# A novel blood collection device minimizes cellular DNA release in blood during sample storage and shipping

M. R. Fernando[1], S. Norton[1], K. Luna[1], J. Lechner[1] and W. L. Ryan[1],

[1]Research and Development Division, Streck, Inc., La Vista, NE 68128, USA

## ABSTRACT

**Background:** Cell-free DNA (cfDNA) naturally occurs in blood and is currently being used for non-invasive prenatal diagnostics and for detection, monitoring, and molecular analysis of cancer biomarkers (1). Due to the rarity of cfDNA targets, it is important to minimize release of genomic DNA (gDNA) following blood draw in order to accurately measure cfDNA concentrations (2). Pre-analytical conditions can affect the release of background gDNA into plasma, decreasing the proportion of specific cfDNA targets and masking their detection in downstream applications. We have evaluated the ability of a novel blood collection device and traditional $K_3$EDTA tubes to minimize cellular gDNA release into plasma when subjected to conditions that can occur during sample storage, shipping and processing.

**Methods:** Blood samples were drawn from healthy donors into $K_3$EDTA and Cell-Free DNA™ BCT tubes. To demonstrate the effect of elevated background gDNA on accurate cfDNA detection, a known concentration of plasmid containing a Y-chromosome specific SRY sequence was spiked into non-pregnant female blood that had been drawn each tube type; tubes were then stored at 22 °C for 14 days. The β-actin assay was used to determine total plasma DNA (pDNA) concentration over time and the SRY assay was used to measure the simulated cfDNA target. To evaluate the result of agitation during the transportation of samples, blood draws were either shaken or unshaken at 22 °C for 24 hours. To assess temperature variations, samples were stored at 6 °C, 22 °C and 37 °C for 14 days. For each experiment, plasma was harvested at various time points by centrifugation. Total plasma DNA (pDNA) was extracted and assayed by quantitative real-time PCR (qPCR).

**Results**: The SRY plasmid spiking experiment illustrated the dramatic effect of elevated background gDNA in plasma on detection of rare cfDNA sequences; 50- and 90-fold increases in β-actin levels were seen in $K_3$EDTA over 7 and 14 days, while detection of SRY levels decreased 3- and 95-fold. In comparison, Cell-Free DNA BCT showed no change in β-actin levels at day 7 and an 8-fold increase at day 14, while detectable SRY levels remained steady throughout the time course. Shaking of blood drawn into $K_3$EDTA tubes showed a significant increase in pDNA over 24 h, whereas no change was seen in Cell-Free DNA BCT tubes. Blood incubated at 6 °C, 22 °C and 37 °C showed significant increases in pDNA isolated from $K_3$EDTA tubes (fold-change, min = 9.5 and max = 233.1) , while pDNA levels from Cell-Free DNA BCT remained stable over 14 days (fold-change, min = 1.3 and max = 6.7).

**Conclusion:** Cell-Free DNA BCT tubes prevent increases in background gDNA levels caused by temperature fluctuations or agitation that can occur during blood sample storage, shipping and processing. This novel blood collection tube provides a method for obtaining high quality stabilized samples for rare DNA target detection and determining accurate cfDNA concentrations.

## INTRODUCTION

Cell-free DNA (cfDNA) naturally occurs in blood and is currently being used for non-invasive prenatal diagnostics and for detection, monitoring, and molecular analysis of cancer biomarkers (1). Due to the rarity of cfDNA targets, it is important to minimize release of genomic DNA (gDNA) following blood draw in order to accurately measure cfDNA concentrations (2). Pre-analytical conditions can affect the release of background gDNA into plasma, decreasing the proportion of specific cfDNA targets and masking their detection in downstream applications. We have evaluated the ability of a novel blood collection device and traditional $K_3$EDTA tubes to minimize cellular gDNA release into plasma when subjected to conditions that can occur during sample storage, shipping and processing.

## RESULTS

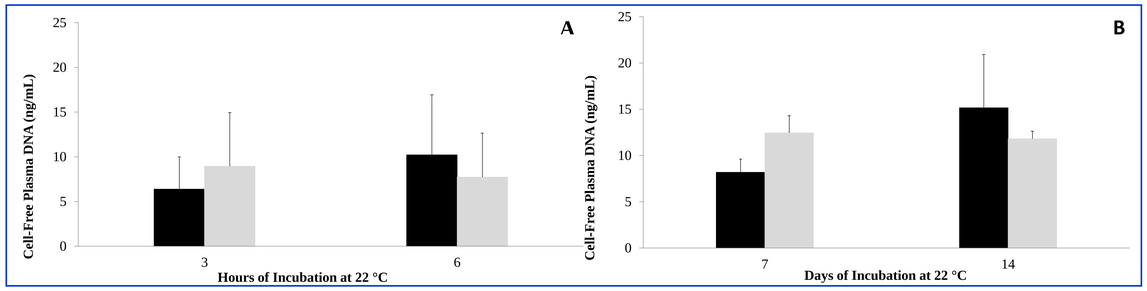


**Figure1.**

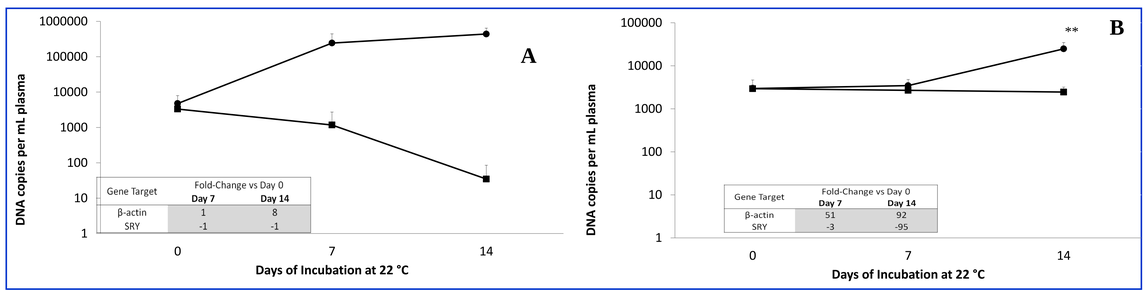


**Figure2.**

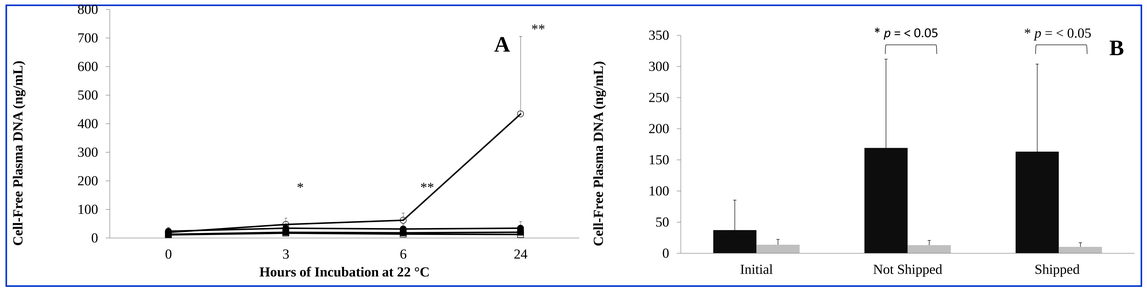


**Figure3.**

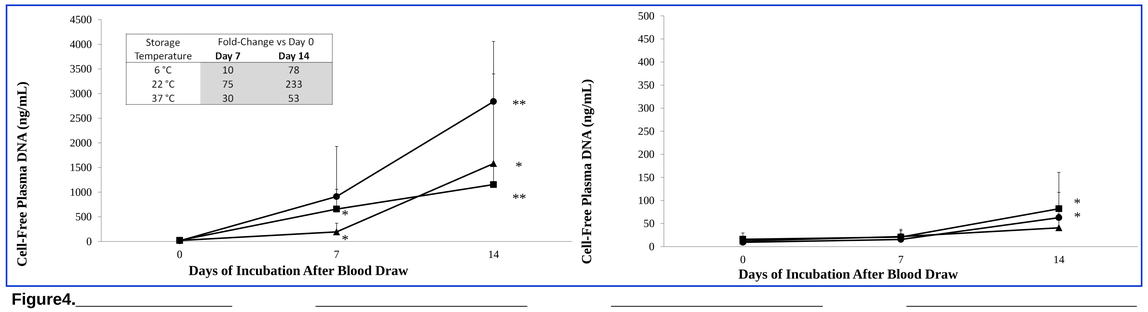


**Figure4.**

## MATERIALS AND METHODS

**Healthy donor recruitment**
Blood donors were recruited with informed consent from Streck Inc., La Vista NE, 68128, USA. Donors were both male and female and presumed to be healthy. All draws were performed using veinipuncture.

**Blood collection**
For each experiment, donor samples were drawn into two different blood collection tubes. Control samples were drawn into10 ml $K_3$EDTA blood collection tubes (BD vacutainer) and compared to samples drawn into a vacuum blood collection tube developed by Streck Inc. (Cell-free DNA™ BCT) which contained chemical compounds that stabilize nucleated blood cells and inhibit plasma nucleases. Blood was mixed well immediately after drawing into the tubes by inverting the tube several times.

**Sample processing**
After phlebotomy blood samples were stored at room temperature (22$^0$C), except where otherwise noted, and plasma was separated at noted time points. For the separation of plasma, blood samples were centrifuged at 22$^0$C at 300 x g for 20 minutes. The plasma layer was carefully removed without disturbing the buffy coat, transferred to a new vial and then centrifuged at 22$^0$C at 5000 x g for 10 minutes to remove residual cells.

**Plasma cell-free DNA isolation**
The QIAamp® Circulating Nucleic Acid Kit (Qiagen, Santa Clarita, CA) was used for the extraction of plasma DNA. The manufacturer's recommended protocol was modified slightly by increasing the duration of the Proteinase K treatment from 30 minutes to 1 hour at 60 $^0$C. Samples were eluted in 50 µL sterile nuclease-free water and stored at -80 $^0$C until analysis by real-time qPCR.

**Real Time quantitative PCR**
Human β-actin primers and probe and primers for the human Y-chromosomal sex determining region (SRY) were prepared as previously described (Chan et al., 2006; Lee et al., 1999). The following probe was designed for the quantification of SRY sequence: 6FAM-ATG GCT CTA GAG AAT CCC AGA ATG CGA AAC TCA GAG A-TAMRA. For each assay, 10-fold dilutions (300,000-30 copies) of plasmid DNA constructs were used to establish qPCR standard curves. Constructs were prepared by cloning a single copy of β-actin or SRY DNA sequence, which produced amplicons of 136 bp or 148 bp, respectively. β-actin genomic equivalents were calculated from qPCR copy number and then converted to nanograms of cfDNA per milliliter plasma (ng/ml) as previously described (Boddy et al., 2005). The final SRY DNA concentration was expressed as DNA copies recovered per milliliter (copies/ml) plasma. All primers were purchased from Integrated DNA Technologies (Coralville, IA). The qPCR probes and TaqMan® Universal PCR Master Mix II were purchased from Applied Biosystems (Foster City, CA).

## CONCLUSION

Cell-Free DNA BCT tubes prevent increases in background gDNA levels caused by temperature fluctuations or agitation that can occur during blood sample storage, shipping and processing. This novel blood collection tube provides a method for obtaining high quality stabilized samples for rare DNA target detection and determining accurate cfDNA concentrations.

## REFERENCES

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