DNA Extraction and Amplification of 10-Day, Room-Temperature Blood Samples

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Abstract

DNA was serially studied in 20 samples of buffy coat stored at room temperature. Each sample was divided into 5 equal volumes, namely D_0, D_3, D_5, D_7 and D_{10}. DNA extraction was performed on days 0, 3, 5, 7 and 10 after blood collection. The mean ratio of OD260/OD280 of the DNA obtained from D_0 to D_{10} ranged from 1.77 to 1.79, and the mean amounts of the DNA obtained from D_0 to D_{10} ranged from 602 to 740 ng/ul. There were no significant differences in the mean ratio and amounts of DNA obtained among these samples ($p > 0.05$). Subsequently, amplification was successfully performed from this template DNA to yield products of 1.4 kb and 142 bp at the sites associated with ß globin and factor VIII genes, respectively. These findings suggest the possibility of sending blood samples for DNA analysis by mail, or no ice is required during transportation.

Key word : DNA Extraction, Buffy Coat

As a result of new technologies in medical science, several diseases can be definitely diagnosed by DNA study. This will enable detection of carriers of various genetic disorders such as thalassemia and haemophilia, so that genetic counseling can be effectively provided to the patients and family members. Either linkage analysis or direct defect detection may be used to identify carriers. Blood samples from patients and family members should be drawn for DNA analysis. However, facilities are extremely limited at Thai university hospitals, and public transportation service is not equipped to transport blood samples on ice. Consequently, patients in rural areas must be transferred to university hospitals, which is costly and inconvenient.

This paper presents the findings of DNA extraction and amplification of 10-day, room-temperature blood samples at the Faculty of Medicine, Ramathibodi Hospital, Bangkok.

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MATERIAL AND METHOD

Ten ml of whole blood mixed with 500 ul of sterile 0.2 M EDTA were collected in a 15-ml sterile polypropylene screw cap tube and centrifuged at 3,000 rpm for 10 minutes. After discarding the plasma and collecting 1,000 ul of buffy coat in a 1.5-ml microcentrifuge tube, the specimen was mixed well and divided into five microcentrifuge tubes, 200 ul each and kept at room temperature. The average room temperature was about 28-33°C. Each microcentrifuge tube was labeled as D_0, D_3, D_5, D_7 or D_{10}. DNA extraction was performed by the "in house" modified method[1] on days 0, 3, 5, 7 or 10 after blood collection.

The dry DNA pellet was dissolved with 30 ul of distilled water. Then, 10 µl of DNA solution was diluted with 990 ul of distilled water and the DNA concentration was measured at OD 260 and OD 280 with a spectrophotometer and 10-mm light path quartz cuvet. The ratio of OD 260/OD 280 was calculated and the DNA obtained was determined by the formula of one OD at 260 nm equal to 50 ug/ml of DNA.

Subsequently, the DNA obtained at two different sites was amplified. The first site studied was the ß globin gene. Primers were 5'-TGTCATC ACTTAGACCTCAC-3' and 5'-GCTTGGACTCA GAATAATCC-3'. The 250 ng of genomic DNA was amplified in 40-ul reaction volumes which consisted of 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$ and 0.01 per cent gelatin; 200 uM dNTPs (Pharmacia), 20 pM of each primer and 1 unit of Taq polymerase (Perkin-Elmer). Following an initial denaturing at 96°C for 10 minutes, thermocycling of 96°C for 2 minutes, 55°C for 1 minute and 72°C for 1 minute for 28 cycles, a final extension at 72°C for 5 minutes was carried out. The amplified DNA contained 1.4 kilobases.

The second site studied was the restriction site polymorphism of *Bcl* I associated with the factor VIII gene. Primers, 5'-TAAAAGCTTTAAA TGGTCTAGGC-3' and 5'-TTCGAATTCTGAAAT TATCTTGTTC-3' were obtained from Kogan et al [2]. The 250 ng of genomic DNA was amplified[3,4] in 50 ul reaction volumes which consisted of 67 mM Tris-HCl pH 8.8, 16.6 mM $(NH_4)_2 SO_4$, 5 mM $MgCl_2$, 10 mM beta-mercaptoethanol, 10 ug/ml bovine serum albumin; 200 uM dNTPs (Pharmacia), 75 ng of each primer and 1 unit Taq polymerase (Perkin-Elmer). Following an initial denaturation at 94°C for 5 minutes, thermocycling of 94°C for 1 minute, 55°C for 1 minute and 72 °C for 2 minutes was carried out for a total of 30 cycles. The amplified DNA contained 142 basepairs.

Table 1. Hematological data of the samples.*

Variables	
Hb (g/dl)	13.85 ± 1.23
Hct (%)	42.55 ± 3.21
WBC (x 10^3/ul)	7.17 ± 1.65
Lymphocyte %	35.56 ± 6.96
Neutrophil %	58.92 ± 7.65
Monocyte %	5.18 ± 2.01

* Mean ± SD

Statistical methods

Statistical analysis was performed using SPSS for Microsoft Windows. Data were analysed by repeated measurement analysis of variance. A p value of less than 0.05 was considered significant.

RESULTS

Twenty specimens of whole blood were collected from normal personnel in Ramathibodi Hospital (11 males and 9 females) aged 21 to 48 yrs. The hematological data are shown in Table 1.

The purity of the DNA was expressed by the ratio of OD260 to OD280. The mean ratios of the DNA obtained from the D_0 to D_{10} samples were similar, ranging from 1.77 to 1.79. In addition, the mean amounts of the DNA obtained from days 0, 3, 5, 7 and 10 were also similar, ranging from 602 to 740 ng/ul (Fig. 1). There were no significant differences in the mean ratio or the amounts of the DNA obtained among these samples ($p > 0.05$). Subsequently, amplification was performed from this template DNA to yield products of 1.4 kilobases and 142 basepairs. The amplified products from the template DNA on days 0, 3, 5, 7 and 10 were similar as shown in Fig. 2.

DISCUSSION

The purity of DNA extracted from 10-day, room-temperature buffy coat samples was consistent. The amounts of DNA obtained were large as well, and amplified products of 1.4 kb and 142 bp were clearly revealed.

ß globin and factor VIII gene studies are crucial, since thalassemia and hemophilia are the

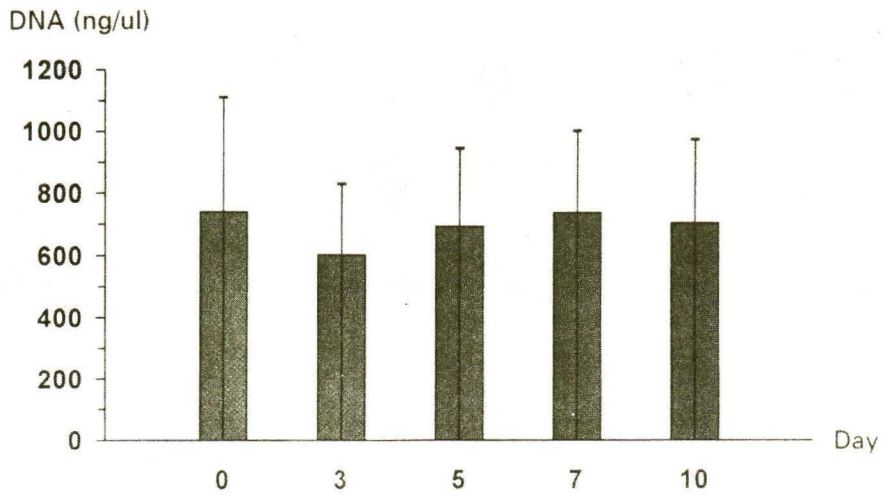

Fig. 1. The mean amounts of the DNA obtained on days 0, 3, 5, 7 and 10.

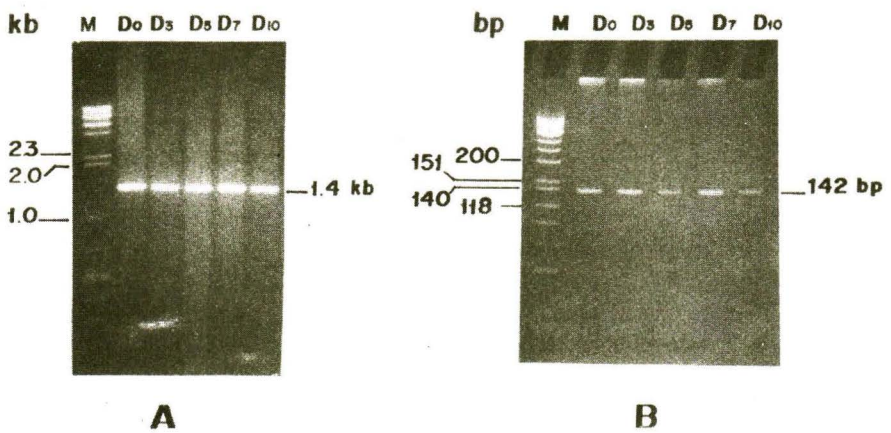

Fig. 2. The electrophoresis of the amplified products of 1.4 kb (A) and 142 bp (B) from the template DNA obtained on days 0, 3, 5, 7 and 10. M = marker, kb = kilobases, bp = basepairs.

two most common hereditary chronic anemia and severe bleeding disorder in Thailand. More accurate diagnosis of the patients and carriers will lead to effective prevention of these hereditary diseases. However, precise pedigrees and accurate identification of the samples are required. Any human error in blood collection procedures will result in incorrect diagnosis.

Recently, there have been several forensic medical reports of DNA extraction and amplification from minute amounts of specimens such as blood stains, saliva, and semen[5]. As the yield of DNA extracted may be small, highly specialized technique are required for this delicate work. DNA amplification from a blood spot on filter paper has been reported[6] but we have found that particles

from the filter paper interfere with the DNA analysis.

We conclude that extraction and amplification of DNA from 10-day, room-temperature blood samples are practical and simple. This suggests the possibility of sending blood samples for DNA analysis by mail, or no ice is required during transportation.

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การสกัดและเพิ่มปริมาณ ดีเอ็นเอ จากเลือดที่เก็บไว้ที่อุณหภูมิห้องเป็นเวลา 10 วัน

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คณะผู้วิจัยได้ศึกษาปริมาณ ดีเอ็นเอ ที่สกัดจาก buffy coat ของคนปกติ 20 ราย ที่เก็บไว้ที่อุณหภูมิห้อง ในวันที่ 0, 3, 5, 7 และ 10 หลังการเจาะเลือด ผลปรากฏว่า ดี เอ็น เอ เหล่านี้มีปริมาณระหว่าง 602–740 ng/μl โดยมีค่าของ OD_{260}/OD_{280} เท่ากับ 1.77–1.79 ซึ่งไม่มีความแตกต่างกันในทางสถิติ ($p > 0.05$) นอกจากนี้ยังได้นำ ดี เอ็น เอ ที่ได้นี้ไปทดสอบการเพิ่มปริมาณโดยวิธี polymerase chain reaction ของยีนที่ควบคุมการสร้าง ß globin ขนาด 1.4 kb และ factor VIII ขนาด 142 bp ก็ให้ผลไม่แตกต่างกันในแต่ละตัวอย่าง แสดงว่าการเก็บตัวอย่างเลือดไว้ที่อุณหภูมิห้องเป็นเวลา 10 วัน มิได้ทำให้ปริมาณและคุณสมบัติของ ดีเอ็นเอ ที่สกัดได้เปลี่ยนแปลง ดังนั้นจึงมีความเป็นไปได้ที่จะส่งเลือดผู้ป่วยจากจังหวัดที่ห่างไกลทางไปรษณีย์โดยไม่จำเป็นต้องแช่น้ำแข็งในระหว่างการขนส่ง เพื่อนำมาศึกษาหาความผิดปกติของ ดี เอ็น เอ ในโรงพยาบาลมหาวิทยาลัยได้

คำสำคัญ : การสกัดดีเอ็นเอ, เม็ดเลือดขาว

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