Norgen's cf-DNA/cf-RNA Preservative Tube Preserves & Prevents the Contamination of Circulating Cell-Free DNA by Cellular Genomic DNA during Blood Collection, Shipping and Storage

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INTRODUCTION

It is well known that cell-free DNA (cf-DNA) can be found freely circulating in the bloodstream. Cell-free DNA of fetal origin is also present in maternal blood and is now used for non-invasive prenatal diagnosis. Cell-free DNA is also typically elevated in cancer patients. A challenge with the use of cf-DNA in diagnostic applications is that fetal or cancer derived cf-DNAs typically represent less than 10% of the total circulating cf-DNA. Accurate and consistent detection of those less abundant alleles therefore requires that blood is processed as soon as possible. This is done to separate the plasma from potential contamination with genomic DNA (gDNA) released by peripheral blood leukocytes as they degrade. Because collection is often done in a resource-limited setting and the samples shipped to a secondary analysis center, it is not usually possible to process blood immediately. Therefore preserving the integrity of cf-DNAs and preventing their contamination with gDNA should be a primary concern for cf-DNA based studies involving blood sample collection, transportation and/or storage. The present study highlights the efficacy of Norgen's cf-DNA/cf-RNA Preservative Tube in preserving and preventing the contamination of cf-DNA with gDNA during blood collection, shipping and storage.

MATERIALS AND METHODS

Sample Collection

Blood samples were collected from six different volunteers recruited by Norgen Biotek Corp. in Thorold, ON Canada. Volunteers were from both sexes and presumed to be healthy. Blood samples from all six donors were drawn onto three different blood collection tubes: K_2EDTA tubes (BD Vacutainer®, Becton Dickinson, Franklin Lakes, NJ), Cell-Free DNA™ BCT tubes (BCT) (Streck Inc., Omaha, NE) and cf-DNA/cf-RNA Preservative Tubes (Norgen Biotek Corp., Thorold, ON Canada). Blood samples were gently mixed immediately after blood collection by inverting 10 times.

Sample Shipping and Storage

Blood was drawn into two of each type of blood collection device from each of the 6 donors (36 tubes total). One Norgen cf-DNA/cf-RNA Preservative tube, one Streck tube and one K_2EDTA tube from each donor was packed in an insulated box and shipped from Thorold, ON via overnight air freight to Winnipeg, MB and then back to Thorold, ON (elapsed time 72 hrs). Upon return an aliquot was collected and the remainder of the preserved sample was stored at room temperature for 7 days before final processing (10 days total since collection). The 18 tubes that were not shipped were left at room temperature for 10 days. An aliquot from each unshipped sample was separated at time 0 and used as a control, and then again after 3 days and 10 days. DNA was subsequently isolated from each aliquot and gene targets assessed by qPCR.

Plasma Preparation

For blood samples collected in K_2EDTA and BCT tubes plasma was separated from each aliquot by centrifuging for 20 minutes at room temperature at 1600 × *g*. For blood samples collected in Norgen's cf-DNA/cf-RNA Preservative Tubes plasma was separated from each aliquot by centrifuging for 20 minutes at room temperature at 425 ×*g*.

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 each plasma fraction. 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

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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 and Purified cf-DNA

Hemolysis was determined by measuring the absorbance of free hemoglobin at 414 nm in the plasma of 6 subjects using a Nanodrop 2000/2000c. The size distribution of the purified plasma cf-DNA was also assessed using an Agilent Bioanalyzer 2100 High Sensitivity DNA Chip.

RESULTS AND DISCUSSION

Measurement of Hemolysis

Hemolysis was determined by measuring the absorbance at 414 nm of plasma collected from 6 individuals in each tube type. Data collected include initial absorbance at day 0, and absorbance 10 days later for shipped and unshipped samples. Mean absorbance and standard deviation are shown **(Figure. 1)**. A paired, two-tailed Student's *t*-test showed that there was no statistically significant difference in the initial mean absorbance at 414 nm and after 10 days (either shipped or unshipped) for blood collected on Norgen's and Streck's preservatives. Blood collected on K_2EDTA however showed a significant difference between mean initial absorbance and mean absorbance after 10 days for shipped samples. A difference was only considered significant if it occurred at $p < 0.05$.

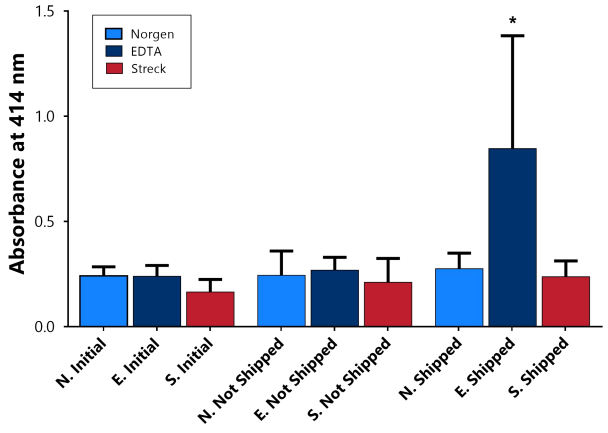

Figure 1. Norgen's cf-DNA/cf-RNA Preservative Tube helps prevent hemolysis of whole blood during shipping and storage (Day 0 and Day 10 data shown).

Size distribution of plasma cf-DNA

Total DNA was isolated from preserved whole blood that was first shipped (elapsed time 72 h) and then stored at room temperature for 7 days (total elapsed time 10 days). Eluate (1 µL) was analyzed on an Agilent Bioanalyzer 2100 using a High Sensitivity DNA chip. Plasma from blood collected on either K_2EDTA or Streck preservative showed an increased amount of high molecular weight DNA **(Figure 2a and Figure 2b)**.

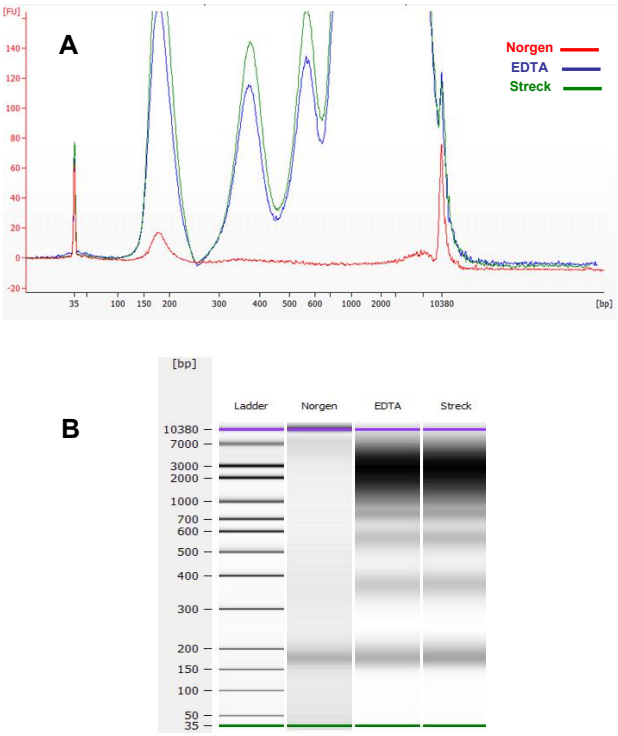

Figure 2. Norgen cf-DNA/cf-RNA Preservative Tube helps prevent the release of high molecular weight gDNA into plasma (Fig. 2A) while also minimizing the accumulation of contaminating apoptotic ladder from dying peripheral blood leukocytes (Fig. 2B).

Effect of shipping on level of high molecular weight DNA and total level of DNA in plasma

Relative levels of total DNA **(Fig.3A)** and relative levels of high molecular weight DNA **(Fig.3B)** were determined by quantitative PCR on different sized amplicons of the β-actin gene. Initial levels were compared to unshipped levels of the same samples 3 days later or against the shipped levels of the same samples after 3 days. In general, shipping caused an increase in DNA levels in all tubes except for Norgen's tubes. This was only significant ($p < 0.05$) between those blood samples initially collected on K_2EDTA and those same samples after 3 days that were shipped.

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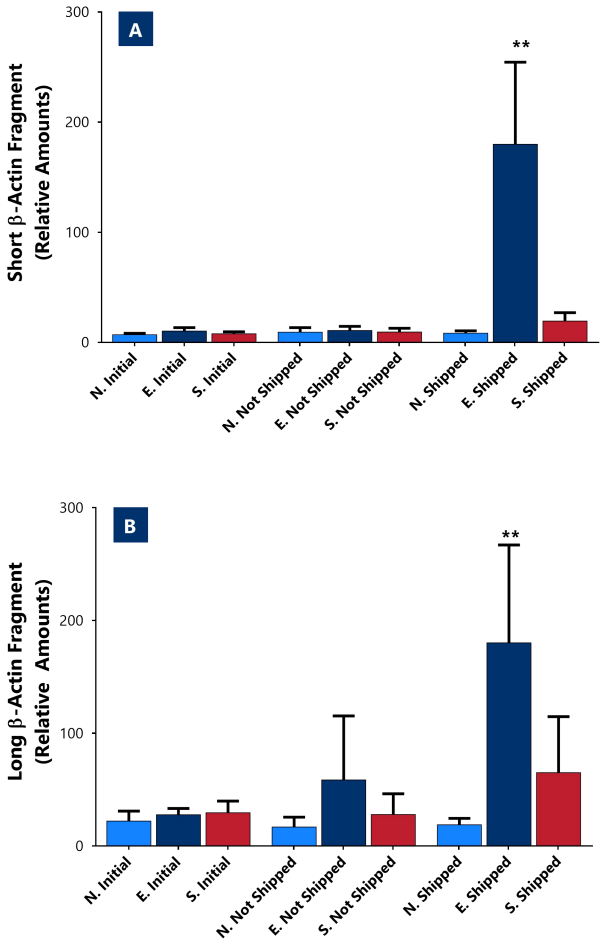

Figure 3. Effect of shipping on (A) total DNA level and (B) level of high molecular weight DNA (>420 bp) (Day 0 and Day 3 Data Shown).

CONCLUSIONS

1. Norgen's cf-DNA/cf-RNA Preservative Tubes help prevent hemolysis and preserve cf-DNA during shipping as measured by free hemoglobin in plasma and Quantitative PCR of short fragment β-actin.
2. Norgen's cf-DNA/cf-RNA Preservative Tubes help prevent the release and subsequent fragmentation of gDNA in whole blood.

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