Assessing the Effect of Simulated Shipping on Cell-Free DNA in Blood Collected in Norgen, Streck and EDTA Tubes

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INTRODUCTION

Non-invasive molecular diagnostics for cancer and prenatal screening have a critical reliance on circulating cell-free DNA (cfDNA). Degradation of and contamination of this important analyte with genomic DNA can occur during blood collection/processing, storage and/or shipping. Therefore, preservation of cfDNA in whole blood is of paramount importance. In this study we determined the effect of simulated shipping on the detectable presence of cfDNA in plasma for blood collected in a novel cfDNA Preservative Tube (Norgen Biotek Corp.), a typical EDTA Tube and a Cell-Free DNA BCT Tube (Streck). Detection by qPCR was done on two size fragments of the β-actin gene. Furthermore, quality of plasma was assessed by measuring hemolysis over time (48 hours) in simulated shipped versus unshipped samples.

MATERIALS AND METHODS

Sample Collection

Blood from one individual was collected into two of each type of tube: cfDNA Preservative Tubes (Norgen Biotek Corp., Thorold Canada), Cell-free DNA BCT Tubes (Streck, Inc., La Vista NE) and K_2EDTA tubes (BD Vacutainer®, Becton Dickinson, Franklin Lakes NJ). Blood samples were gently mixed immediately after blood collection by inverting 10 times.

Sample Processing and Plasma Separation

All whole-blood samples were immediately separated into 2 mL aliquots. Half the aliquots were mounted to a shaker and continuously inverted at room temperature at a rate of approximately 18 inversions per minute. The other half were stored at room temperature without shaking to serve as a control. After 0, 3, 6, 24 and 48 hours plasma was separated by the single spin protocol (425 x g for 20 min) from the aliquots that were shaken and from the unshaken control aliquots.

Cell-free DNA Isolation from Plasma

The Plasma/Serum Cell-Free Circulating DNA Purification Mini Kit (Cat# 55100, Norgen Biotek Corp., Thorold, ON Canada) was used to isolate the DNA present in 0.2 mL of plasma at each time point. Isolation was performed according to the manufacturer's instructions. Samples were eluted in 50 µL and stored at −20°C until analysis by quantitative PCR.

Quantitative PCR

The level of total plasma DNA was assessed by amplifying a short DNA fragment (136 bp) from the human β-actin gene (forward primer 5′-GCG CCG TTC CGA AAG TT-3′; reverse primer 5′-CGG CGG ATC GGC AAA-3′). The level of contaminating genomic DNA was assessed by amplifying a longer β-actin fragment (420 bp) (forward primer 5′-CCG CTA CCT CTT CTG GTG-3′; reverse primer 5′-GAT GCA CCA TGT CAC ACT G-3′). The DNA template input volume was 3 µL amplified in a final volume of 20 µL using 2X Real-Time PCR Master Mix (Norgen Biotek Corp., Thorold, ON Canada). Data analysis was done on GraphPad Prism, version 6.01.

Quality of Plasma as Determined by Hemolysis

Hemolysis was determined by measuring the absorbance of free hemoglobin in the plasma of all samples at 414 nm using a Nanodrop 2000/2000c.

RESULTS AND DISCUSSION

Measurement of Hemolysis

Hemolysis was determined by measuring the absorbance at 414 nm of each plasma aliquot. The absorbance distribution over time for each sample is shown in Figure 1. Individual absorbance curves are plotted for each sample in Figure 2. A paired, two-tailed Student's *t*-test showed that there were no statistically significant changes between shaken and unshaken samples collected on either Norgen's Plasma CF-DNA preservative or Streck's CF-DNA Preservative ($p < 0.05$). The same test however showed that there was a statistically significant increase ($p = 0.0285$) between shaken and unshaken samples collected in K_2EDTA tubes.

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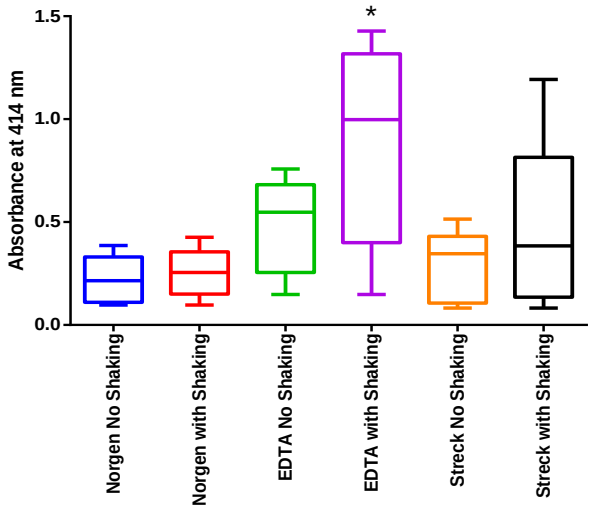

Figure 1. Hemoglobin distribution over 48 hours

The whiskers represent the maximal and minimal values (Time 0 and 48 hours respectively). The line inside the box represents the median value. The limits of the box indicate the 10th and the 90th percentiles.

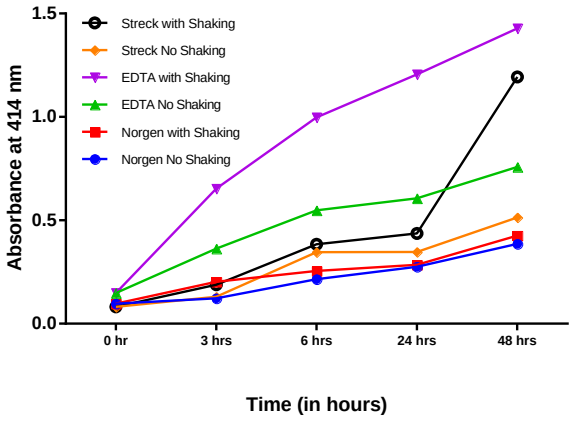

Figure 2. Hemoglobin Increases over Time

Plasma recovered from blood collected on Norgen's cfDNA Preservative Tube showed a minimal increase in absorbance after 48 hours of shaking as compared to the unshaken sample. Similarly plasma recovered from blood collected in Streck's BCT DNA tube showed a minimal increase in absorbance at 414 nm for up to 24 hours as compared to the unshaken Streck tube. However by 48 hours the absorbance had suddenly increased by over two fold. Plasma from blood collected on K_2EDTA and stored unshaken at room temperature started to increase in absorbance after just 3 hours and continued linearly until 48 hours. The shaken K_2EDTA tube also followed a similar trend, however the rate of increase was much higher.

Effect of Shaking on Levels of High Molecular Weight DNA and Total Levels of DNA in Plasma

Relative levels of total DNA (Fig.3B) and relative levels of high molecular weight DNA (Fig.3A) were determined by quantitative PCR on different sized amplicons of the β-actin gene. For all the aliquots collected on Norgen's cfDNA Preservative tube, the short and the long β-Actin fragments were not affected by shaking as compared to the unshaken, room temperature controls. The blood collected on Streck's Cell-Free DNA BCT tube showed a similar trend to Norgen for up to 24 hours, but then by 48 hours started to show indications that stability was lacking. As for the aliquots collected on EDTA, both fragments increased at a much higher rate when shaken as compared to those aliquots that were stored at room temperature without shaking.

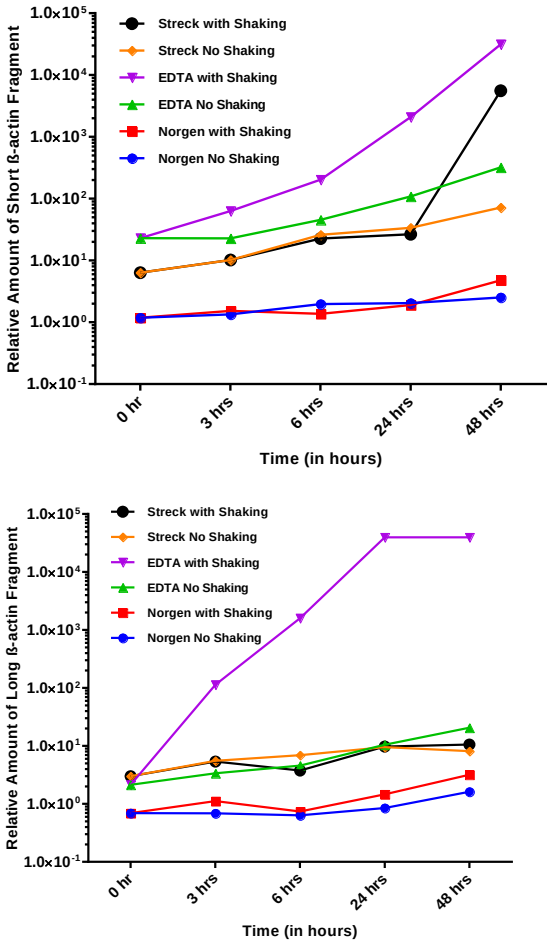

Figure 3. Effect of shaking on (A) relative level of high molecular weight DNA and (B) relative level of total DNA in plasma.

CONCLUSION

Our results demonstrate that Norgen's cfDNA Preservative Tubes prevent hemolysis and aid in the preservation of circulating cfDNA during simulated shipping conditions.

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