

Long-range PCR amplification of DNA from DNAgard[®] Blood Tubes

Steven Wilkinson, Angela Stassinopoulos, Josh Bliesath, Winnie Huang, Lutfunnessa Shireen, Scott Whitney and Rolf Muller

Experimental design:

Blood from five different human donors was collected in DNAgard[®] Blood Tubes, K₂-EDTA Vacutainers (Becton Dickinson, 2 samples per donor) and in a competitor's blood collection tubes for DNA stabilization in whole blood. One K₂-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 K₂-EDTA Vacutainer replicate per donor 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 DNAgard Blood Tubes and K₂-EDTA Vacutainers using the QIAampDNA 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.

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.

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

Long-range PCR: An equivalent amount of input DNA (250 ng) from each of the four blood storage conditions for each donor was used as the template for amplification of a 22 kbp region of the tissue plasminogen activator (tPA) gene using the Manual PCR Extender System (5 Prime).

Results:

The integrity of genomic DNA recovered from blood stored in each of the four storage conditions was analyzed by long-range PCR (**Figure 2**), a technique sensitive to ssDNA nicks and dsDNA breaks. Amplification of a 22 kbp region of the tPA gene was more efficient in DNAgard® Blood Tube samples from all five donors than in the other blood storage conditions. These data suggest that the DNA is more protected from ssDNA nicks and dsDNA breaks in DNAgard Blood samples compared with the other storage methods, including frozen storage of blood. These data do not rule out the possibility that heme or other contaminants are more efficiently removed from DNAgard Blood samples or that the DNA co-purifies with a PCR enhancer.

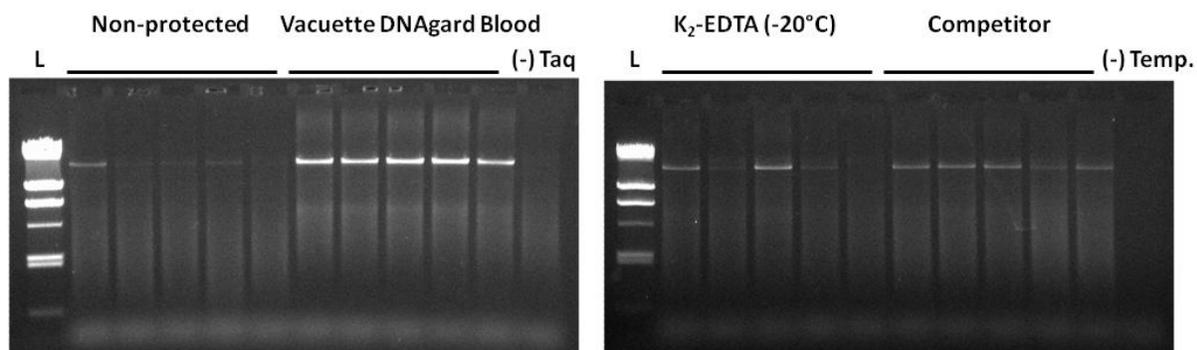


Figure 2. DNA integrity after the shipping simulation as measured by long-range PCR. DNA recovered from blood stored in the four storage conditions was analyzed for each of the five blood donors. Equal input DNA (250 ng) was used in each amplification reaction using primers to amplify a 22 kbp amplicon of the tPA gene. Control reactions with all components except the Taq polymerase ((-) Taq) were run for each DNA sample to demonstrate that the high molecular weight band represents the desired amplicon (one representative sample is shown). An additional control reaction without the DNA template ((-) Temp) was also run for the same purpose.

Conclusions:

Genomic DNA integrity is maintained in human whole blood stored in DNAgard® Blood Tubes even during exposure to temperature extremes as shown by efficient long-range PCR amplification, a technique sensitive to ssDNA nicks and dsDNA breaks. Amplification of a 22 kbp fragment of the tPA gene from 5 different human blood donors was more efficient from DNA recovered from DNAgard® Blood samples than from DNA isolated from the competitor's blood tubes or from K₂-EDTA Vacutainers.