Comparative Analysis of Select Liquid Biopsy Blood Collection Tubes

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INTRODUCTION

In recent years, liquid biopsy has paved a more accessible path for cellular and molecular diagnostic studies and assays. A simple blood draw can reveal key biomarkers such as circulating tumor cells (CTCs) and cell-free DNA (cfDNA). These fragile analytes are present in low quantities and are prone to rapid degradation. Preservation of these key analytes over time at ambient and non-ambient temperatures is essential for the accuracy of downstream tests. This study compares three commercial liquid biopsy blood collection tubes: the common industry anticoagulant tube (EDTA), Streck Cell-Free DNA BCT (Streck), and the new Biomatrica LBgard® Blood Tube (LBgard). In this report, we assess 1) levels of total plasma DNA, cfDNA, and genomic DNA (gDNA), 2) stability of a cancer-specific cfDNA fragment, 3) hemolysis, 4) compatibility with Next Generation Sequencing, 5) DNA methylation and 6) cell recovery (of CTC and white blood cells, WBC) in each type of tube over a variety of temperatures and incubation times post-blood draw of normal blood and clinical samples.

METHODS

Blood Samples. Blood from healthy donors and blood from Stage IV colorectal cancer (CRC) patients were collected in EDTA, Streck, or LBgard tubes. Blood samples were incubated as indicated. At each time-point, plasma was isolated and stored at -80°C or cells (CTCs and WBCs) were isolated and stained for flow cytometry.

Plasma DNA Quantification. DNA from plasma samples was isolated using the QIAamp CNA kit (Qiagen) and quantified by Quant-iT (ThermoFisher) or by qPCR using RPS18 primer probes (ThermoFisher).

Droplet Digital PCR (ddPCR). A DNA fragment bearing the KRASG12D mutation (cfDNA mimic; Horizon) was spiked into blood samples and incubated for up to 14 days at 25°C. Plasma DNA was isolated and analyzed by ddPCR using the KRASG12D Mutation Assay and QX200 ddPCR instrument (BioRad).

Bioanalyzer. cfDNA and gDNA isolated from plasma were characterized using the High Sensitivity DNA Analysis kit and the 2100 Bioanalyzer (Agilent).

Hemolysis. Plasma hemolysis was assessed visually over 14 days.

DNA Methylation and Next Generation Sequencing (NGS). Healthy donor blood was collected in EDTA, LBgard or Streck tubes, and plasma DNA was isolated on Day 0 and Day 7. Targeted libraries containing specific methylation sites were constructed from extracted DNA by bisulfite conversion, enrichment and amplification. Libraries underwent targeted NGS using Ion Torrent PGM™.

Cell Recovery. VCaP cells (CTC mimics) were spiked into healthy donor blood and incubated at specified temperatures for 4 days. EpCAM+ VCaP cells and CD45+ WBCs were quantified by flow cytometry (Novocyte; ACEA). CTC and WBC recoveries were normalized to Day 0 counts.

RESULTS

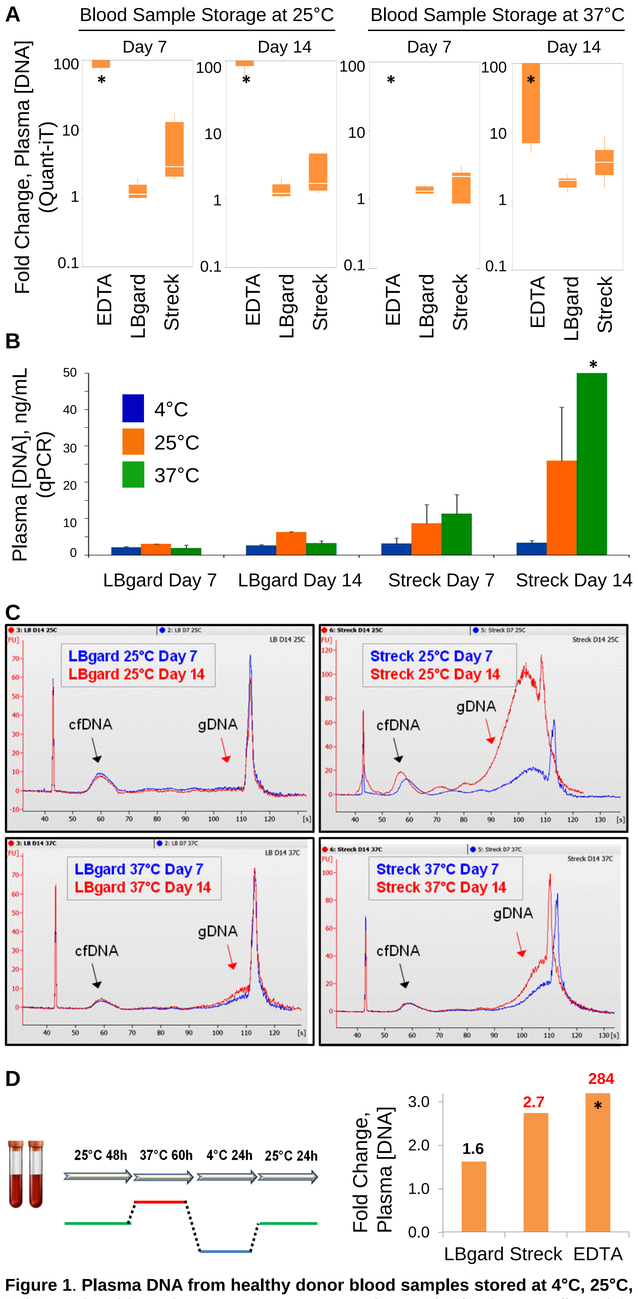

Figure 1. Plasma DNA from healthy donor blood samples stored at 4°C, 25°C, 37°C, and subjected to temperature excursions. A) Blood was collected in EDTA, LBgard, or Streck tubes and incubated at 25°C or 37°C for up to 14 days. Plasma DNA was extracted and quantified by Quant-iT. Fold change = Day 7 [DNA] / Day 0 [DNA] or Day 14 [DNA] / Day 0 [DNA]. **B)** Blood was collected in LBgard or Streck tubes, incubated at 4°C, 25°C, 37°C for up to 14 days. Plasma DNA was extracted and quantified by qPCR with RPS18 primers. **C)** Plasma DNA was characterized for cfDNA vs gDNA by Bioanalyzer. *cfDNA peak = black arrow; gDNA peak = red arrow.* **D)** Blood was subjected to temperature cycling, representing temperature excursions experienced during shipping/storage. Plasma DNA was analyzed as in A). *asterisks (*) = off-scale values.*

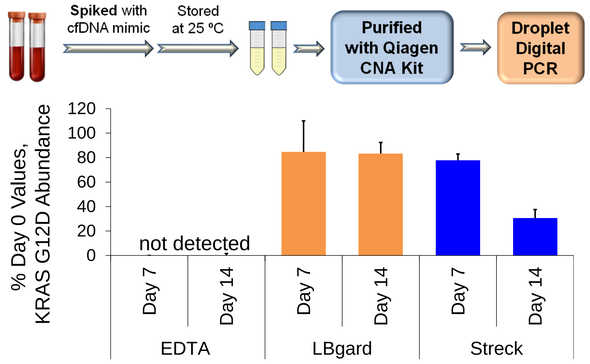

Figure 2. Tumor allele abundance determined by ddPCR from samples stored for up to 14 days at 25°C. Healthy blood samples were collected in EDTA, LBgard, or Streck tubes and spiked with a ~160 bp DNA fragment bearing KRASG12D. Blood samples were incubated at 25°C for up to 14 days. DNA was extracted from plasma, and the fractional abundance of KRASG12D was determined by ddPCR.

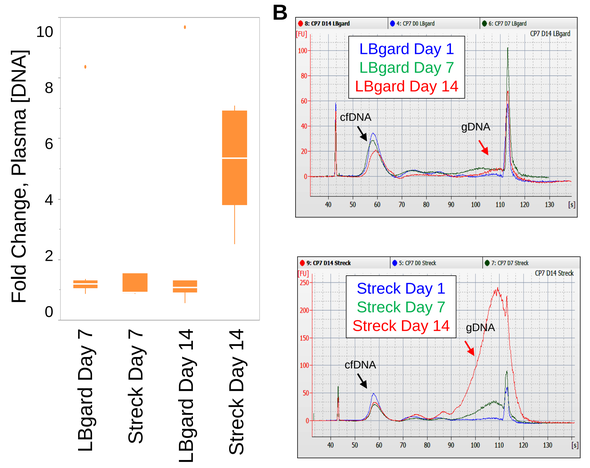

Figure 3. Plasma DNA isolated from the blood of Stage IV Colorectal Cancer patients stored for up to 14 days at 25°C. Blood was collected from Stage IV CRC patients in LBgard or Streck tubes and incubated for up to 14 days at 25°C. **A)** Plasma DNA was extracted and quantified by Quant-iT. Fold change = Day 7 [DNA] / Day 1 [DNA] or Day 14 [DNA] / Day 1 [DNA]. **B)** Plasma DNA was characterized by Bioanalyzer; traces are from a representative patient sample. *cfDNA peak = black arrow; gDNA peak = red arrow.*

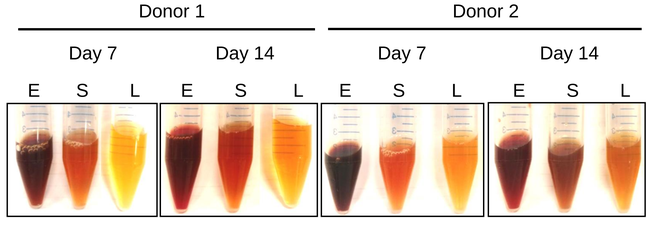

Figure 4. Hemolysis of healthy donor blood samples stored for up to 14 days at 25°C. Blood was collected in EDTA (E), Streck (S) or LBgard (L) tubes and incubated for 7 or 14 days at 25°C. Images are of plasma from two representative healthy donors.

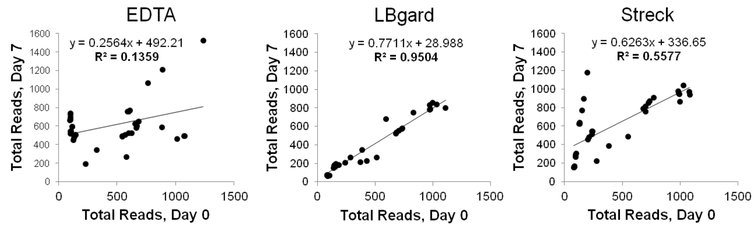

Figure 5. LBgard tubes are compatible with Next Generation Sequencing. Total reads (unique reads) for each tube type, comparing Day 0 to Day 7 samples. An R^2 value of 1 and a slope of 1 indicate no change in number of reads over time.

CpG Position	Chr18:35041724	Chr18:35041752	Chr18:35041784	Chr18:35041842	Chr18:35041844	Chr3:36988763	Chr3:36993418	Chr3:36993441	Chr3:36993465	Chr3:36993473	Chr3:36994134	Chr3:36994398	Chr3:36994438	Chr3:36994442	Chr3:36994446	Chr3:36996744	Chr1:6466344	Chr1:6466339	Chr1:6466337	Chr1:6466317	Chr1:6466311	Chr11:1277708	Chr11:1277689	Chr11:1277685	Chr11:1277675	Chr11:1277666
Gene	MAPRE2	MAPRE2	MAPRE2	MAPRE2	MAPRE2	MLH1	MLH1	MLH1	MLH1	MLH1	MLH1	MLH1	MLH1	MLH1	MLH1	MLH1	TNFRSF25	TNFRSF25	TNFRSF25	TNFRSF25	TNFRSF25	TOLLIP	TOLLIP	TOLLIP	TOLLIP	TOLLIP
EDTA																										
LBgard																										

Figure 6. Methylation status of plasma DNA from healthy donor samples assessed using Ion Torrent™ NGS. Change in methylation status for Day 7 samples, as compared to standard (EDTA Day 0 samples). ■ corresponds to minimal change (0.5-2.0-fold of standard); ■ corresponds to increase in methylation (> 2.0-fold of standard); ■ corresponds to decrease in methylation (< 0.5-fold of standard); ■ corresponds to sites not expected to be methylated for healthy donors.

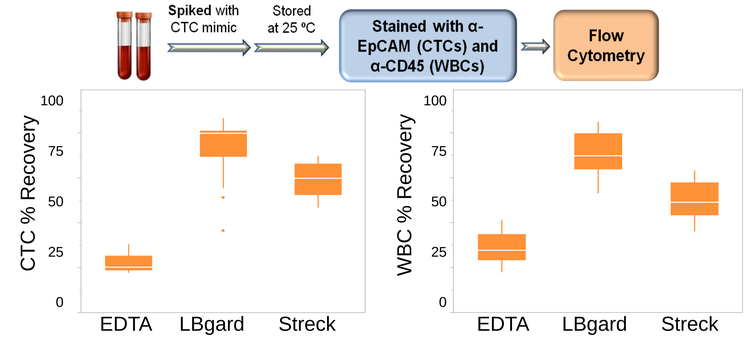

Figure 7. CTC and WBC Recovery from blood samples stored for up to 4 days at 25°C. CTC-spiked healthy blood samples collected in LBgard, Streck, or EDTA tubes were incubated for 4 days at 25°C. CTCs (left) and WBCs (right) were stained and quantified by flow cytometry on Day 0 and Day 4. Day 4 percent recoveries are normalized to Day 0 absolute cell counts.

CONCLUSION

LBgard tubes stabilize cfDNA and prevent gDNA release for up to 14 days at 25°C and at 37°C. Significantly more gDNA is released in Streck and EDTA tubes than in LBgard tubes in both healthy donor blood and CRC patient blood by Day 14 post-draw. The fractional abundance of a circulating tumor DNA mimic (spiked mutant DNA) was maintained in LBgard tubes but not in Streck or EDTA tubes. Hemolysis is lower in LBgard tubes compared to EDTA and Streck tubes. NGS coverage is stable in LBgard tubes over 7 days. DNA methylation levels in cfDNA derived from LBgard samples show minimal change over 7 days. Finally, cell recovery (CTC mimics and WBCs) is significantly higher after 4 days at 25°C from blood stored in LBgard tubes than in Streck tubes.

Overall, LBgard® Blood Tubes provide robust ambient stabilization of cfDNA, CTCs and WBCs by a variety of metrics.