TFLB is designed to stabilize cfDNA, cfRNA, total RNA, intact cells, and proteins in a single tube.

Abstract

The pre-analytical stability of whole blood is a critical factor for the reliability of liquid biopsy assays. This technical note evaluates the performance of the TAG FLEX-LB (TFLB) Blood Collection Tube (BCT), a novel stabilization technology, against market-leading Streck BCTs and standard K2EDTA tubes. Key performance metrics including cell-free DNA (cfDNA), cell-free RNA (cfRNA), and total RNA stability were assessed over 7 days under various storage and thermal stress conditions (4°C, ambient, 37°C). Results demonstrate that TFLB provides cfDNA stability equivalent to Streck BCTs while offering superior preservation of miRNA integrity, evidenced by a minimal 4% change in the miRNA:Small RNA ratio over 7 days compared to 19-30% shifts in 5 days for Streck. Furthermore, TFLB prevents the cold-induced (4°C) hemolysis observed in competitor tubes and preserves total RNA integrity under significant heat stress (37°C) that resulted in unusable RNA yields from Streck BCTs with a common silica-column extraction method. These findings position TFLB as a robust and reliable single-tube solution for preserving multiple analyte types, enhancing data quality and workflow flexibility for liquid biopsy research.

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

- **Eliminates Sample Degradation Challenges by Stabilizing Multiple Biomarkers in One Tube, Reducing Variability and Enhancing Reliability**: Stabilizes cfDNA, cfRNA, total RNA, extracellular vesicles, proteins, and intact cells.
- **Thermal Stability**: Maintains sample stability for 7 days across a range of temperature variations typical of sample transport including: ambient. 4°C, and transient heat spikes. This supports reliable results, reducing the risks of typical sample collection, shipment, and lab processing.
- **Cost-Effective for High-Quality Results at Scale**: Excellent performance at a price significantly lower than other stabilizing BCTs.

Table 1: Summary Table of Results

Feature / Metric	TAG FLEX-LB (TFLB)	Streck BCTs	Standard EDTA
cfDNA Stability (Ambient)	Stable up to 7 days	Stable up to 7 days	Unstable; gDNA contamination after 2 hours
cfRNA/miRNA Integrity	**Exceptional:** Stable ratio (4% change in 7 days)	**Unstable:** Ratio shifts 19-30% in 5 days, compromising normalization	Rapid degradation; Unsuitable for cfRNA analysis
Hemolysis Control (4°C)	**Superior:** Prevents cold-induced hemolysis, protecting sample integrity	**Compromised:** Significant hemolysis releases inhibitors & contaminants	High risk of hemolysis upon storage or delay
Thermal Resilience (37°C)	**Robust:** Preserves total RNA after 12hr heat stress	**Unstable:** Yields unusable RNA with common silica-column extraction methods	Unstable; Requires immediate processing at 4°C
Workflow Efficiency	**Streamlined:** Direct plasma isolation	**Multi-Step:** Requires Proteinase K digestion (20 min **incubation)**	Requires immediate processing (within 2 hours)

TAG FLEX-LB BCT 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 thermally stabilize whole blood while preserving circulating cell-free nucleic acids (cfDNA, cfRNA), extracellular vesicles, proteins, and intact cells for 5 or more days.

Table 2: Comprehensive Performance Matrix of TFLB vs. Competitor BCTs

Analytex	Condition	Time Point	Metric	TFLB Result	Streck BCTs Result	EDTA Result	Source Figure
cfDNA	Ambient (20-22 °C)	7 Days	Stability	Stable	Stable	Unstable (>2h)	Fig 2
cfRNA/miRNA	Ambient (20-22 °C)	7 Days	miRNA:Small RNA Ratio	4% change	19-30$ change (in 5d)	N/A	Fig 3B
Whole Blood	Refrigerated (4°C)	6 Days	Visual Hemolysis	Minimal	Prominent	High Risk	Fig 4B
cfDNA	Refrigerated (4°C)	6 Days	Yield Stability	Stable	Decreased	N/A	Fig 5B
Total RNA	Heat Stress (37°C)	12 Hours	RNA Yield (ng/ul)	27 ng/ul	<7 ng/ul (Unusable)	Unstable	Fig 8B
Spiked-in cfDNA	Ambient (20-22 °C)	7 Days	Amplicon Coverage	Higher retention	Lower retention	N/A	Fig 12

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). Plasma from TFLB BCTs maintained usable volume as RBCs were protected from significant hemolysis over 7 days similar to Streck cfDNA and Streck NA BCTs. CellSave BCTs struggled to maintain plasma volume over 7 days, likely due to RBC hemolysis, which releases water and hemoglobin, affecting plasma volume (Figure 1). TFLB is optimal at maintaining plasma volume, which is critical for the initialization of any downstream applications.

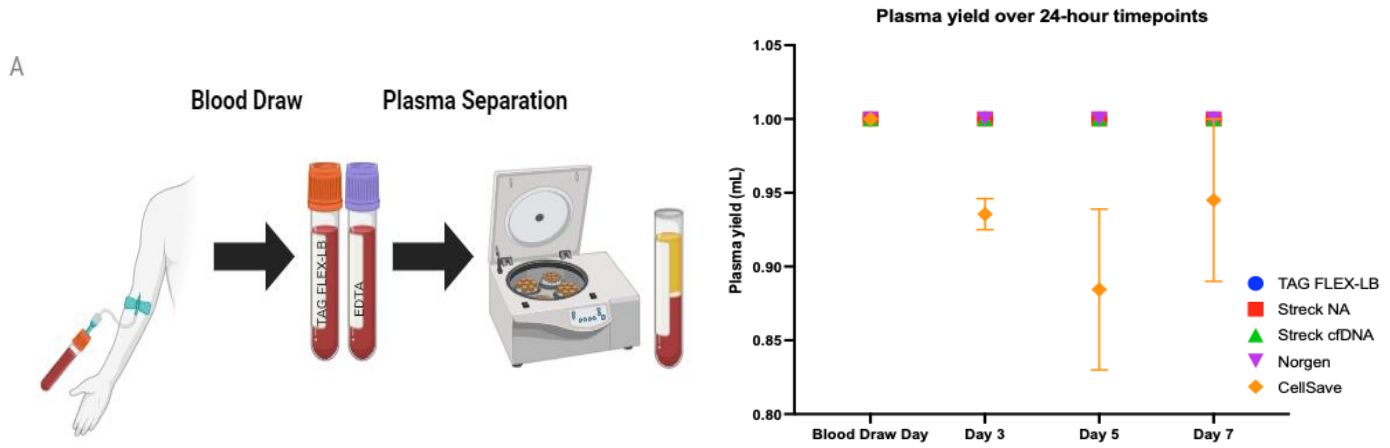

Figure 1. TFLB preserves plasma quantity for 7 days at ambient temperature. A) Blood was collected from a healthy donor into TFLB, Streck cfDNA, Streck NA, Norgen, and CellSave BCTs. Plasma was centrifuged at 24-hour time points, at ambient temperature, with a double spin protocol. Plasma volume was measured after centrifuging 2 mL of whole blood.

Robust cfDNA Stabilization with TFLB: Reducing Signal Loss and Noise

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 (ProK), while Streck cfDNA and Streck Nucleic Acid BCTs did use ProK (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 cfDNA recovery was analyzed by cfDNA ScreenTape (Agilent 4150 TapeStation). From the point of collection through transport to the lab, BCTs are often subject to unwanted temperature changes. Thermal stress causes hemolysis and apoptosis of the cells, weakening or destroying the targeted biomarker signal. However, samples stabilized in TFLB BCTs offer strong cfDNA signal recovery, losing only about 10% cfDNA throughout the 7-day timepoint (Figure 2b). This trend is also seen with PAXgene and both Streck BCTs (Figure 2b). Norgen BCTs show up to 5 days of stabilization (Figure 2b). EDTA and CellSave show stabilization for Day 1, however, the cfDNA recovery significantly decreases by Day 3 for both BCTs. Further, EDTA BCTs show time-dependent increases in plasma DNA concentration due to lysis, while CellSave BCTs failed to maintain sufficient cfDNA showing less than 1 ng/ul of cfDNA. TFLB BCTs demonstrated stable DNA and cfDNA concentrations and comparable results to PAXgene and Streck BCTs (Figure 2C).

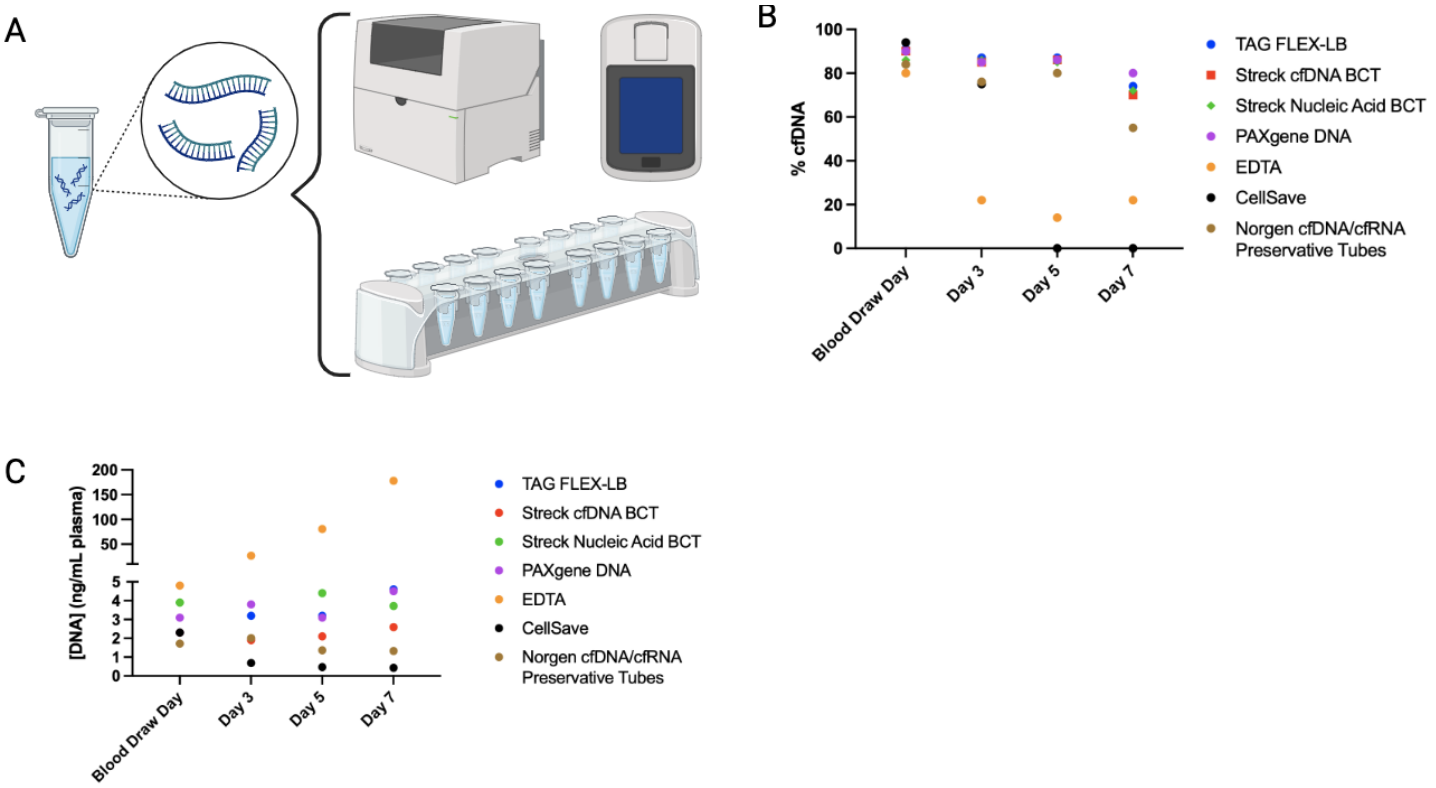

Figure 2. Cell-free DNA recovery is stable in TFLB for 7 days. A) cfDNA recovery used bead extraction and analysis. B) cfDNA analysis used cfDNA ScreenTape and C) cfDNA concentration was analyzed by QUBIT dsDNA HS assay.

TFLB maintains micro- and circulating cell-free RNA for 7 days while limiting apoptosis and hemolysis 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). MicroRNA QUBIT demonstrates that TLFB BCTs has no substantial concentration rise from blood draw day (Day 0) to Day 7. In comparison, the Streck RNA Complete BCT and the Streck Nucleic Acid (NA) BCTs also show no significant gain throughout the time points. (Figure 3A). TFLB offers a stable miRNA / Small RNA ratio over the 7-day time points, with only a 4% difference. While blood drawn in Streck RNA BCTs changes over 30% from Day 0 to Day 5. This trend is similar in the Streck NA BCTs, with a 19% difference between Day 0 to Day 5 (Figure 3B). Additionally, the electropherogram from Day 0 to Day 7 has no differences in integrity for TFLB (Figure 3C). Interestingly, Streck RNA Complete and NA BCTs also show similar integrity throughout the time points. 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 BCTs show a slight downward trend of degradation over time, but no hemolytic activity is detected (Figure 3D). Both Streck RNA Complete and Streck NA BCTs show high hemolytic activity with an upward C_t trend on Day 3, to a drop in C_t value through Day 7 (Figure 3D). Stabilization of RNA is crucial to the field of liquid biopsy as it enables the identification of important cancer biomarkers that enter the bloodstream.

The stabilization of whole blood in TFLB BCTs offers 7-day stability of miRNA, with minimal hemolysis, which is optimal for liquid biopsy techniques.

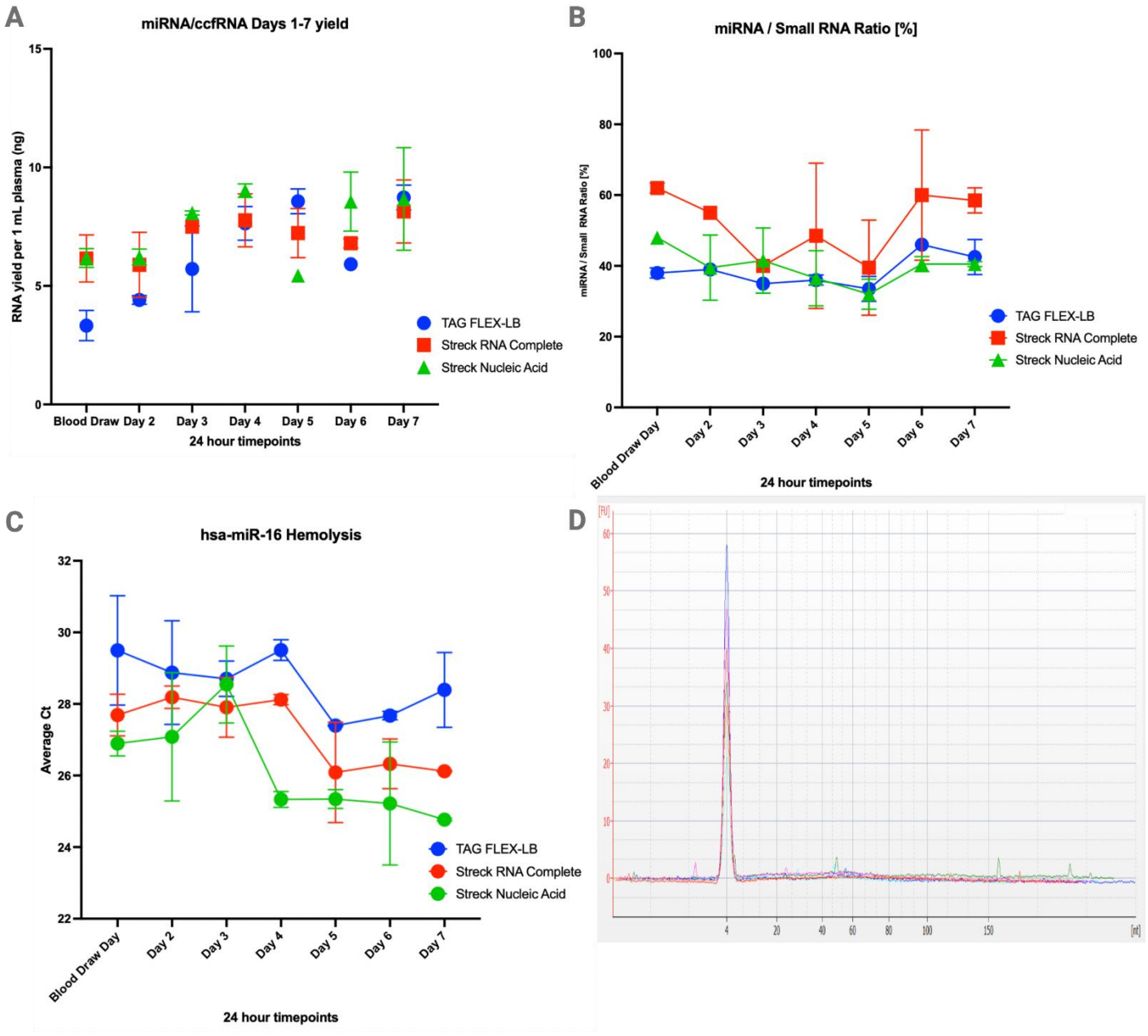

Figure 3. miRNA concentration and integrity is preserved in TFLB for 7 days. A) micro and circulating cell-free RNA yield. B) miRNA / Small RNA Ratio [%] utilizing Small RNA Bioanalyzer to evaluate noise. C) Electropherograms for miRNA for TFLB (light blue and dark green), Streck RNA Complete (red and orange), and Streck Nucleic Acid (light green and dark blue). D) Streck BCTs show a progressive decrease in Ct values from Day 3 to Day 7, indicating increasing levels of the hemolysis marker hsa-miR-16.

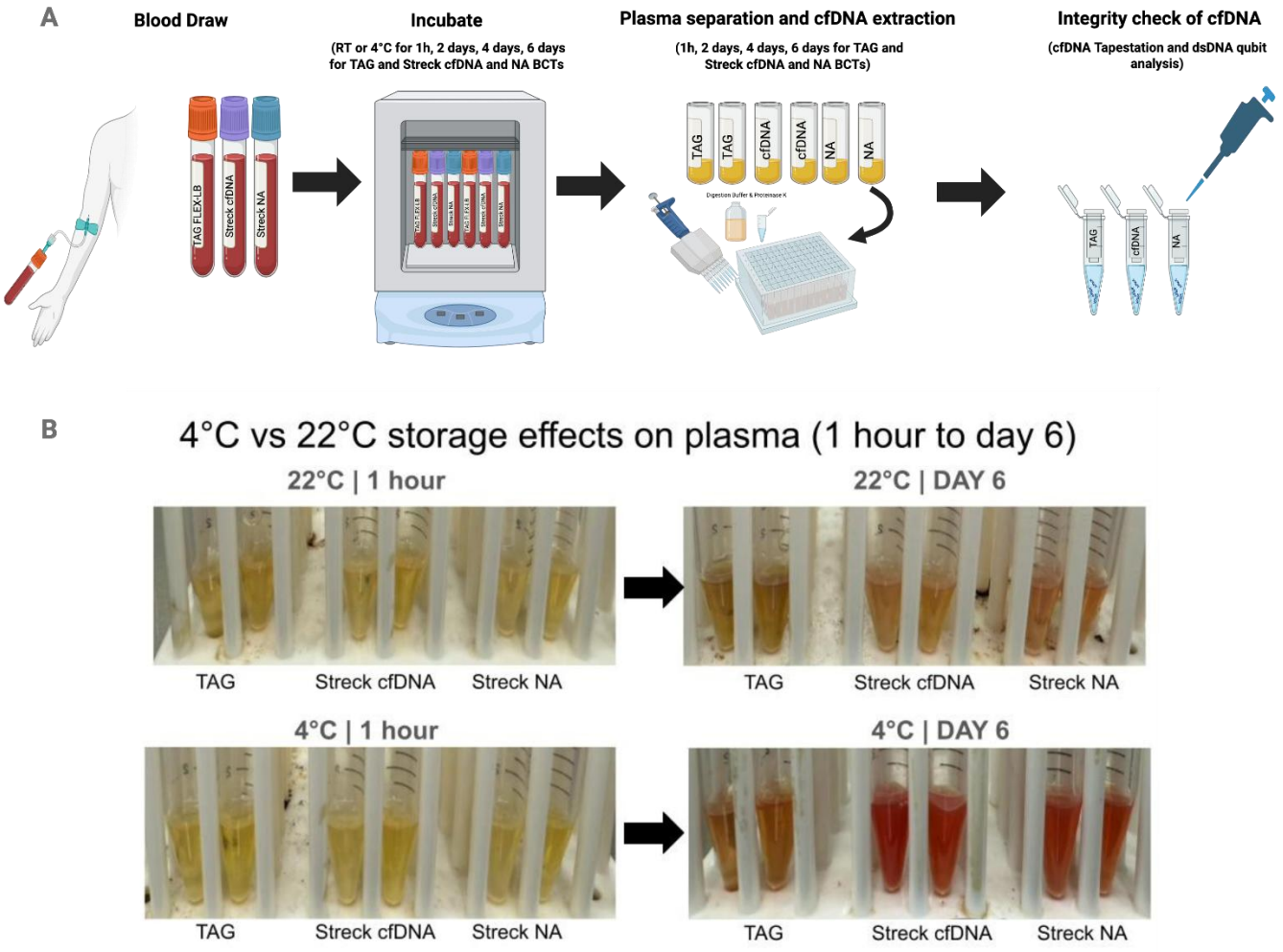

Figure 4. **Storage at ambient vs 4°C in TFLB, Streck cfDNA, and Streck NA BCTs over 6 days.** A) Workflow for whole blood storage and extraction of cfDNA from TFLB, Streck cfDNA, and Streck NA BCTs**.** B) Storage temperature at 4°C vs 21°C affects plasma over a 6-day time point in TFLB and Streck BCTs.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

Blood was collected into TFLB, Streck cfDNA, and Streck NA BCTs and stored at ambient (20- 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 Biosystem 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 blood collection 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 ambientfor 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 BCTs can be stored at 21°C and 4°C, with minimal hemolysis occurring compared to other stabilizing BCTs.

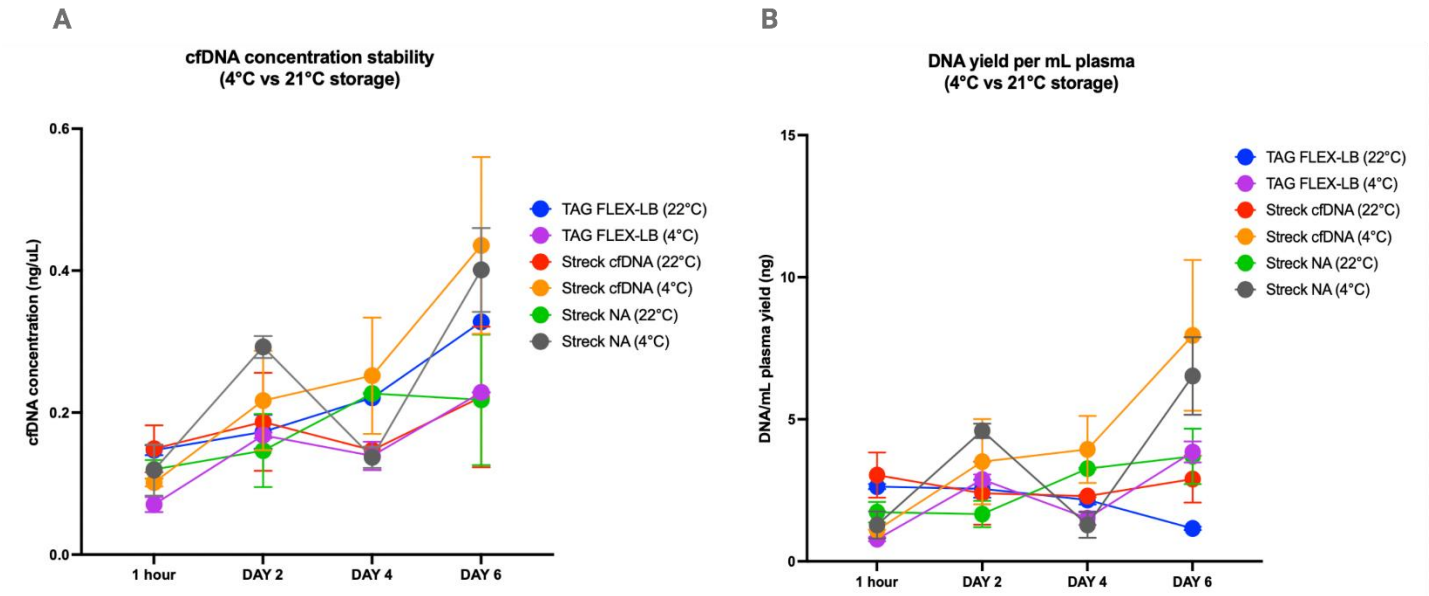

Figure 5. TFLB maintains cfDNA concentration and yield during refrigerated storage, while competitor BCTs show signs of degradation. A) Comparison of cfDNA concentration from plasma in TFLB, Streck cfDNA, and Streck NA BCTs at 22°C and 4°C. B) Comparison of cfDNA yield per 1 mL Plasma of TFLB, Streck 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 5-day timepoints. Streck cfDNA at 22°C (red line) and Streck cfDNA at 4°C (orange line) for 1-hour, 2-, 4-, and 5-day timepoints. Streck NA at 22°C (green line) and Streck NA at 4°C (black line) for 1-hour, 2-, 4-, and 5-day timepoints.

Superior cfDNA Stability and Yield During Refrigerated Storage (4°C)

cfDNA yield for TFLB BCT samples stored at 22°C and 4°C was consistent from 1-hour up to day 6 post-blood draw (Figure 5A). cfDNA concentration of samples drawn in TFLB and stored at 22°C or 4°C also remained consistent for the 1-hour up to 6-days. Streck cfDNA and NA BCTs had increased concentration, specifically when stored at 4°C compared to ambient temperature (Figure 5B). The increase in concentration in Streck BCTs is likely due to increased hemolysis. Streck cfDNA and NA BCTs had decreased cfDNA yield in 4°C conditions from 1-hour to 6-days post-blood draw compared to the samples held at ambient temp (22°C). DNA yield in TFLB BCTs was maintained over the 6-day time point at ambient and 4°C. The results suggest that cfDNA is stabilized in TFLB at 4°C from 1 hour up to 6 days post-blood draw. Streck BCTs have increased hemolysis and are less stable under 4°C storage conditions. The findings from this experiment demonstrate that samples drawn into TFLB BCTs can be stored at 4°C or ambient temperature, maintaining cfDNA integrity

and concentration. However, Streck cfDNA and Streck NA BCTs struggle to maintain cfDNA concentration and integrity at 4°C storage conditions.

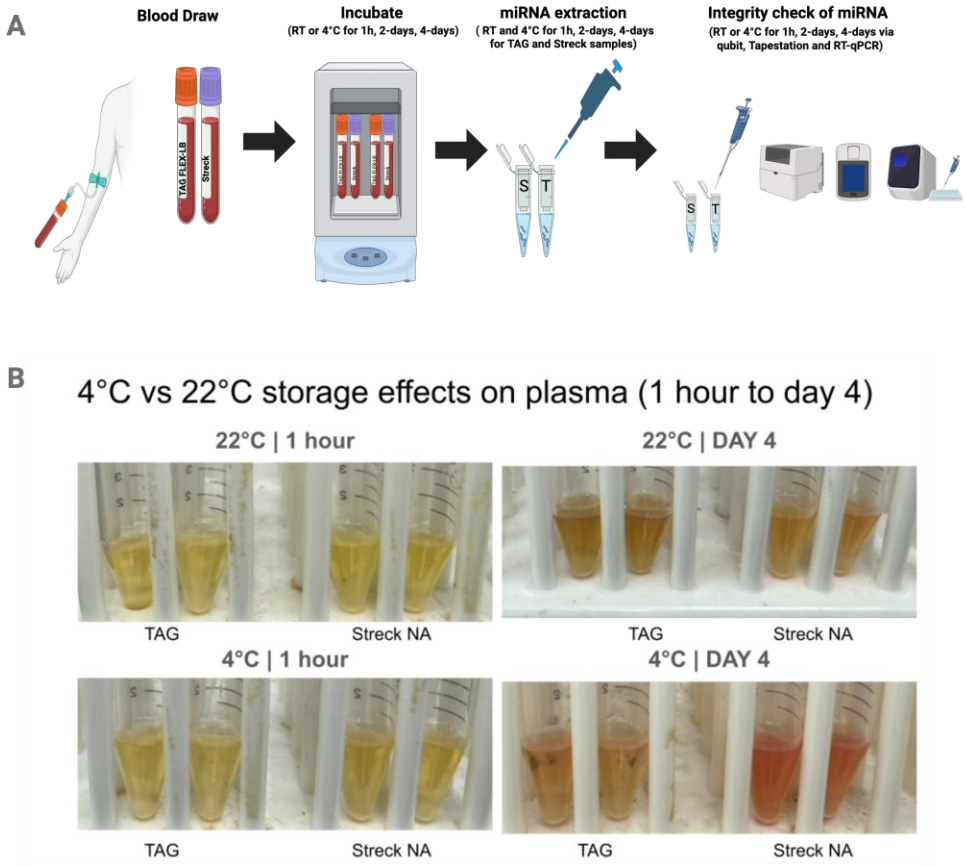

Figure 6. miRNA stability at 22°C and 4°C in TFLB and Streck NA BCTs over 4 days. A) Workflow for whole blood storage and extraction of miRNA from TFLB and Streck NA BCTs. B) Effects of storage temperature at 4°C vs 22°C on plasma over a 4-day time point in TFLB and Streck BCTs.

TFLB BCTs stabilize plasma at ambient and 4°C compared to Streck BCTs.

Whole blood was collected into TFLB and Streck NA BCTs and stored at ambient 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 2mL whole blood stored at ambient and 4°C for 1 hour, 2-, 4 days post-blood draw (Figure 6A). Plasma color is similar for all blood collection BCTs under ambient and 4°C storage at 1 hour. A slight color change is seen in TFLB and Streck BCTs at ambient 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 BCTs. The color change in Streck NA BCTs may be indicative of RBC-induced hemolysis (Figure 6B). Blood drawn in TFLB BCTs at ambient and 4°C had minimal changes in plasma appearance compared to Streck NA BCTs over 4

days. Samples drawn into TFLB BCTs have thermostability from 4°C to 21°C with minimal hemolysis, which is not offered in Streck BCTs.

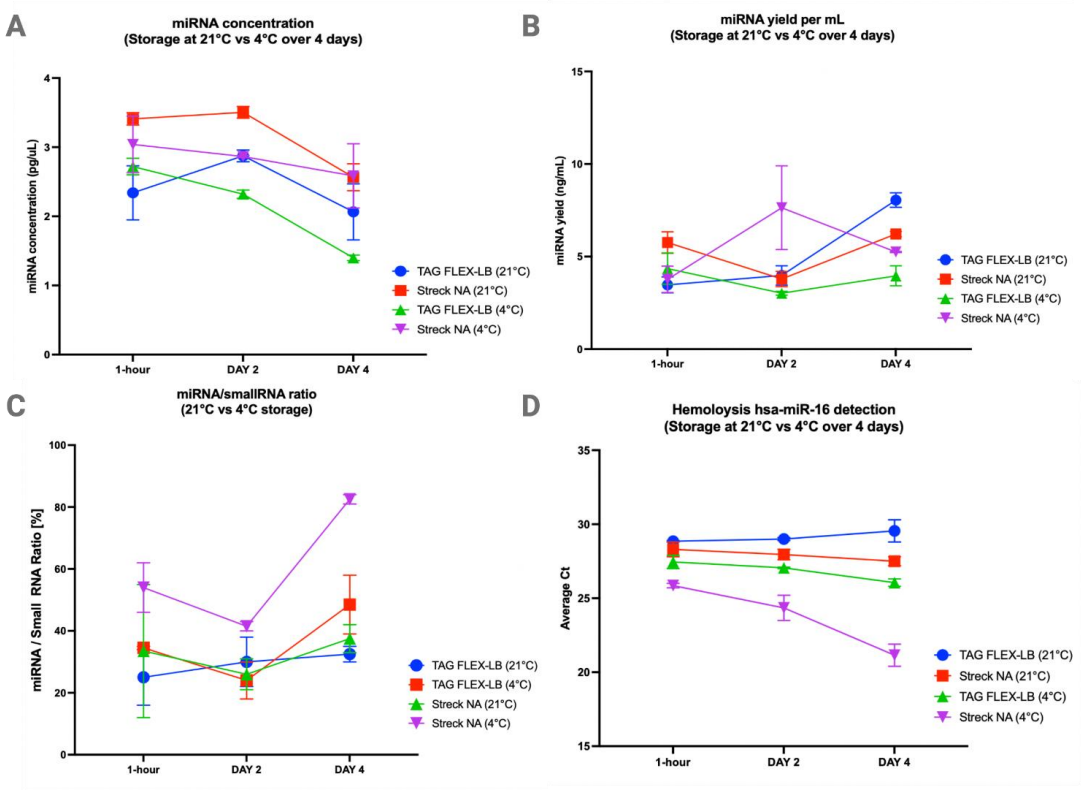

Figure 7. Concentration stability, miRNA/small RNA ratio, and hemolytic analysis is maintained in TFLB and Streck NA BCTs. A) RNA concentration from plasma in TFLB and Streck NA BCTs at 21°C and 4°C**.** miRNA concentration (ng/uL). B) Comparison of miRNA concentration from plasma in TFLB and Streck 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 timepoints. miRNA concentration from Streck cfDNA at 21°C (red line) and Streck NA at 4°C (purple line) for 1-hour, 2-, and 4-day timepoints.

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

miRNA concentration in blood drawn in TFLB at ambient and 4°C was maintained over 4 days. There was a small decrease in miRNA concentration in TFLB and Streck NA samples held at 4°C, likely due to degradation over time (Figure 7A). miRNA yield for TFLB samples stored at ambient and 4°C was consistent from 1 hour to 4 days post-blood draw (Figure 7B). The percentage of miRNA/small RNA in TFLB is maintained at ambient and 4°C, while Streck NA BCTs have a 40% decrease in miRNA/small RNA ratio after four days at 4°C compared to samples held at ambient (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 are maintained at ambient and 4°C, suggesting minimal hemolysis over time. In Streck NA samples, the Ct values of miR-16 are significantly decreased compared to samples held at ambient which may be due to

miRNA degradation at colder temperatures over 4 days (Figure 7D). The findings from this experiment demonstrated that samples drawn into TFLB BCTs can be stored at 4°C or ambient temperature, maintaining miRNA integrity, concentration, and reduced hemolysis over 4 days compared to Streck NA BCTs.

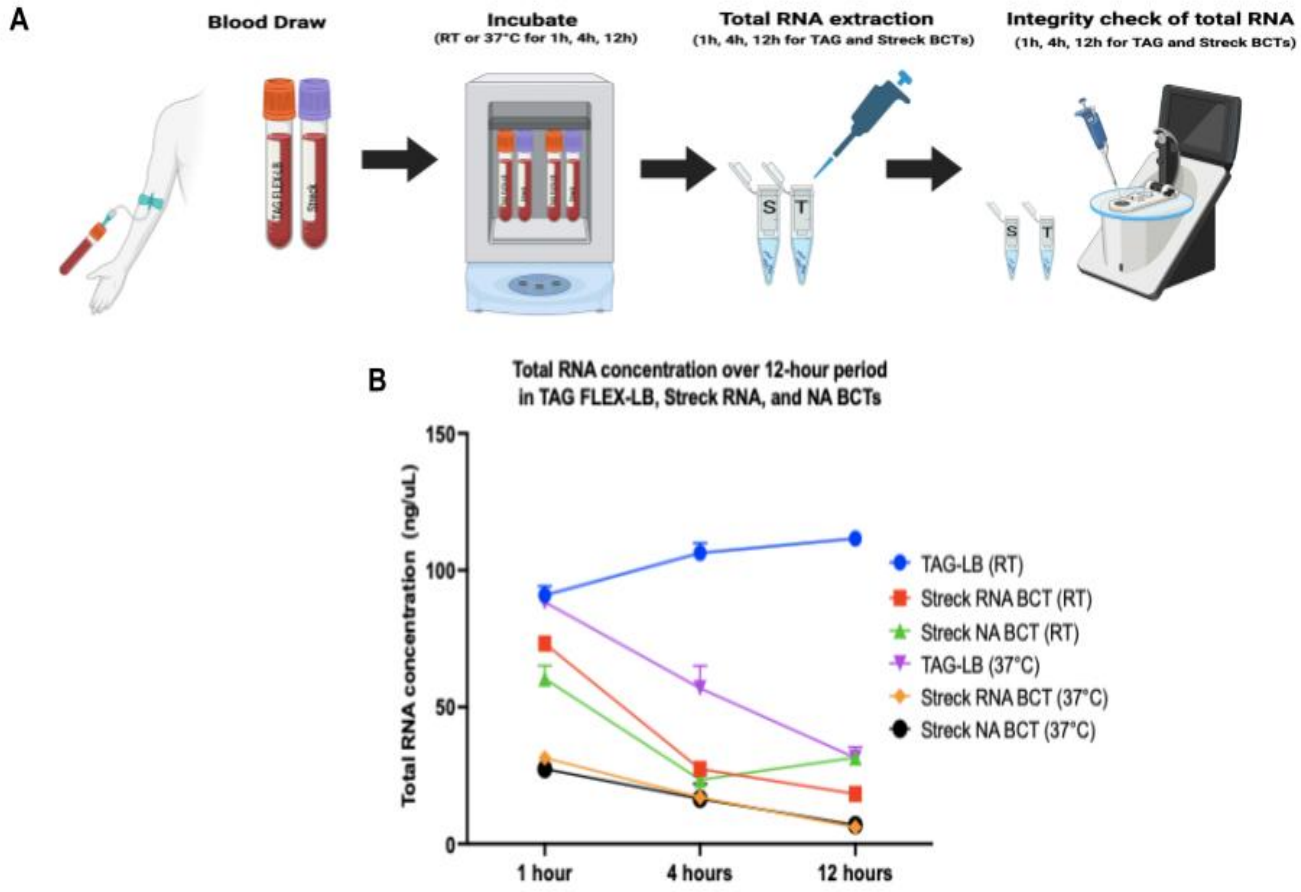

Figure 8. Total RNA concentration is maintained under ambient and 37°C temperatures for TFLB for over 12 hours. A) Schematic of experimental design for blood draw, incubation, and total RNA extraction of TFLB, Streck RNA, and Streck NA BCTs. B) RNA concentration (ng/uL) changes for over 12 hours TFLB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

TFLB ensures consistent RNA integrity under thermal stress conditions typical of real world biosample transport logistics.

Heat Shock Proteins (HSPs) act as thermal stress markers. TFLB effectively preserves the total RNA under heat stress with such exceptional fidelity that this subtle biological response can be accurately quantified, ensuring sample reliability as measured by HSP response. Thermal stress causes blood samples in BCTs to degrade during shipping and storage. RNA degradation due to heat stress causes integrity, stability, and quantity issues of total RNA, resulting in failed test results for patients.

Blood was drawn into TFLB, Streck RNA, and Streck NA BCTs and incubated at ambient temperature (20-22°C) or 37°C. Total RNA for both temperatures is extracted using Qiagen's Total RNA blood mini kit (52304) for 1-, 4-, and 12- hours. Concentration and purity were measured for all samples using nanodrop recommended by the manufacturer of the extraction kit. Total RNA concentration at ambient temperature is consistent in TFLB for 12 hours, maintaining a concentration of 111 ng/uL (Figure 8b). As the temperature increased to 37°C, there was a linear decrease in total RNA concentration over the 12 hours, ranging from 88 ng/uL to 27 g/uL (Figure 8B). Due to the sensitive structure of RNA, the loss of total RNA is expected as temperature increases, likely attributed to cellular degradation and apoptosis. However, total RNA concentration at 37°C was sufficient for RT-qPCR, demonstrating that the integrity and quantity of RNA are stable for TFLB BCTs at ambient and 37°C. Streck RNA and Streck NA BCTs at ambient temperature (20-22°C) demonstrate a linear decrease in total RNA concentration ranging from 74.4 ng/uL to 17 ng/uL (RNA Complete) and 64.4ng/uL to 22.7 ng/uL (NA). Total RNA concentration yield for Streck BCTs is far less than TAG at ambient and at 37°C. Under 37°C heat, both Streck BCTs yielded insufficient total RNA recovery, with concentrations ranging from 31.6 ng/uL to 6.5 ng/uL (RNA Complete) and 28.1 ng/uL to 7.2 ng/uL (NA) (Figure 8B). Streck RNA and Streck NA BCTs show an overall decrease in total RNA concentration at ambient temperature (20°C) and 37°C compared to TFLB BCTs at 37°C.

The Qiagen Total RNA blood mini kit, a common silica-column-based method, failed to recover sufficient total RNA from Streck RNA and Streck NA BCTs after 12 hours of heat stress at 37°C. This resulted in unusable RNA yields for downstream analysis. While this suggests an incompatibility between the Streck preservative and silica-based extraction under these conditions, TFLB BCTs were fully compatible and yielded sufficient RNA. Further investigation with non-silica-based extraction methods may elucidate the exact mechanism of failure for the competitor tubes. Since low total RNA concentration and inadequate purity were yielded from Streck RNA and Streck NA, we could not proceed with RT-qPCR to monitor gene expression changes of Heat Shock Protein 27 (HSP27) and Heat Shock Protein 70 (HSP70). Therefore, the rest of the experiment was conducted with TFLB samples only for over 12 hours at ambient temperature and 37°C. Overall, TFLB exhibited thermal stability for whole blood BCTs, essential for protein expression and shipping in hot environments. In contrast, neither Streck BCTs yielded sufficient total RNA concentration at ambient or 37°C to

assess HSP levels.

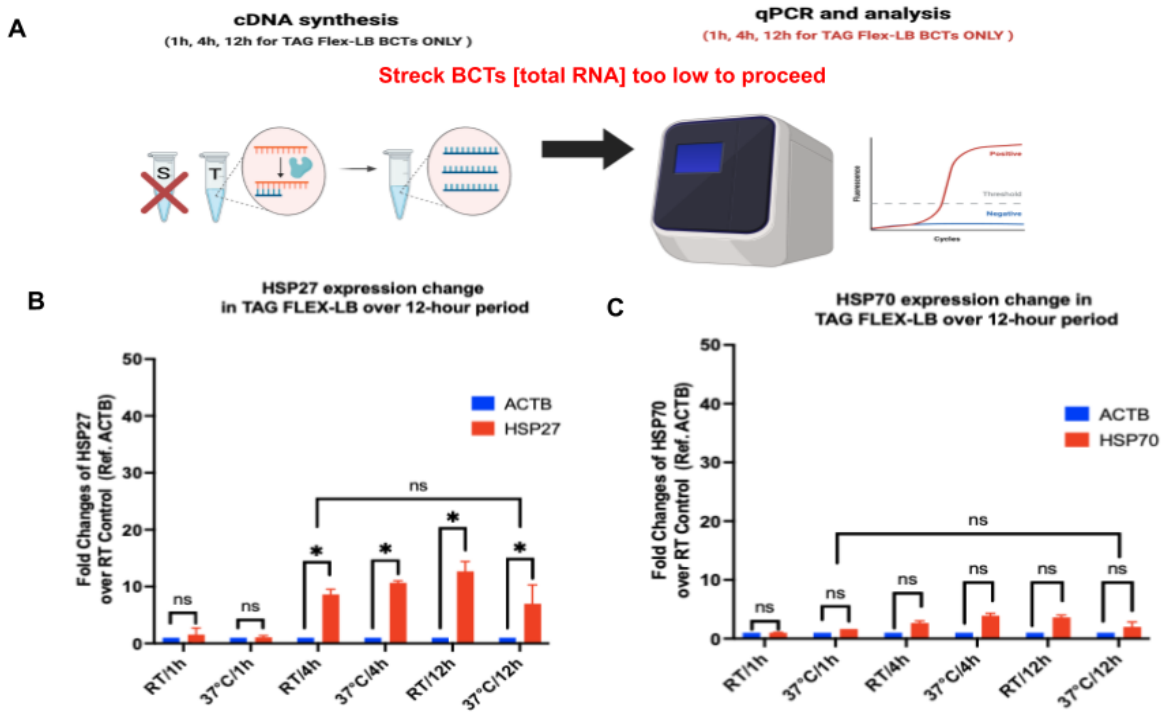

Figure 9. Gene expression changes for HSP27 and HSP70 in TFLB under heat stress at 37°C over 12 hours. A) Schematic of experimental design for cDNA synthesis, RT-qPCR, TFLB, Streck RNA, and NA BCTs analysis. **B)** Fold-expression changes of HSP27 (red) normalized over the control, ACTB, at ambient temperature (reference gene; blue) **C)** Fold-expression changes of HSP70 (red) normalized over the control, ACTB, at ambient temperature (reference gene; blue).

HSP27 and 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 BCTs (Figure 9a). Gene expression changes for HSP27 were not significantly different at ambient temperature (20-22°C) and when heat-stressed at 37°C after 1 hour. As expected, HSP27 gene expression is increased after 4 and 12 hours at ambient temperature and 37°C compared to our reference gene, ACTB (Figure 8b). The increase in HSP27 expression is a typical response to environmental temperature changes, since HSP27 is a small and highly activated protein for an early response to heat-induced stress. However, the gene expression changes of HSP27 at each time point (i.e.,1-, 4-, and 12-hours) at ambient and 37°C did not demonstrate any significant changes over time, indicating that TFLB BCTs can offer thermal protection of whole blood over 12 hours at 37°C (Figure 9b). Gene expression changes for HSP70 are not significantly different at ambient temperature 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 ambient temperature, and 37°C was unchanged and is likely not altered by minor environmental temperature changes. HSP27 is considered an early responder of heat stress, functioning upstream of HSP70, explaining the slight increase in HSP27 expression compared to HSP70. The cumulative RT-qPCR results further indicate that TFLB BCTs 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 BCTs

Variability in storage and transport of samples can significantly 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. The purpose was to assess the performance of TFLB BCTs 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 work proposed does not involve the collection of identifiable human specimens and is limited to in vitro evaluation of biospecimen stabilization technologies for NGS. By validating TFLB BCTs in this context, the proposed study seeks to contribute to improved standardization and reliability of liquid biopsy workflows, which are critical for advancing the clinical translation of NGS-based cancer diagnostics.

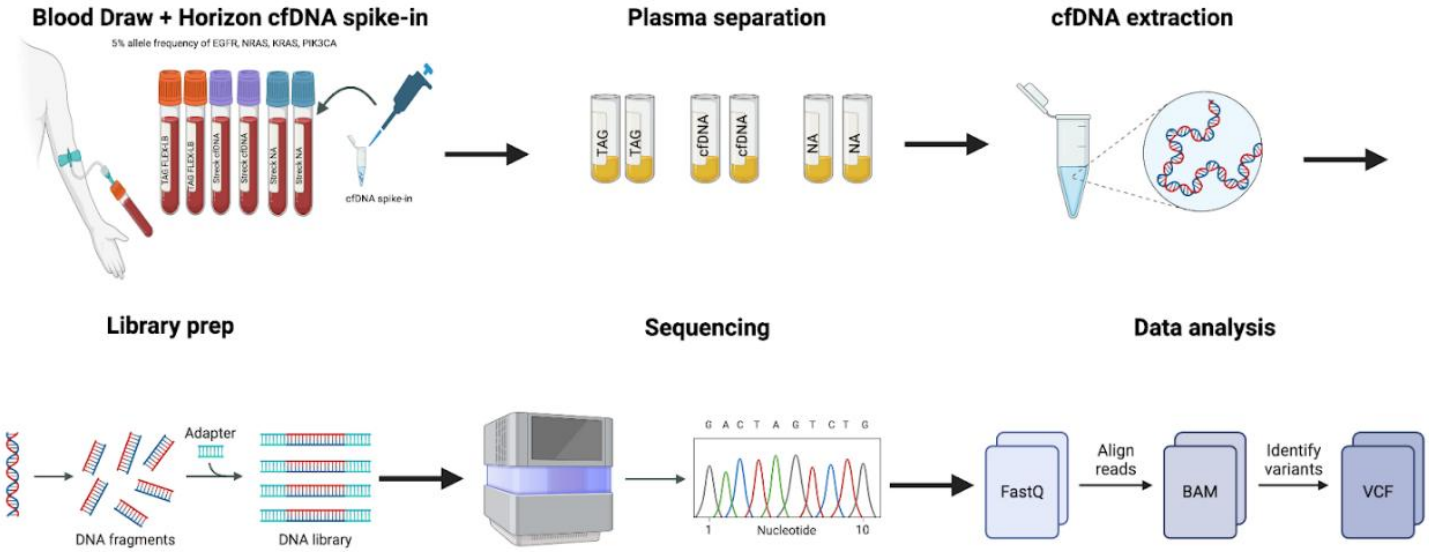

Figure 10. Workflow for Cancer Spike-in project in TFLB and Streck BCTs. Blood samples were taken from two study participants and randomly drawn into TFLB, Streck cfDNA, and Streck NA BCTs. Synthetic cfDNA carrying EGRF, PIK3CA, KRAS, and NRAS variants at 5% allele frequency were added to the blood samples to a final concentration of 11.25 ng/mL. 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 (20-22°C) temperature. cfDNA was extracted from the plasma samples and sequenced using the Illumina TruSight Tumor 15 panel.

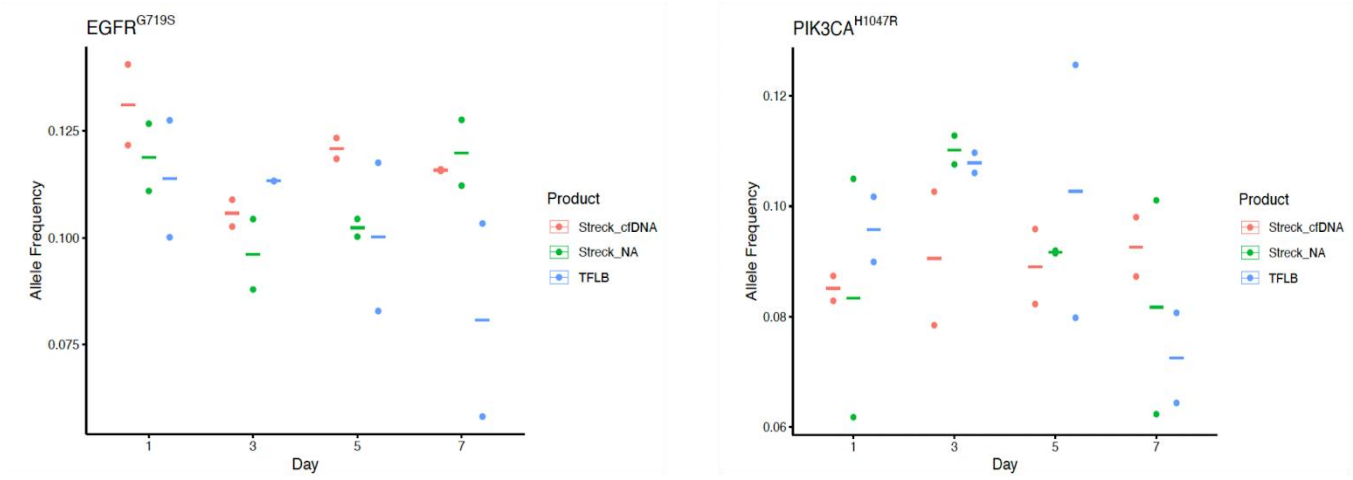

Figure 11. EGFR and PIKC3A mutations are identified in TFLB up to 7 days. TFLB BCTs (blue dots) identified EGFR and PIKC3A mutations from day 1 (blood draw day) to day 7. Streck cfDNA (red dots) and Streck NA (green dots) BCTs identified mutant alleles from day 1 to day 7.

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 represent the product mean of the two biological replicates for each storage time. Overall, the performance of TFLB BCTs with respect to variant discovery was comparable to those of the Streck BCTs over 7 days. In TFLB BCTs we could also identify PIK3AC over 7 days. Allele frequency detection ranges as low as 0.06 and as high as 0.120. Detection of PIK3A is similar to Streck NA and Streck cfDNA BCTs. After seven days, the observed allele frequency was often comparable to the allele frequency measured on the day of the initial blood draw (Day 1). The findings from our NGS spike-in project allowed us to identify key cancer markers over 7 days using TFLB BCTs.

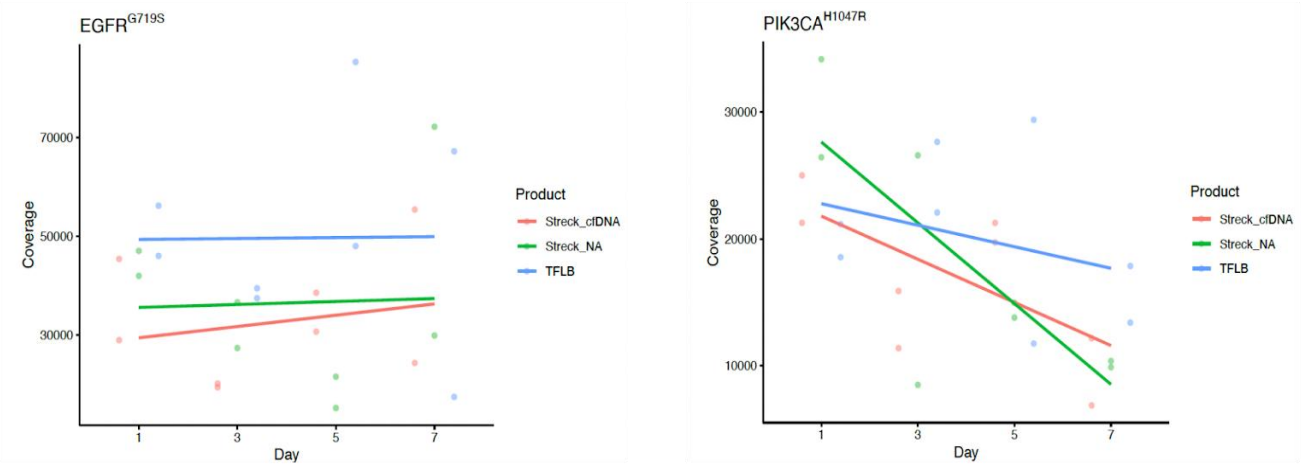

Figure 12. TFLB has higher amplicon coverage of EGFR and PIKC3A mutations than Streck BCTs. TFLB (blue dots) identified EGFR and PIKC3A mutations from day 1 (blood draw day) to day 7. Streck cfDNA (red dots) and Streck NA (green dots) BCTs identified mutant alleles from day 1 to day 7.

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 significant cfDNA degradation occurred during storage, a decrease in sequencing coverage of the target amplicons would be expected. Coverage of TFLB for EGFR mutations is ~1000 to 70000 reads. Coverage is more stable for TFLB BCTS over 7 days than Streck BCTs. Coverage of TFLB for PIK3AC mutations is ~10000 to 30000 reads. Coverage is more stable for TFLB BCT over 7 days than Streck BCTs. Amplicon sequencing coverage declined over time for all products. However, TFLB BCTs displayed higher coverage at Day 7 for the amplicons overlapping the spike-in variants. The results suggests that TFLB BCT may preserve cfDNA integrity for amplification in the TruSight Tumor 15 workflow better than Streck BCTs products. These results from two healthy donors are promising and suggest TFLB may offer enhanced cfDNA stability for NGS applications; larger cohort studies are planned to further validate these findings.

Conclusion

Integrating TFLB BCTs into liquid biopsy workflows empowers researchers to overcome challenges in biomarker preservation, ensuring reproducible results and advancing breakthroughs in molecular diagnostics and oncology research. TFLB BCT stabilization performance evaluation versus other stabilizing BCTs, such as PAXgene DNA and Streck cfDNA and Streck NA BCTs demonstrate equivalent or better performance in stabilizing circulating nucleic acids (cfDNA, cfRNA) for up to 7 days at 4°C, 21°C, and 37°. Preliminary NGS experiments indicate that the TFLB BCTs perform comparably with the Streck cfDNA and Streck NA BCTs based on an analysis of variant discovery and variant-specific amplicon sequencing coverage over 7 days. Furthermore, TFLB demonstrated superior retention of amplicon coverage at Day 7, suggesting enhanced

preservation of cfDNA integrity that is critical for the sensitivity and reliability of NGS applications. Labs aiming to optimize usable plasma volume, ensure quality, integrity, and quantity of extracellular vesicles, circulating cell-free nucleic acids, and total RNA -mitigating hemolysis and apoptosis during sample transportation can benefit by incorporating TFLB BCTs into their research and development.

1. Superior cfRNA/miRNA Integrity: Uniquely maintains a stable miRNA:Small RNA ratio, crucial for assay normalization and reproducibility.
2. Exceptional Hemolysis Control: Prevents cold-induced (4°C) hemolysis that compromises competitor tubes, ensuring sample integrity during refrigerated storage.
3. Robust Thermal Resilience: Preserves total RNA after heat stress (37°C) that causes catastrophic sample loss in competitor tubes when using common silica-column extraction methods.
4. Streamlined Workflow: Compatible with direct plasma isolation, saving time and resources compared to multi-step protocols required for other BCTs.

Limitations and Future Work

The findings presented in this note are highly encouraging; however, some limitations should be noted. The preliminary Next-Generation Sequencing (NGS) data was generated from samples from two healthy donors. While the results suggest TFLB may offer enhanced cfDNA stability for NGS applications, larger cohort studies are planned to further validate these findings and establish statistical significance. Future work will also include expanded proteomics analysis and a comprehensive evaluation of Circulating Tumor Cell (CTC) stabilization

Operational and Economic Implications for High-Throughput Laboratories

Beyond the analytical performance benefits, the stabilization technology within the TFLB BCT offers significant operational and economic advantages for clinical and research laboratories.

- **Increased Workflow Efficiency and Flexibility:** The elimination of the mandatory Proteinase K digestion step required for Streck BCTs with certain common extraction kits saves 20 minutes of incubation time per batch. For a laboratory processing a full 96-well plate of samples, this translates to over 32 hours of cumulative saved incubation time. More importantly, the 7-day ambient stability decouples sample collection from processing, eliminating the need for immediate processing of samples drawn late in the day or before a weekend, thereby improving staff allocation and reducing overtime costs.
- **Reduction of Pre-Analytical Error and Sample Rejection:** The superior control of cold-induced hemolysis demonstrated by TFLB directly mitigates a common cause of sample rejection. Fewer rejected samples significantly reduce costs and logistical burdens associated with patient re-draws, while also improving turnaround time and client satisfaction.

- **Lower Per-Sample Processing Costs:** The streamlined workflow reduces hands-on time and may lower reagent costs by removing the use of Proteinase K. Combined with a reduction in failed runs due to hemolysis or RNA degradation, TFLB can contribute to a lower total cost-per-result, enhancing the economic viability of large-scale liquid biopsy testing.

Learn more about the TAG FLEX-LB RUO BCT 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 precision diagnostics with TFLB BCTs.

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

1) **Request a TFLB BCT Evaluation Kit today**
   - email: tflb@tagreagents.com
   - call: +1-775-237-8799

2) **Schedule a detailed call with TAG scientists**
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