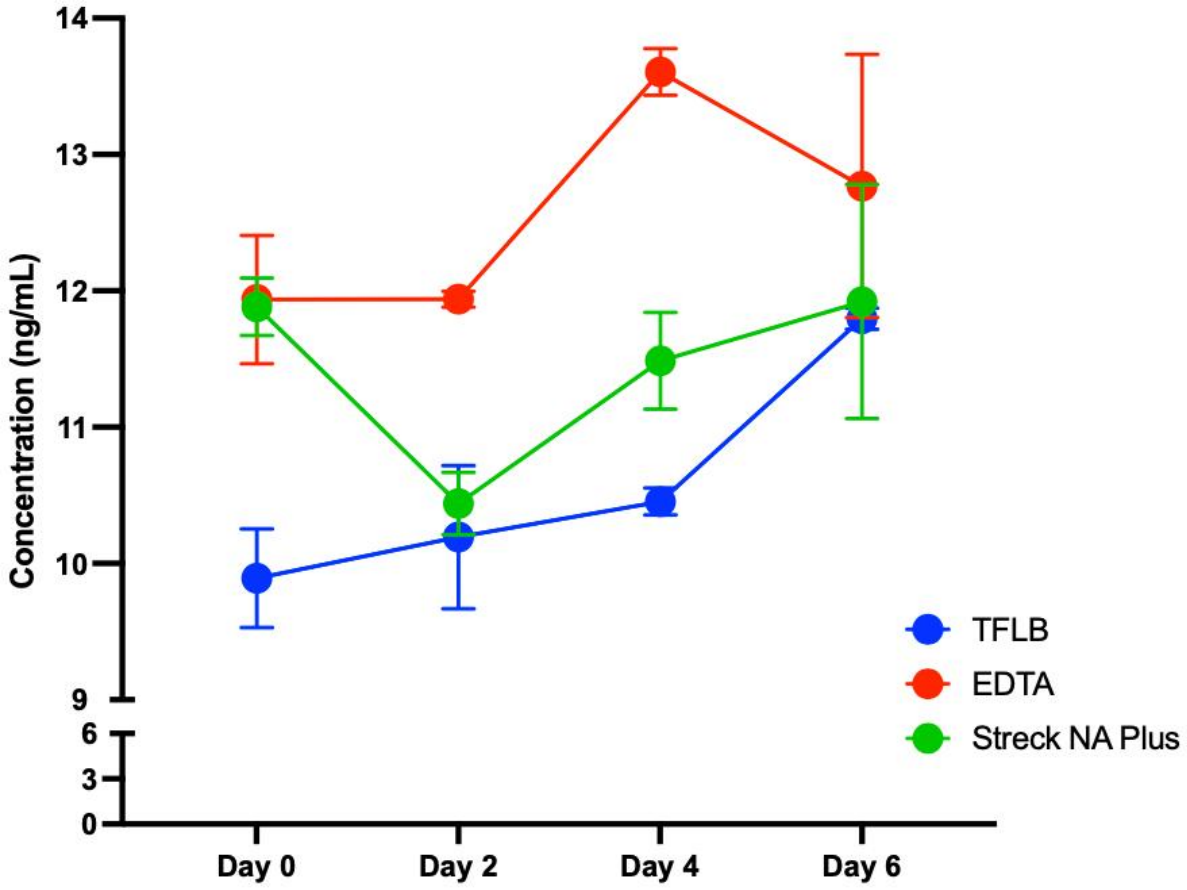
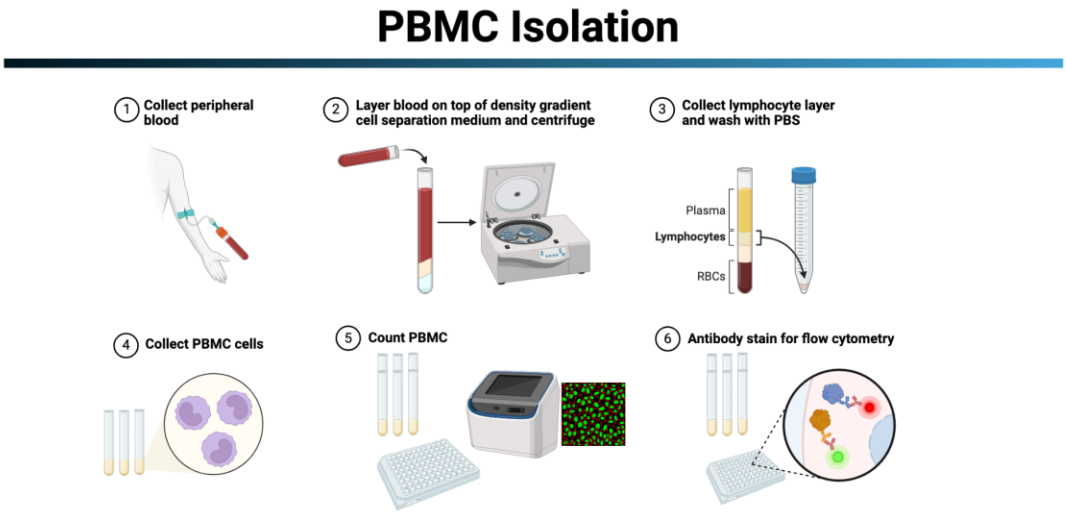
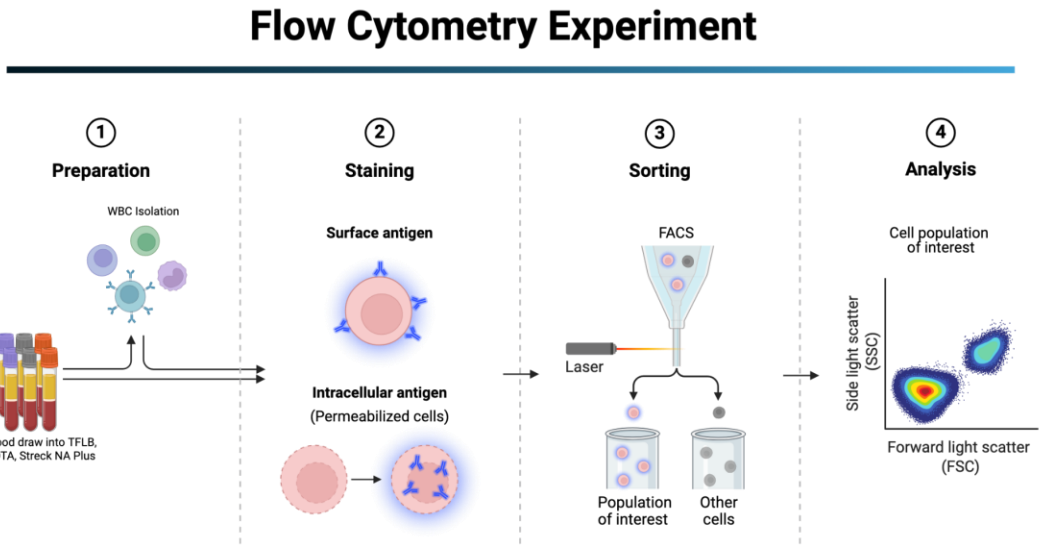



**Figure 14. Longitudinal Bradykinin Concentration over a 6-Day Ambient Hold (Exploratory Marker).** Bradykinin concentration was measured in TFLB (blue line), $K_2$EDTA (red line), and Streck NA Plus (green line) in healthy donor plasma for 6 days at ambient temperature.

**Assessment of small peptide stress responses via bradykinin stability (Exploratory marker).**

Bradykinin immunoreactivity was quantified using a competitive ELISA (Novus NBP2-69969, range from 1.56 to 100 ng/mL). On Day 0, TFLB plasma yielded baseline levels of 9.9 ng/mL, compared to 11.9 ng/mL in both $K_2$EDTA and Streck NA Plus. By Day 6, concentrations across all conditions converged between 11.7 and 12.8 ng/mL. Ex vivo bradykinin generation is driven by contact system activation and countered by enzymatic cleavage; observed levels reflect both collection chemistry and processing variables. Given that these values exceed physiological reference intervals for intact bradykinin measured via LC-MS/MS in protease-inhibited plasma (2.3 to 6.8 pg/mL) by three orders of magnitude, likely due to antibody cross-reactivity with related kinin metabolites, bradykinin is categorized as an exploratory marker. Definitive quantification of intact peptide requires specialized contact-activation inhibition paired with mass spectrometry, and future matrix validation will include synthetic spike-recovery assays to determine if TFLB additives influence colorimetric signal.




**Evaluation of Leukocyte Viability and CD45+ Preservation in TFLB**

Pre-analytical stabilization of leukocytes is essential in liquid biopsy applications to prevent post-draw cell lysis and subsequent genomic DNA (gDNA) contamination of plasma fractions. In this longitudinal study, the stabilization performance of TFLB was evaluated against standard anticoagulant $K_2$EDTA and commercial Streck Nucleic Acid Plus (Streck NA Plus) tubes across ambient hold times of 2, 24, and 48 hours. Biological duplicates (n=2) were processed via density gradient centrifugation followed by spectral flow cytometry acquisition on a Cytek Aurora platform.

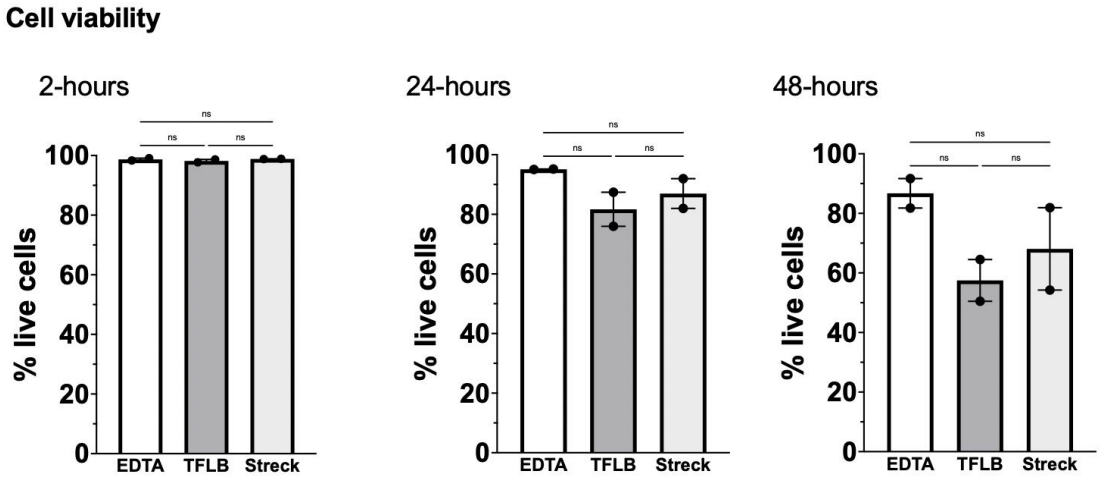



**Figure 15. Flow Cytometric Assessment of cell viability over 48 hours at ambient temperature.** Quantification of the total viable cells was evaluated via gating of the % live single-cell population across 48-hours. Values reflect mean ± SD across biological duplicates (n=2).

Exploratory flow cytometry of recovered leukocytes through 48 hours

Blood from two healthy donors was drawn into TFLB, $K_2$EDTA, and Streck Nucleic Acid Plus BCTs (formerly Protein Plus BCTs), 10 mL per tube, and held at 21 °C. At 2, 24, and 48 hours, mononuclear cells were prepared by density gradient centrifugation and counted on a Countess 3 cell counter. Red-cell lysis buffer was applied at the 24- and 48-hour points only, so the 2-hour counts are not comparable with the later points. Cells were stained with anti-CD45 (eFluor450) and LIVE/DEAD NIR, acquired on a Cytek Aurora with unstained TFLB and Streck controls at each time point, and analyzed with one gating template for all time points. The live-cell fraction was about 98% in all tubes at 2 hours post-blood draw. At 24 hours, it was about 95% in $K_2$EDTA, 87% in Streck Nucleic Acid Plus, and 82% in TFLB. At 48 hours, it was about 87%, 68%, and 57% in the respective BCTs (Figure 15). The CD45+ live fraction was about 2% to 4% of events in every tube at 2 hours. At 48 hours, it was about 12% in TFLB, 8% in $K_2$EDTA, and under 1% in Streck Nucleic Acid Plus (Figure 16); the unstained TFLB control placed about 2% of events in the same gate. Streck Nucleic Acid Plus tubes still yielded 3.1 and 9.0 million cells per mL at 48 hours, against 4.8 and 9.0 million in TFLB (Figure 16B), so the low CD45+ fraction in Streck did not come from a shortage of recovered cells (paired, n = 2 donors).

These results are exploratory. The flow percentages are fractions of acquired singlet events, with no scatter gate, and dye exclusion reports membrane integrity, not viability or function. CD45+ was the only leukocyte marker, and $K_2$EDTA had no unstained control. The low CD45+ fraction in the Streck tube, with cell counts similar to TFLB, could reflect leukocyte loss with other events taking their place, or reduced CD45 antibody binding after crosslinking; this experiment does not separate these possibilities.

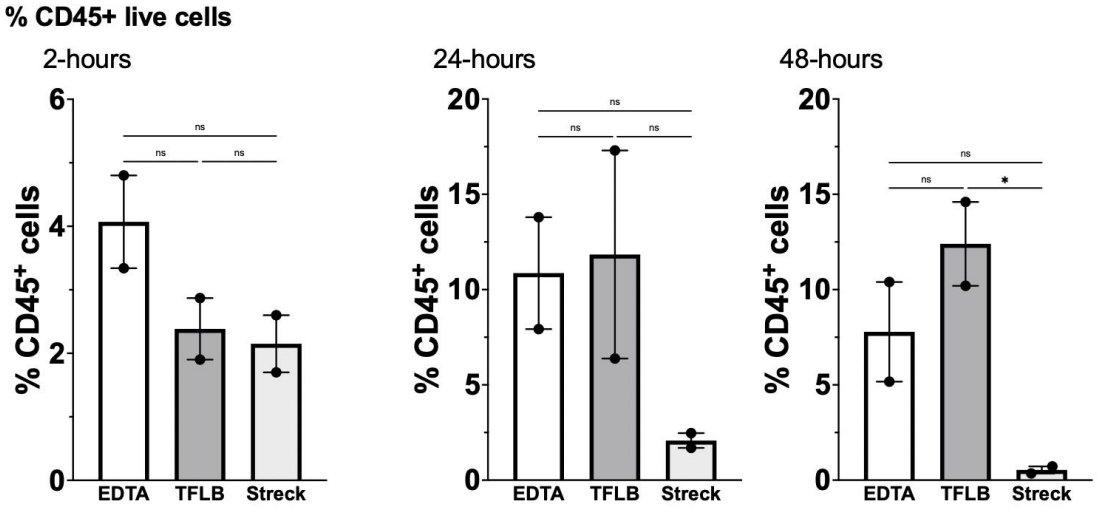



**Figure 16. Flow Cytometric Assessment of Leukocyte Viability.** Quantification of the viable leukocyte fraction was evaluated via gating of the % CD45+ live single-cell population across extended transit windows. Values reflect mean ± SD across biological duplicates (n=2).

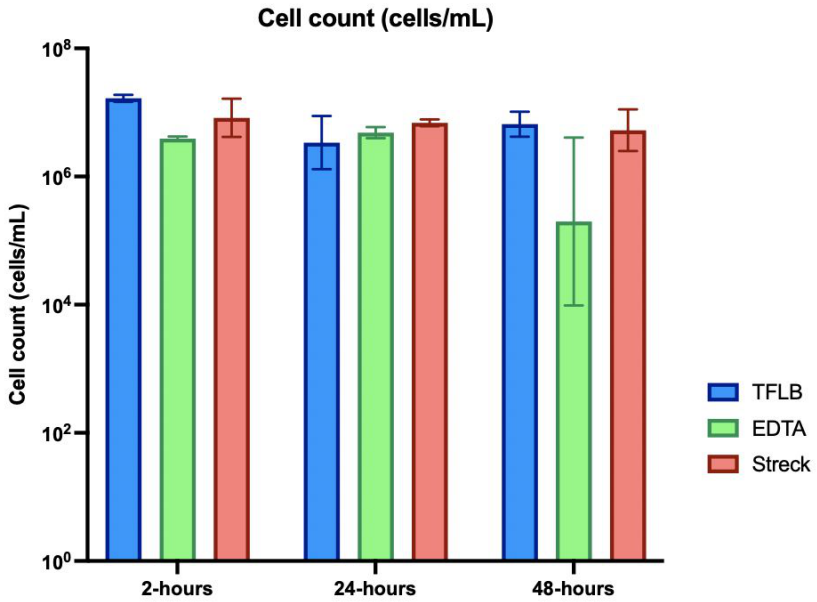



**Figure 16B.** Mononuclear cells count over 48 hours at ambient temperatures. Cells were counted on a Countess 3 after density gradient isolation and are plotted on a log scale as cells/mL, as read by the counter. Red-cell lysis buffer was applied at 24 and 48 hours only, so the 2-hour counts are not comparable with later points. Bars show the geometric mean and geometric SD of two donors (paired, n = 2). At 48 hours, one $K_2$EDTA donor read 23,500 cells/mL and the other 1.68 million, which produces the wide $K_2$EDTA error bar.

**Genomic DNA from the cell fraction through Day 7**

Tumor-informed and germline-filtered ctDNA assays compare plasma DNA with DNA from the patient's blood cells. We asked whether genomic DNA (gDNA) could still be recovered from the cell fraction of the same tubes after an ambient hold. Blood from 2 healthy donors was drawn into TFLB, Streck Cell-Free DNA and Streck Nucleic Acid BCTs and held at 20 to 21 °C. gDNA was extracted from 2 mL whole blood with GeneJet Whole Blood Genomic DNA Mini-Kit (K0782), eluted in 50 uL, quantified by dsDNA Qubit, and sized on the Agilent TapeStation on Days 1, 3, 5, and 7. Every tube was extracted with the same protocol. TFLB returned 67.5 to 52.3 ng of gDNA per uL of blood, with a DNA Integrity Number (DIN) of 9.1 and 9.4 on Day 1 and 8.5 to 8.8 on Days 5 and 7. Extractable gDNA from both Streck tubes decreased by about 90% between Day 3 and Day 5 (Figure 17; paired, n = 2). The Day 7 TFLB value was higher than earlier days; we have not determined why. Intact but scarce DNA is the pattern expected when DNA remains held in crosslinked cells. Published work shows that a decrosslinking step improves long-read sequencing of buffy-coat DNA from Streck tubes (Chrysanthou 2025). We did not test a decrosslinking step here. With the standard extraction used, TFLB did not need this step.

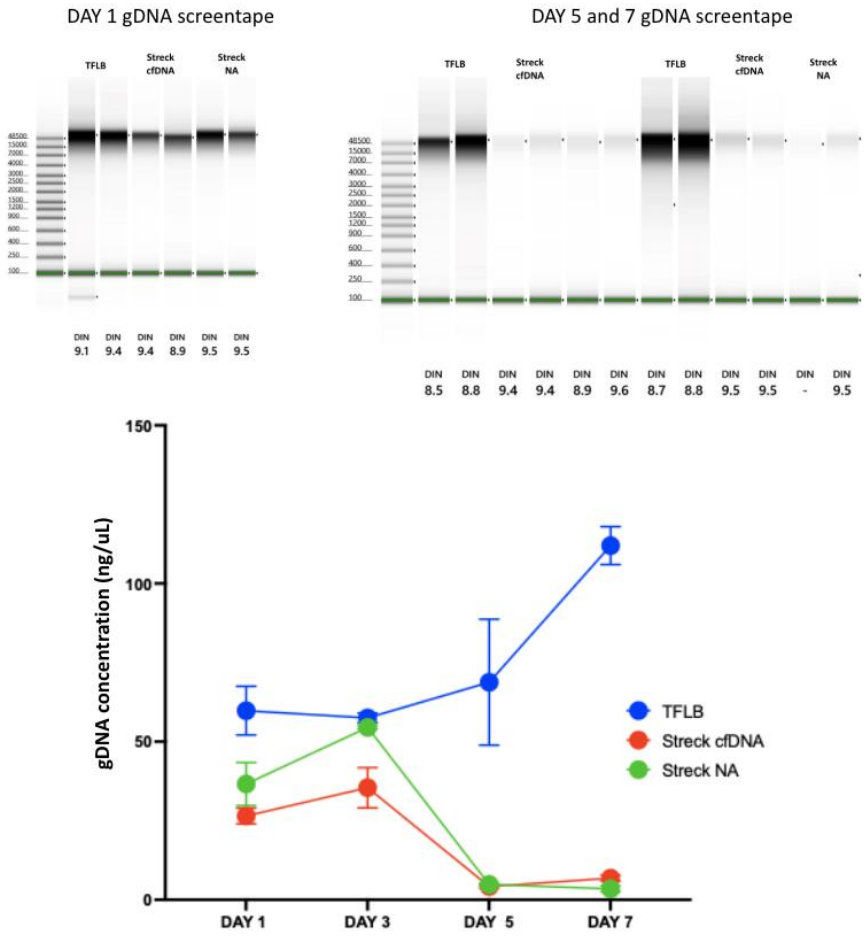



**Figure 17. Genomic DNA (gDNA) extraction yields and structural integrity over a 7-day ambient hold.** Whole blood collected in TFLB, Streck cfDNA, and Streck NA tubes were stored at ambient temperature and subjected to gDNA extraction

at days 1, 3, 5, and 7. A) Representative TapeStation electrophoretic gel panels demonstrating high-molecular-weight structural integrity. DNA Integrity Number (DIN) scores. B) Longitudinal quantification of gDNA concentration (ng/µL). Data are presented as mean ± SD of biological duplicates (n=2).

**Genomic DNA Recovery Across Extended Ambient Hold Times**

Quantification of extraction yields and structural fragment sizing across biological duplicates (N=2) underscores the fundamental divergence between non-formaldehyde stabilization and conventional fixative chemistries under ambient conditions (20–21°C)**.** TFLB displayed consistent gDNA recovery, sustaining stable concentration metrics (~50–70 ng/µL) through 3 days post-collection. At the 7-day terminal timepoint, observed yields increased beyond 100 ng/µL. Streck Nucleic Acid and Streck cfDNA tubes showed moderate baseline recoveries at initial processing, followed by rapid degradation by Day 3 and decreased levels of extractable gDNA by Day 7. Progressive methylene cross-linking irreversibly entrapped target nucleic acids within an insoluble cellular matrix. Electrophoretic sizing on a TapeStation platform confirmed preserved DNA Integrity Number (DIN) values across TFLB preparations. By pairing structural leukocyte preservation, TFLB provides a versatile baseline for downstream applications. Laboratories can utilize a unified blood collection device for paired flow cytometric surface profiling (e.g., CD45+ leukocyte gating) and downstream genomic workflows.

Through 48 hours at ambient temperature, CD45+ leukocytes stayed detectable by flow cytometry in TFLB and $K_2$EDTA and fell to about 1% of acquired events in Streck Nucleic Acid Plus, although the TFLB and Streck tubes yielded millions of cells per mL. Membrane integrity was highest in $K_2$EDTA. With cfDNA, cfRNA and cell-fraction genomic DNA from the same draw, these results support evaluating TFLB where a laboratory needs plasma analytes and leukocytes from one tube. They do not yet establish PBMC subset or functional performance.

## Conclusion

In these feasibility-scale comparisons, TFLB kept plasma DNA free of the genomic DNA rise seen in $K_2$EDTA for 7 days at ambient temperature, on the same course as PAXgene DNA and Streck cfDNA tubes. It held the miRNA share of small RNA within the narrowest band of the three tubes tested, returned total RNA suitable for RT-qPCR after 12 hours at 37 °C, carried spiked EGFR and PIK3CA variants through Day 7, kept CD45+ leukocytes detectable by flow cytometry through 48 hours, and in a pilot comparison limited the ex vivo rise of plasma TGF-β1 seen in $K_2$EDTA. It does so without the Proteinase K digestion the MagMAX cfDNA workflow specifies for Streck cfDNA tubes. The studies are small, and the next section states what they do not show.

- Cell-free DNA: total plasma DNA stayed under 5 ng/mL through Day 7, against 180 ng/mL in $K_2$EDTA (Figure 2).
- Cell-free RNA: the narrowest miRNA ratio band of the three tubes tested over 7 days (Figure 3).
- Heat: total RNA recoverable for RT-qPCR after 12 hours at 37 °C (Figure 8).
- Workflow: no Proteinase K digestion with the MagMAX cfDNA kit.

## Limitations and Future Work

Every comparison in this note comes from one or two healthy donors, so the results are directional. Published tube validations have used up to 60 donors (Medina Diaz 2016). In the 37 °C experiment, Streck tubes were extracted with a silica-column kit without the Proteinase K digestion Streck specifies, so that result describes compatibility with that kit, not Streck tube performance under Streck's protocol. The 4 °C experiments held Streck tubes below their labeled range to model an ice-pack excursion, and plasma appearance was judged visually. Hemolysis was inferred from hsa-miR-16 Ct alone. In the NGS study, observed allele frequencies ran above the nominal 5 percent of the reference material. The protein data are a pilot: platelet activation was not measured, and the bradykinin immunoassay reads three orders of magnitude above mass-spectrometry values, so it is exploratory. The flow data are exploratory: event fractions for one leukocyte marker, with cell counts by Countess, and they do not measure function. The genomic DNA comparison used one extraction protocol without a decrosslinking step. Planned work: larger donor sets, circulating tumor cell evaluation, PBMC subset and functional testing, mass spectrometry confirmation of bradykinin, and a multiplex plasma protein study.

## Operational and Economic Implications for High-Throughput Laboratories

**Fewer steps per cfDNA run.** With the MagMAX Cell-Free DNA Isolation Kit, Streck Cell-Free DNA BCT plasma requires a Proteinase K digestion (15 uL per mL of plasma, 20 minutes at 60 °C); TFLB plasma does not. Per run, that removes one 20-minute incubation and, at US list price, about $2.30 to $3.25 of Proteinase K per sample for 3 to 4 mL of plasma. PAXgene DNA, Norgen and CellSave tubes also skip the digestion on this kit.

**Collection decoupled from processing.** Like other preservative tubes, TFLB does not need the 2- to 4-hour processing window of $K_2$EDTA (Leest 2024), so samples drawn late in the day or before a weekend can wait for the next scheduled run.

## Learn more about TFLB and how it can improve your liquid biopsy results and workflow, while reducing costs.

We invite researchers to partner with TAG in exploring the future of research with TFLB.

TAG welcomes questions and can share additional data and samples on request to support liquid biopsy innovation and accelerate the future of molecular diagnostics.

**Let's discuss your applications and needs, and the potential fit for TAG FLEX-LB**

**Schedule a detailed call with TAG scientists**

- email: tflb@tagreagents.com
- call: +1-775-237-8799