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Effect of Storage Temperature on the Quality and Quantity of DNA Extracted from Blood

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Abstract: The present study was undertaken to study the effect of temperature during storage of blood on DNA (deoxyribonucleic acid) extracted from it or on DNA stored as such. Samples of whole blood were divided into three groups containing 10 samples each. One group was processed immediately (group A, fresh). The other two groups were stored at 4°C (group 8) or at -20°C (group C). The DNA extracted from fresh samples was divided into 3 equal portions and stored for 60 days at either: i) room temperature, ii) 4°C, iii) or at -20°C. The concentration of DNA was measured spectrophotometrically and quality of DNA from each sample was examined by electrophoresis on agarose mini gels (1.5%) stained by ethidium bromide. Average DNA yields from fresh samples was 26.55 ± 6.4 μg/ml higher ($P<0.05$) than yield of DNA from the blood stored at 4°C (18.84 ± 3.5 μg/ml of blood) and at -20°C (16.81 ± 3.18 μg/ml of blood). The storage temperatures for blood (4 and -20°C) yielded similar amount of DNA. The quality of all DNA was good with average 260/280 OD ratios ranging from 1.79 to 1.96. Results of gel electrophoresis indicated that DNA stored at -20°C was most intact and most probably had higher molecular weight and less shearing than the DNA samples stored at 4°C or at room temperature. The results can be used to advantage in storing DNA samples and then using them for molecular diagnosis of genetic diseases or other genetic disorders.

Key words: Blood, DNA, storage, isolation, quality and quantity

Introduction

Recombinant DNA Technology is increasingly important for the detection of genetic diseases (Hodgkinson and Scambler, 1984; Kazazian, 1985; Steel, 1984), solving forensic problems (Dodd, 1985; Gill *et al*., 1985; Jeffreys *et al*., 1985) and for investigations of specification (Jeffreys, 1984; Higuchi *et al*., 1984). Tissue or DNA that have been stored for very long time may be needed for these studies. In addition, blood, the most accessible source is generally mailed to diagnostic laboratories and thus, may require proper preservation during collection and storage, to be used as a source for higher molecular weight DNA. The standard procedure for this purpose involves freezing anticoagulated blood samples in liquid nitrogen and shipping samples at very low temperature. Cultured cells remained viable in liquid nitrogen for at least 20 years (Hull, 1983) and therefore DNA may remain intact for long times (Towne and Devor, 1990). However, this is highly specialized procedure for preserving or storing DNA that necessitate sample preparation, special freezing, liquid nitrogen, special care in thawing the samples. Clearly, it is not appropriate as a routine procedure for preserving DNA. The impact of other storage procedures on the quality or quantity of DNA of blood samples has largely been ignored. Infect, the optimal conditions for storage of blood and of extracted DNA are not yet clear. This study was conducted to evaluate the quality and quantity of DNA stored under various storage conditions as blood samples or purified genomic DNA.

Materials and Methods

Blood samples were collected from ten healthy individuals in 5-ml evacuated EDTA tubes, and immediately placed on ice. Samples, from each individual were divided into three equal parts. One part was immediately processed to evaluate DNA yield and quality. The remaining two parts from each individual were stored for 95 days at either 4 or 20°C.

Anticoagulated whole blood was mixed with 4 volumes of chilled lysis solution (0.32 M sucrose, 10 mM Tris HCl, 5 mM $MgCl_2$ and 1 percent Triton X-100) in a 20 ml disposable plastic centrifuged tubes and centrifuged at 2,500 rpm for 15 min at 4°C. The pellet that contains nucleated cells was again suspended with 2 volumes of lysis solution, centrifuged again for 10 win. Lysates were treated with proteinase K (50 μM of 20 mg/ml) and incubated at 37°C for over night. Samples were then extracted with phenol/chloroformisoamyl alcohol and precipitated with ammonium acetate and ethanol. The DNA was dissolved in 100 μl of TE buffer (10 mM Tris HCl pH 8.0 and 1 mM EDTA).

DNA extracted from fresh whole blood was dividid into 3 equal portions and stored at i) room temperature, ii) 4°C and iii) -20°C for 95 days. DNA was quantitated spectrophotometrically at 260 nm and purity was evaluated by examining the average ratio of UV absorbance at 260 and 280 nm. DNA quality was evaluated after separation by electrophoresis in 1.5 percent TBE agarose gel. DNA was stained with ethidium bromide and visualized under UV light. The student's t test was used to make pair wise comparisons between selected-means.

Results and Discussion

The isolated DNA was dissolved readily in TE buffer and did not have any pigments as is commonly eitien in blood DNA isolates. Fresh blood yielded the highest ($P<0.05$) amount of DNA (26.55 ± 6.44 μg/ml of blood), compared with the blood stored at 4°C (18.84 ± 3.50 μg/ml of blood, -29.05%) or at -20°C (18.81 ± 3.18 μg/ml of blood, -36.70%). Thus, blood samples stored at 4°C yielded 10 percent higher DNA than samples stored at -20°C. However, the amount of DNA extracted from the blood samples was sufficient for most of the molecular biology techniques. The 95 percent confidence limit of three amounts of DNA showed that the amount of DNA after storage of blood at 4°C and -20°C did not significantly differ from each other. However, they are significantly ($P<0.05$) different from the amount obtained from fresh blood. So the yield of DNA is about the same at two temperattires. The quality of all DNA was satisfactory; i.e. it's average O. D. 260/280 ratio was 1.87 ± 1.01 and there was no contamination of RNA present as determined by gel electrophoresis analysis, and all DNA samples can readily be used for most applications.

There was no significant ($P<0.05$) difference among DNA samples

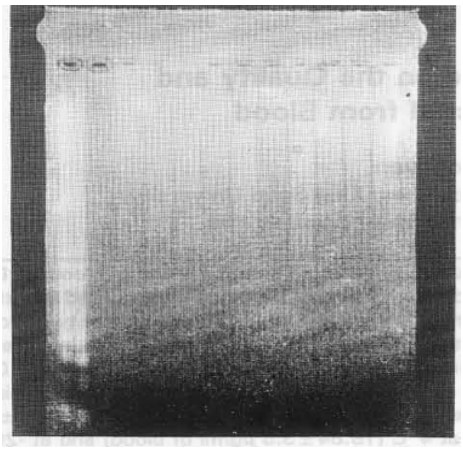

Fig. 1: Ethidium bromide stained agarose mini gel for DNA extracted from fresh blood samples. Lanes 2-11 are for fresh DNA samples and lane 1 is for DNA Ladder).

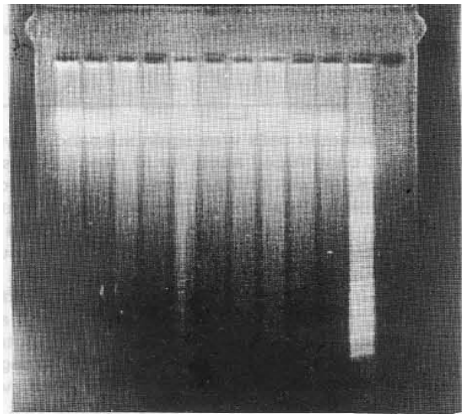

Fig. 2: Ethidium bromide stained agarose mini gel for DNA extracted from blood samples stored at 4°C. Lanes 1-10 are for samples stored at 4°C and lane 11 is for DNA Ladder).

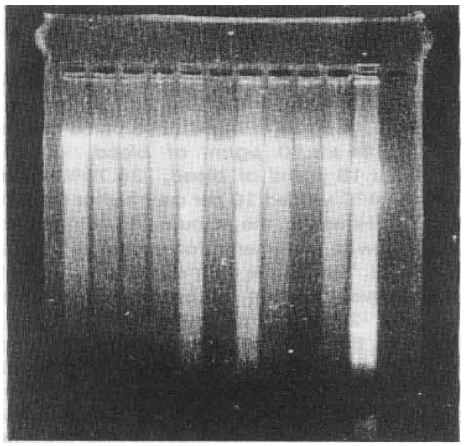

Fig. 3: Ethidium bromide stained agarose mini gel for DNA extracted from blood samples stored at -20°C. Lanes 1-10 are for samples stored at -20°C and lane 11 is for DNA Ladder).

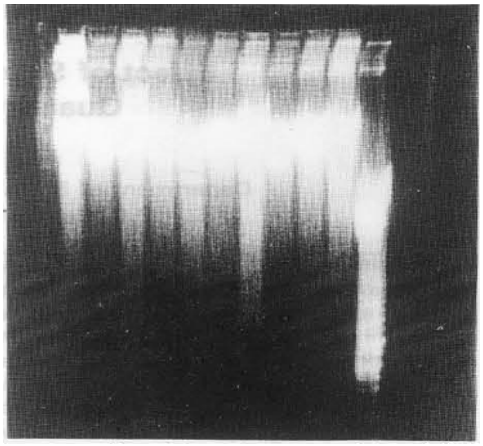

Fig. 4: Ethidium bromide stained agarose mini gel for DNA samples stored at 4°C. Lanes 1-10 are for samples stored at 4°C and lane 11 is for DNA Ladder).

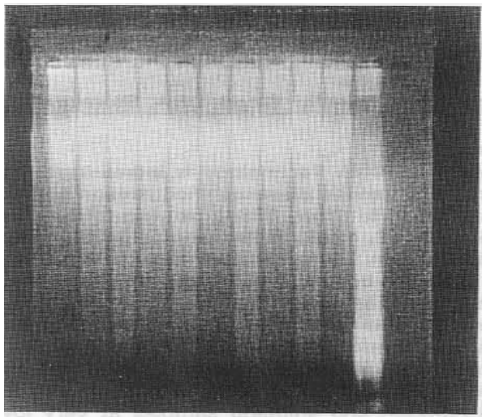

Fig. 5: Ethidium bromide stained agarose mini-gel for DNA samples stored at -20°C. Lanes 1-10 are for samples stored at 4°C and lane 11 is for DNA Ladder).

stored at various temperatures. The mean DNA values for the samples stored in TE buffer at room temperature, at 4°C or at -20°C were 26.20 ± 6.56, 27.32 ± 4.23 and 25.96 ± 7.15 respectively.

Electrophoresis on agarose gel, showed that the freshly extracted DNA from blood samples was more stable and intact than the DNA extracted from blood stored at 4°C or -20°C (Fig. 1, 2, 3). Purified human genomic DNA samples stored at -20°C are more stable and larger than the DNA stored at 4°C (Fig. 4, 5).

It is common knowledge that the best way to isolate high molecular weight DNA is to use fresh tissue as the source material. In a number of situations, however, fresh tissue cannot be obtained and one has to rely on already collected and stored samples. When blood is the source tissue, it is often frozen immediately and stored or transported at low temperatures. The extract ability of DNA from whole blood depends upon the viability of the leukocytes. The viability of granulocyte (McCullough *et al.*, 1974) in whole blood decreased about 14 percent in 24 hr at 4°C and several studies (McCullough *et al.*, 1978; Glasser *et al.*, 1985) recommended 22°C as optimum temperature for storage of granulocytes.

However, it has been suggested by Madisen *et al*. (1987) that low yields of DNA may result, despite the high white blood cell counts, because of the use of insufficient extraction solution. The type and concentration of anticoagulant used or other additives may affect DNA extraction. The presence of EDTA as an anticoagulant resulted in high yield of good quality DNA (Madisen *et al*., 1987) In the present study a 37 percent decrease of DNA was noted when blood was frozen at -20°C and a 29 percent decrease when stored at 4°C. The yield of DNA decreased by 30-40 percent if blood is frozen at -20°C or stored at 4°C. Our work shows that the quantity of extractable DNA in the blood falls when stored at 4°C or -20°C, but its quality remain unaffected. The yield of DNA after storage of blood at 4°C and -20°C did not significantly differ from each other. However, they are significantly ($P<0.05$) lower from the amount obtained from fresh blood. Therefore the yield of DNA is about the same at both the temperatures. These are in line with those reported by Gustincich *et al*. (1991).

In summary, extraction of DNA should be done as quickly as possible from whole blood. If the immediate preparation of DNA cannot be done, the whole blood may be stored at 4°C or - 20°C once the DNA is extracted, it can be stored at any of the above-mentioned temperatures. However, to have the DNA intact with higher molecular weight -20°C is recommended to store the purified DNA. Based on the preceding results the some of the observations have been noted; i) if DNA is to be extracted with in 7-8 weeks, store blood samples at 4°C ii) if blood is frozen at -20°C, expect a general decline in quantity of DNA iii) it is important to minimize exposure of blood and DNA samples to warmer temperature.

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