Five donor study shipping simulation of blood stored in DNAgard® Blood Tubes

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Experimental design:

Blood from five different human donors was collected in DNAgard® Blood Tubes, K_2-EDTA Vacutainers® (Becton Dickinson, 2 samples per donor) and in a competitor's blood collection tubes for DNA stabilization in whole blood. One K_2-EDTA vacutainer per donor was placed into a -20°C freezer within 30 minutes of blood collection. DNAgard Blood tubes, competitor's blood tubes and one K_2-EDTA Vacutainer replicate per donor were subjected to a temperature cycle mimicking a worst-case shipping scenario (**Figure 1a**). Blood tube storage temperatures were cycled for 9.5 days over a temperature range of -20°C to 45°C. At the end of the temperature cycle blood samples were equilibrated to room temperature prior to sample processing for DNA isolation. DNA was extracted from DNAgard Blood Tubes and K_2-EDTA Vacutainers using the QIAamp® DNA Blood Maxi Kit (QIAGEN) with a 1 ml elution volume. DNA was extracted from the competitor's blood tubes according the manufacturer's standard protocol with a 1 ml rehydration volume.

Blood DNA analysis:

Gel analysis: The integrity of 1 µl the DNA recovered for each of the five donors in each of the four storage conditions was analyzed by agarose gel electrophoresis (0.8 %; 1xTAE; 125 volts).

Absorbance spectroscopy: The absorbance profile from 200 – 320 nm was measured for each DNA sample. Data was used to quantify DNA yield, absorbance 260/ 280 ratios and absorbance 260/ 230 ratios.

PicoGreen quantification: DNA yield was quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies).

Results:

Visual inspection of the DNA isolated from DNAgard Blood Tubes and K_2-EDTA tubes for each of the five donors after the shipping simulation revealed clear eluate from the purification columns and no indication of insoluble precipitates. However, the DNA purified from blood from each of the five donors stored in the competitor's blood tubes contained significant amounts of insoluble debris (**Figure 1b**).

A. **B.**

Temperature	Time
25°C	2 days
37°C	2 days
25°C	2.5 days
-20°C	1 day
45°C	2 days

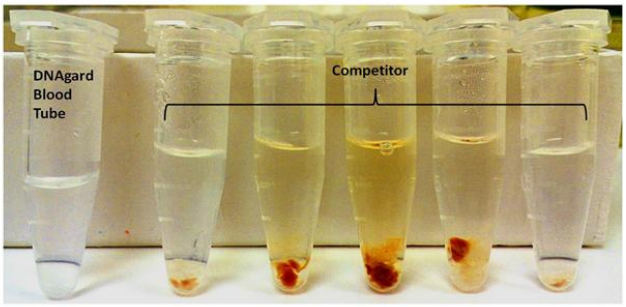

Figure 1. (**A**) Temperature cycle conditions for blood samples during the simulated shipping experiment. (**B**) Contaminant co-purification with DNA was assessed visually. Shown are rehydrated DNA samples recovered from blood from all five donors stored in the competitor's blood collection tubes. Purified DNA from a single donor recovered from a DNAgard Blood Tube is shown for comparison.

Genomic DNA integrity from blood stored in each of the four storage conditions was compared by agarose gel electrophoresis (**Figure 2**). DNA degradation in all fived donors, as indicated by smearing below the high molecular weight gDNA band, is apparent in blood that was stored in K_2-EDTA Vacutainers and subjected to the shipping simulation temperature cycle (non-protected). In contrast, the gDNA recovered from DNAgard Blood Tube samples is equivalent in integrity to that recovered from the gold-standard method of blood storage (frozen at -20°C in K_2-EDTA Vacutainers). The purified gDNA is of high molecular weight with no apparent DNA degradation. The integrity of the gDNA recovered from the competitor's blood tubes was also high, though care was taken to avoid the insoluble precipitates when loading the gel.

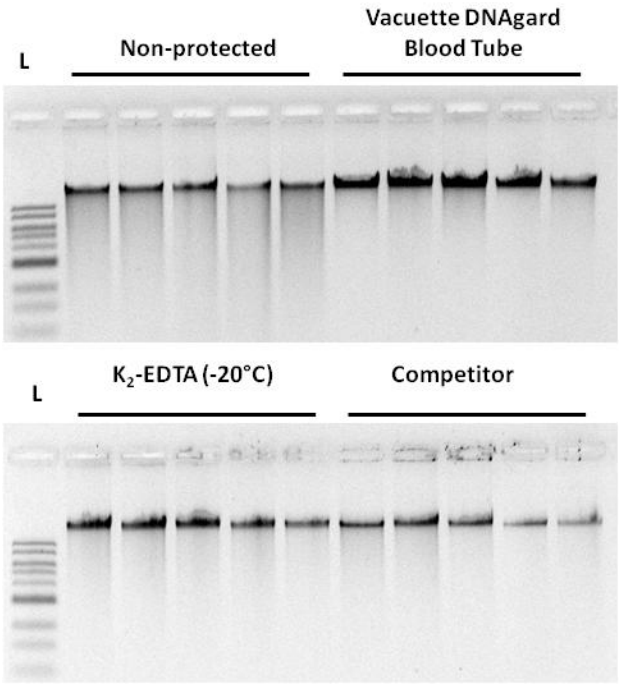

Figure 2. Equivalent volumes of gDNA purified from blood samples were analyzed by agarose gel electrophoresis. DNA recovered from each of the five donors was loaded sequentially on the gel for each of the four blood storage conditions. L = 1 kbp DNA Ladder (New England Biolabs).

DNA yield was measured by fluorescence quantitation (**Figure 3A**). The mean DNA yield per ml of blood from the five blood donors was highest for DNAgard Blood Tubes and in the competitor's blood tubes (17.8 µg/ ml and 16.3 µg/ ml, respectively). The mean DNA yield from K_2-EDTA Vacutainers was slightly lower (13.4 µg/ ml for tubes subjected to the shipping simulation and 11.7 µg/ ml for tubes stored frozen at -20°C). (DNA yield measurements by absorbance spectroscopy were within 15% of the picogreen measurements (data not shown)).

DNA purity as analyzed by UV-Vis spectroscopy revealed absorbance profiles characteristic of highly pure DNA for DNAgard Blood DNA Tube samples and K_2-EDTA Vacutainer samples (**Figure 3B**). Absorbance maxima at 260 nm, absorbance minima at 230 nm and low background absorbance at wavelengths above 300 nm were measured in these samples. An elevated absorbance peak at 215 nm was observed in DNA samples isolated from the competitor's blood tubes despite taking care to only analyze the soluble fraction of the extracted DNA sample. This elevated absorbance resulted in a mean A260/ A230 ratio of 1.4 in the competitor's samples, which is below the optimal minimal threshold value of 2.0 for this metric (**Figure 3C**). The mean A260/ A230 ratios of the DNAgard Blood Tube and K_2-EDTA Vacutainer samples were 2.6 and 2.5, respectively, indicative of highly pure DNA. The A260/ A280 ratios for DNA recovered from all four blood storage conditions were in the optimal range of 1.8 - 2.0.

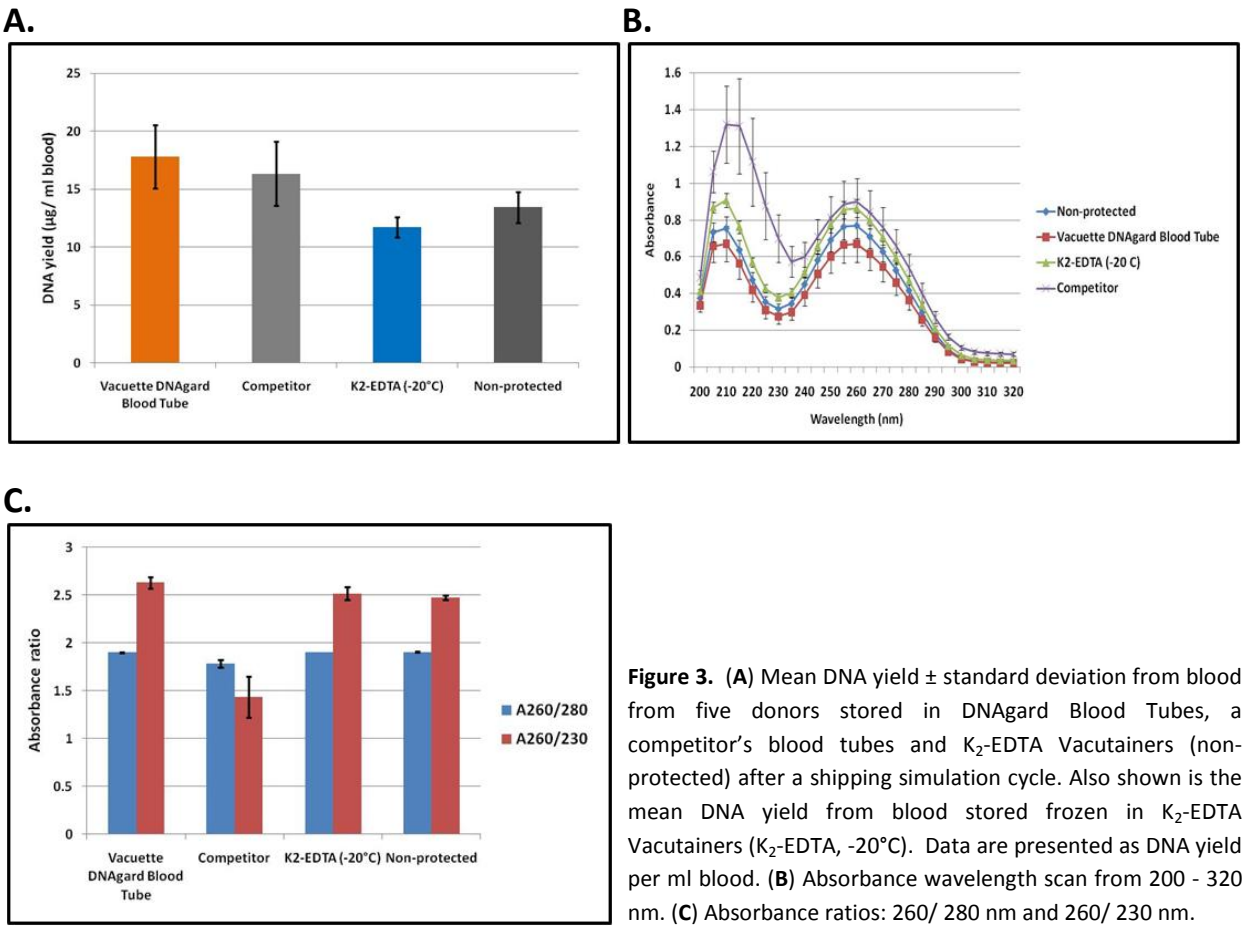

Figure 3. (**A**) Mean DNA yield ± standard deviation from blood from five donors stored in DNAgard Blood Tubes, a competitor's blood tubes and K_2-EDTA Vacutainers (non-protected) after a shipping simulation cycle. Also shown is the mean DNA yield from blood stored frozen in K_2-EDTA Vacutainers (K_2-EDTA, -20°C). Data are presented as DNA yield per ml blood. (**B**) Absorbance wavelength scan from 200 - 320 nm. (**C**) Absorbance ratios: 260/ 280 nm and 260/ 230 nm.

Conclusions:

Genomic DNA yield, purity and integrity in blood samples subjected to extreme temperature conditions are optimized in DNAgard Blood Tubes with the QIAamp DNA Blood Maxi Kit. Genomic DNA from DNAgard Blood Tubes displays optimal A260/ A280 and A260/ A230 ratios, indicative of high purity, and is free of insoluble debris that co-purified with DNA from the competitor's blood tubes. Genomic DNA yields from DNAgard Blood Tubes ranged from 10 - 24 µg/ ml across the five donor study (mean 17.8 µg/ ml), and slightly exceeded yields from blood stored in K_2-EDTA Vacutainers. Whereas genomic DNA was denatured in blood stored in K_2-EDTA Vacutainers during the shipping simulation, genomic DNA integrity from DNAgard Blood samples is equivalent to the conventional, gold-standard cold-storage method of shipping and storing blood as demonstrated by agarose gel electrophoresis.