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Optimizing blood collection tubes and timing for plasma processing to facilitate multicenter studies focused on cell free DNA analysis

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ABSTRACT:

OBJECTIVE: To investigate the suitability of Streck Blood Collection Tubes (BCT) on cell-free DNA (cf-DNA) levels in plasma and establish a protocol for transporting blood samples prior to plasma processing.

METHODS: Blood was collected in EDTA and BCT tubes from pregnant women carrying male fetuses (number suppressed per NCS disclosure guidelines). One set of each tube from each subject was processed to plasma immediately (standard cf-DNA protocol) whereas the other set was shipped by air courier and then processed. Total and fetal DNA concentrations were measured by multiplexed quantitative real-time PCR (TaqMan).

RESULTS: No significant differences were observed in total cf-DNA in plasma between immediately processed EDTA (control) and immediately processed BCT samples. Shipped EDTA tubes yielded significantly increased levels of total DNA compared to control and a lowered fetal fraction. Shipping blood in BCT tubes resulted in a slight increase of total DNA but no significant change of fetal fraction. When samples were divided into groups according to transport temperature no differences were observed for BCT samples shipped at room temperature; whereas, a significant increase of cf-DNA was observed in BCT samples transported at 4°C, leading to a significantly decreased fetal fraction.

DISCUSSION: The ability to batch process would be a tremendous advantage for a multi-center study such as the National Children's Study. BCT tubes are suitable for transporting whole blood prior to plasma processing. NCS recruiters are utilizing BCT tubes in this current pilot formative project. To date, fifty NCS samples have been drawn in BCT tubes and stored at room temperature for up to a week in order to facilitate weekly batch processing. However, these data suggest care should be taken to ensure that samples are not exposed to extreme temperatures during transportation.

Figure 1:

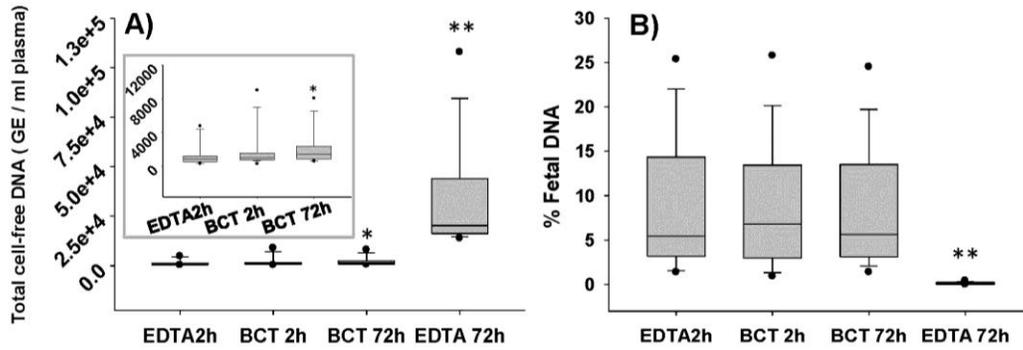


Figure 1: Panel A) Total cell free DNA in all samples as determined by RNaseP real-time PCR method and expressed as Genome Equivalents cf-DNA /ml plasma. The inset in panel A shows the same data but on a different y-axis scale. Panel B) Depicts the fetal percentage of the total cf-DNA based on SRY/RNaseP real-time PCR assays. In both plots the median is shown as a line within the gray bar which depicts the 25th percentile closest to the median line and 75th percentile farthest from the median line. The 10th and 90th percentiles are depicted by whiskers and the 5th and the 95th percentile outliers are shown as dots. EDTA 2h and BCT 2h are data from immediately processed samples. EDTA 72h and BCT 72h samples are all the shipped samples in the study including samples shipped with both RT and frozen gel-packs. Asterisks indicates significantly different from the current gold standard which is EDTA 2h samples (*= $p \leq 0.05$, **= $p \leq 0.001$).

Figure 2:

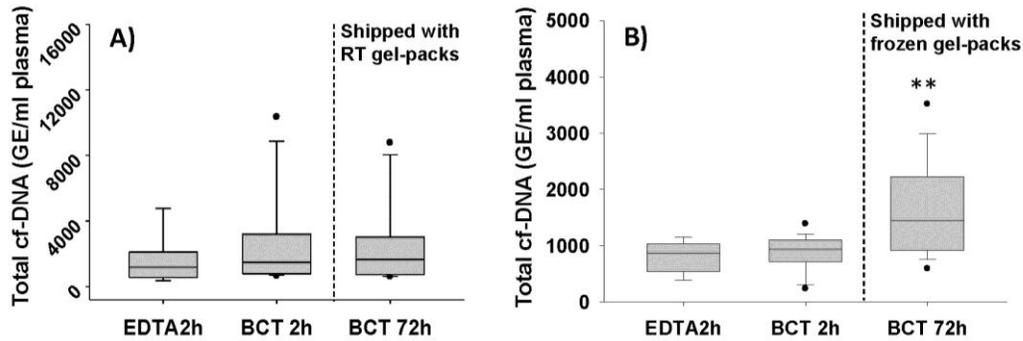


Figure 2: Total cell free DNA as determined by RNaseP real-time PCR method and expressed as Genome Equivalents DNA/ ml plasma. Panel A) shows data from immediately processed samples collected in EDTA and BCT tubes and their corresponding aliquot collected and shipped with room temperature gel-packs. Panel B) shows data from immediately processed samples collected in EDTA and BCT tubes and their corresponding aliquots collected in BCT tubes and shipped with frozen gel-packs. Medians are shown as a line within the gray bar which depicts the 25th percentile closest to the median line and 75th percentile farthest from the median line. The 10th and 90th percentiles are depicted by whiskers and the 5th and the 95th percentile outliers are shown as dots. Asterisk indicates significantly different from the current gold standard which is EDTA 2h samples (**= $p \leq 0.001$).