Abstract
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Comparison of Liquid Biopsy Blood Collection Tubes

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INTRODUCTION

Critical biomarkers such as cell-free DNA (cfDNA) derived from tumors and circulating tumor cells (CTCs) can be detected from a simple blood draw. These analytes are fragile, prone to degradation, and often present in extremely low quantities which may impact the accuracy of downstream tests. Preservation of key analytes at ambient temperature also removes the need for cold chain transport and storage, decreasing cost and increasing accessible market size. This study compares five commercial liquid biopsy blood collection tubes: EDTA, Biomatrica LBgard® Blood Tube (LBgard), Streck Cell-Free DNA BCT (Streck), PreAnalytiX PAXgene Blood ccfDNA Tube (PAXgene) and Roche Cell-Free DNA Collection Tube (Ariosa). Concentration of plasma DNA, levels of genomic DNA (gDNA), hemolysis, and cell recovery (CTC and white blood cell/WBC) were assessed over a variety of temperatures and incubation times post-blood draw from healthy blood donors or stage IV colorectal cancer (CRC) patients.

METHODS

Blood Samples. Blood was collected in EDTA, Streck, PAXgene, Ariosa or LBgard tubes. Blood samples were incubated as indicated and at each time-point, plasma was isolated and stored at -80°C.

Plasma DNA Quantification. DNA from plasma samples was isolated using Qiagen CNA kit and quantified by Quant-iT or by qPCR using RPS18.

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

CTC Recovery. VCaP cells (CTC mimics) were spiked into healthy donor blood and incubated at 25°C for 4 days. EpCAM+ VCaP cells and CD45+ WBCs were quantified by flow cytometry (Novocyte; ACEA). CTC and WBC recoveries were determined by the ratio of Day 4 to Day 0 absolute counts.

Hemolysis. The degree of plasma hemolysis was determined using the Cripps method for quantification of oxygenated hemoglobin (see reference), as well as by visual assessment.

RESULTS

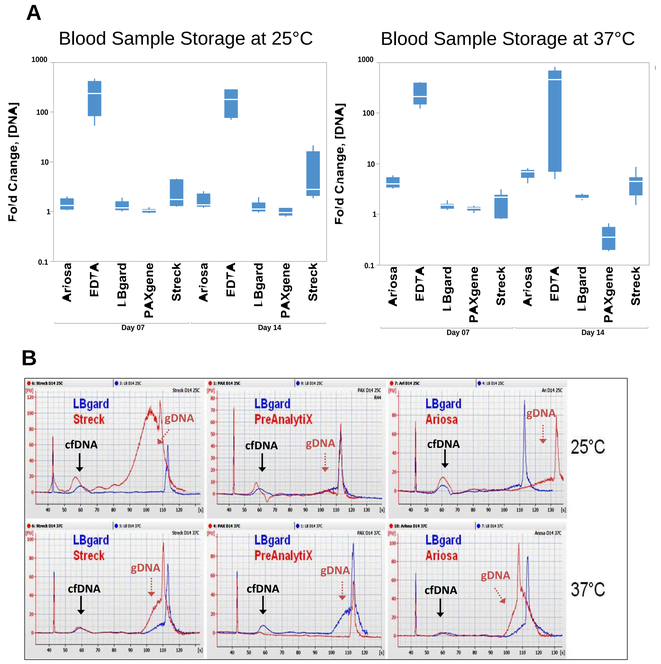

Figure 1. Plasma DNA concentration in healthy blood samples stored for up to 14 days at 25°C and 37°C. A) Blood samples were collected in Ariosa, EDTA, LBgard, PAXgene (PreAnalytiX) or Streck tubes and incubated at 25°C or 37°C for up to 14 days. Plasma DNA was extracted and quantified by Quant-iT. The fold change represents change from Day 0. B) Plasma DNA was characterized for cfDNA (black arrows) and gDNA (red arrows) by Bioanalyzer. Day 14 traces are shown.

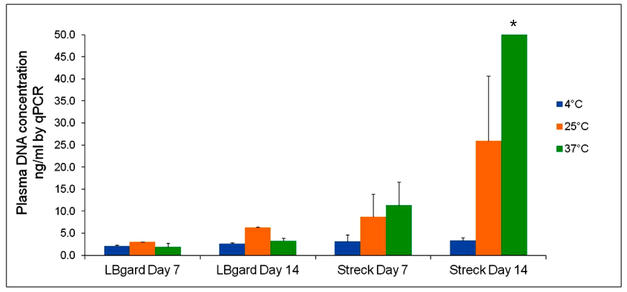

Figure 2. Plasma DNA concentration determined by qPCR in healthy blood samples stored for up to 14 days at 4°C, 25°C and 37°C. Blood samples were collected in LBgard or Streck, incubated at 4°C, 25°C and 37°C for up to 14 days. cfDNA was extracted and quantified by qPCR with RPS18 primer/probes. Asterisk (*) means value is above 50 ng/ml (118 ng/ml).

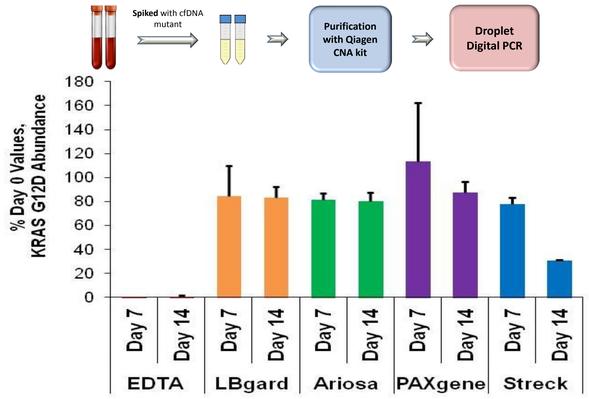

Figure 3. Circulating tumor DNA (ctDNA) allele concentration determined by ddPCR of blood samples stored for up to 14 days at 25°C. Healthy blood samples were collected in EDTA, LBgard, Ariosa, PAXgene or Streck tubes and spiked with a ~160 bp DNA fragment bearing KRASG12D. Blood samples were incubated at 25°C for up to 14 days. DNA was extracted from plasma, and the fractional abundance of KRASG12D was determined by ddPCR.

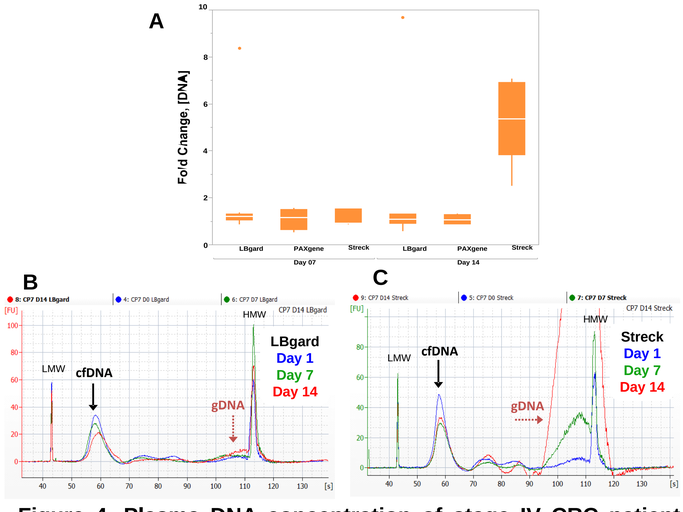

Figure 4. Plasma DNA concentration of stage IV CRC patients blood samples stored for up to 14 days at 25°C. Blood samples from stage IV CRC patients were collected in LBgard, PAXgene or Streck and incubated for up to 14 days at 25°C. A) Plasma DNA was extracted and quantified by Quant-iT. The fold change represents changes versus time 0. B) and C) Bioanalyzer traces from one particular patient blood sample collected in LBgard and Streck tubes. *cfDNA = black arrows; gDNA = red arrows; LMW = low molecular weight marker; HMW = high molecular weight marker.*

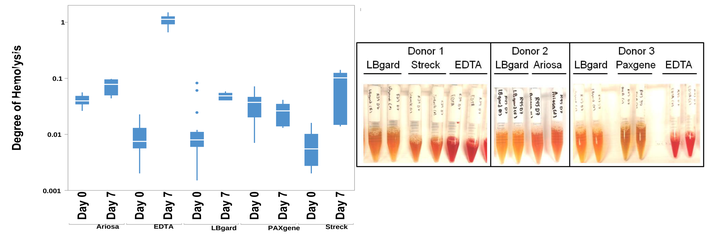

Figure 5. Hemolysis assessment in healthy blood samples stored for up to 7 days at 25°C. Blood was collected in Ariosa, EDTA, LBgard, PAXgene or Streck tubes and incubated for up to 7 days at 25°C. Degree of hemolysis was assessed using the method described by Cripps, *et al*. (left) and by visual inspection (right).

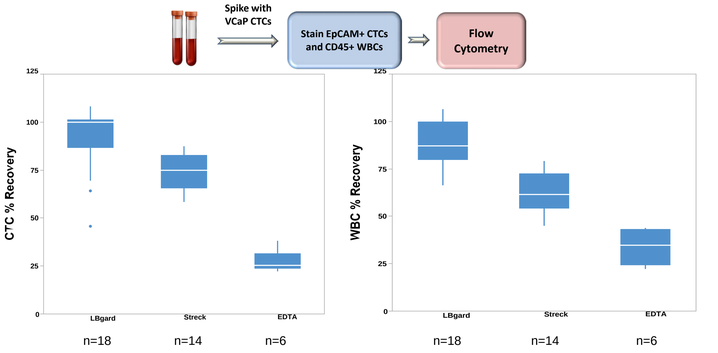

Figure 6. CTC and WBC levels in blood samples stored for up to 4 Days at 25°C. CTC-spiked healthy blood samples collected in LBgard, Streck or EDTA were incubated for 4 days at 25°C. CTCs (left) and WBCs (right) were stained and quantified by flow cytometry on Day 0 and Day 4, and percent cell recoveries on Day 4 were quantified.

CONCLUSION

Our results show that the choice of blood collection tube is critical as significant variation was observed for several biomarker levels depending on the storage conditions of blood samples.

Blood collected in LBgard, PAXgene and Ariosa tubes stabilize cfDNA and prevent gDNA release for up to 14 days at 25°C. However, under stressed conditions at 37°C, more gDNA is released in both Ariosa and Streck tubes than LBgard tubes. At 37°C, cfDNA is lost in PAXgene tubes. Plasma DNA concentrations from CRC patient blood were less stable/more variable at Day 14 in Streck tubes than in LBgard or PAXgene tubes. The fractional abundance of spiked mutant DNA was maintained in LBgard, PAXgene and Ariosa tubes but not in Streck tubes. And finally, cell recovery was higher after 4 days at 25°C in LBgard than in Streck.

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

REFERENCE

Cripps CM *et al.*, J Clin Pathol 1968 Jan;21(1):110-2.