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Sequencing and methylation analysis of blood genomic DNA after extreme temperature exposure in VACUETTE® DNAgard® Blood Tubes

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Experimental design:

Blood from five different human donors was collected in VACUETTE® DNAgard® Blood Tubes and K_2-EDTA Vacutainers® (Becton Dickinson). The K_2-EDTA samples were placed into a -20°C freezer within 30 minutes of blood collection. VACUETTE DNAgard Blood tubes were subjected to a temperature cycle mimicking a worst-case shipping scenario (**Figure 1**). 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 VACUETTE DNAgard Blood Tubes and K_2-EDTA Vacutainers using the QIAamp® DNA Blood Maxi Kit (QIAGEN) with a 1 ml elution volume.

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. Temperature cycle conditions for blood samples during the simulated shipping experiment.

Blood DNA analysis:

PicoGreen quantification: DNA yield from blood samples was quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies) and validated by absorbance spectroscopy.

Sequencing: A 2 kbp region of the GAPDH gene was amplified from 100 ng sample DNA recovered from the VACUETTE DNAgard Blood Tubes and K_2-EDTA Vacutainers (-20°C). Chain-termination sequencing was conducted with a primer internal to the amplicon and the quality and read length of the sequences were determined with Sequencher software. Sequences alignment was conducted by BLAST (NCBI).

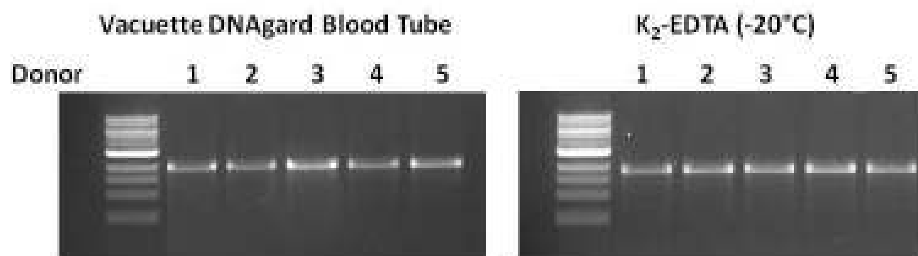
DNA methylation analysis: The methylation status of 20 promoter loci were quantified using the EpiTect® Methyl Signature qPCR Array for Human Cytokine Production (QIAGEN). DNA recovered from

VACUETTE DNAgard Blood Tubes and K_2-EDTA Vacutainers (-20°C) from two donors were analyzed. Data was quantified using the EpiTect Data Analysis software (QIAGEN).

Results:

The amplification efficiency of a 2 kbp region of the GAPDH gene was equivalent when using input DNA from VACUETTE DNAgard Blood Tube and K_2-EDTA (-20°C) samples from each of the five donors (**Figure 2A**). There were no differences in read lengths or sequences of the GAPDH gene in the post-shipping simulation DNAgard and frozen control samples for each of the five donors (**Figure 2B**) demonstrating that DNAgard Blood is equivalent to the conventional method of frozen blood storage in maintaining sequence integrity.

A.



B.

Figure 2. (**A**) Amplification of a 2 kbp region of the GAPDH gene from VACUETTE DNAgard Blood and K_2-EDTA (-20°C) samples for each of the five donors. (**B**) Chromatogram sequence alignment of a representative 115 bp portion of the GAPDH gene from sequencing reactions from VACUETTE DNAgard Blood and K_2-EDTA (-20°C) samples from a single donor. The sequence, quality and length of read were identical in VACUETTE DNAgard Blood and K_2-EDTA (-20°C) samples for each of the five donors.

The DNA methylation patterns in DNA extracted from VACUETTE DNAgard Blood Tube samples from two different donors were compared with the methylation patterns in the corresponding frozen control blood specimens (**Figure 3**). The methylation profiles of 20 promoter loci were quantified in this assay. For each donor, the degree of methylation at each locus was equivalent in the VACUETTE DNAgard Blood Tube and frozen control samples. Loci that were hypomethylated (green and yellow boxes) or quantitatively more methylated (orange and red boxes) in frozen control samples maintained approximately the same methylation status in the VACUETTE DNAgard Blood Tube samples even after exposure to extreme temperature fluctuations.

Symbol	DGB-1	DGB-2	BD -20°C-1	BD -20°C-2	p value
BRCA2	0.1%	0.1%	0.2%	0.1%	0.73
CCNB1	1.1%	0.1%	1.1%	0.3%	0.88
CCND1	0.2%	0.0%	0.1%	0.0%	0.64
CCNE1	0.1%	0.1%	0.2%	0.0%	0.77
CCNF	0.1%	0.0%	0.3%	0.1%	0.57
CDK2	0.2%	0.2%	0.3%	0.3%	0.03
CDK4	0.0%	0.0%	0.1%	0.0%	0.34
CDK5RAP1	0.2%	0.0%	0.2%	0.0%	0.98
CDKN1A	8.8%	1.7%	11.2%	3.9%	0.69
CDKN1B	0.2%	0.0%	0.3%	0.1%	0.62
GADD45A	4.7%	0.8%	7.7%	2.4%	0.55
MCM2	0.2%	0.3%	0.2%	0.1%	0.19
MCM4	0.2%	0.1%	0.3%	0.4%	0.25
MRE11A	0.1%	0.0%	0.2%	0.4%	0.20
RAD17	0.1%	0.1%	0.1%	0.0%	0.77
RAD51	0.2%	0.0%	0.3%	0.1%	0.57
RAD9A	8.3%	8.7%	7.8%	9.2%	0.98
RBL1	0.1%	0.0%	0.1%	0.0%	0.46
RBL2	0.1%	0.0%	0.2%	0.0%	0.80
TP53	0.5%	0.1%	0.3%	0.2%	0.97

Figure 3. The methylation status of 20 promoter loci quantified using the EpiTect Methyl Signature qPCR Array for Human Cytokine Production. DNA isolated from VACUETTE DNAgard Blood (DGB) and frozen K_2-EDTA Vacutainer (BD -20°C) samples from two donors were analyzed.

Conclusions:

VACUETTE DNAgard Blood Tubes maintain DNA sequence integrity in blood samples even under extreme temperature conditions potentially encountered during worst-case shipping scenarios. DNA methylation, the most common epigenetic modification, is also preserved in blood stored in VACUETTE DNAgard Blood Tubes under extreme temperature conditions.