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DNA Isolation by a Rapid Method from Human Blood Samples: Effects of $MgCl_2$, EDTA, Storage Time, and Temperature on DNA Yield and Quality

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The isolation of DNA from whole blood by a modified rapid method (RM) was tested using various detergents and buffer conditions. Extraction of DNA with either NP-40 or Triton X-100 gave a high yield of undegraded DNA in less than an hour. The concentration of magnesium ion in the buffers was critical to obtaining intact, high molecular weight (HMW) DNA. Greater than 10 mM $MgCl_2$ led to degradation. Addition of EDTA to the buffer inhibits this degradation. Preparation of DNA from blood stored at room temperature or incubated at 37°C for 24 hr resulted in the same amount and quality of DNA as from samples frozen at −70°C. DNA from blood samples that had undergone more than four freeze-thaw cycles was found to be partially degraded. The modified RM can be applied to extract DNA from as little as 10 μl of blood (340 ng of DNA) and from dried blood samples. DNA samples remained intact and undegraded for longer times when DNA was dissolved in higher concentrations of EDTA.

KEY WORDS: high molecular weight DNA; $MgCl_2$; integrity of DNA; rapid method; DNA banking.

INTRODUCTION

The application of genetics to study human disease (Anderson, 1992; Miller, 1992) and to analyze gene function *in vivo* depends upon the quality of DNA samples. Intact and good-quality DNA is also essential for screening DNA

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samples to study polymorphisms. However, these studies often require analysis of a large number of DNA samples, which must be of sufficient quality for Southern blot analysis. Recently, a fast, safe, and economical method (RM) was developed to extract DNA from whole blood (Lahiri and Nurnberger, 1991). The main advantage of the RM is that it is a nonenzymatic and inorganic procedure. There are two important aspects of DNA preparation: yield, and degree of degradation of DNA samples. The present report deals with various factors that influence the integrity of DNA. We show that the concentration of $MgCl_2$ is crucial to obtain intact, high-yield DNA from whole blood. We present a simplified version of the RM that extracts DNA with fewer steps and with less manipulation. We have examined the effect of blood storage time and temperature on DNA yield and quality and have studied the state of extracted DNA in different buffers.

MATERIALS AND METHODS

Brij-35, digitonin, nonidet P-40 (NP-40), Sarcosyl, Triton X-100, Tween-20, and Tween-80 were bought from Sigma Chemical Co. Other chemicals used were analytical grade (Sigma). Whole blood was collected in a Vacutainer tube (purple-stoppered) containing 100 μl of 15% EDTA. The simplified version of the RM is described below. One milliliter of blood was treated with an equal volume of low-salt buffer containing 10 m*M* Tris–HCl, pH 7.6, 10 m*M* KCl, 2 m*M* EDTA (TKE) containing 4 m*M* $MgCl_2$ (TKM). Twenty five microliters of NP-40 was added and the cells were lysed by inverting several times. The suspension was centrifuged at 1000*g* for 10 min at room temperature (RT). The pellet of mostly leukocytes was saved and washed two more times with TKM buffer. The final pellet was resuspended in 0.2 ml of TKM buffer. Fifteen microliters of 10% sodium dodecyl sulfate (SDS) was added, and the whole suspension mixed thoroughly and incubated for 5 min at 55°C. After adding 75 μl of saturated NaCl (~ 6 *M*), the tube was mixed well and centrifuged at 12,000*g* for 5 min. The supernatant contained DNA, which was precipitated using ethanol. DNA was redissolved in 0.5 ml of 10 m*M* Tris–HCl, 1 m*M* EDTA, pH 8.0 (TE). The yields of DNA were calculated from the absorbance at 260 nm (Maniatis *et al.*, 1982) and by comparison with a molecular weight DNA standard on an agarose gel stained with ethidium bromide.

RESULTS

Effect of Different Detergents in the Extraction of DNA from Blood Samples

Two kinds of detergents were used to study their effects in the extraction of DNA. One group lysed the red cells, resulting in a clear solution; the other

did not lyse the cells, yielding a cloudy solution and a larger pellet of cells. When whole blood was washed with TKM buffer containing NP-40 or Triton X-100, there was a complete lysis of red cells. A gradual increase in DNA yield was observed with an increasing amount of either NP-40 or Triton X-100. Both detergents gave the highest yields at concentrations greater than or equal to 1.2% in TKM buffer (25–32 μg DNA/ml blood) (data not shown). The addition of Brij-35, digitonin, Sarcosyl, and Tween-20/-80 had no effect on lysis. No particular trend was noted when DNA yield was measured after the addition of various amounts of these detergents (data not shown), and the lowest amount of DNA was obtained with sarcosyl (3 μg DNA/ml blood).

Effect of $MgCl_2$ and EDTA in the Extraction of DNA

We extracted DNA at various concentrations of $MgCl_2$ (from 0 to 25 m*M*) in TKM buffer. When equal amounts of DNA from each preparation were compared, a gradual degradation was observed. The degradation was more pronounced after 10 m*M* $MgCl_2$, and at 20 m*M* $MgCl_2$ DNA was mostly degraded (Fig. 1A). Below 10 m*M* $MgCl_2$ the yield of DNA increased gradually with increased $MgCl_2$ but it was not linear. We obtained a maximum amount of DNA in the range of 6–10 m*M* $MgCl_2$ (data not shown).

To confirm that degradation of DNA was caused solely by excess $MgCl_2$, we wanted to see if this effect could be inhibited by the addition of EDTA. The result of extraction of DNA with varying amounts of EDTA indicated that its concentration had an inverse effect on the degradation, but not yield, of DNA. When we extracted DNA by the RM at 10 m*M* $MgCl_2$ in the presence of increasing amounts of EDTA, the degradation of DNA was gradually inhibited (Fig. 1B). DNA prepared at 10 m*M* $MgCl_2$ was partially degraded but this could be inhibited by the addition of an equal concentration of EDTA. EDTA (2 m*M*) yielded undegraded DNA at 4 m*M* $MgCl_2$.

Effect of NaCl in the Extraction of DNA

In the original RM procedure (Lahiri and Nurnberger, 1991) the addition of NaCl was required in two of the four steps of DNA extraction: lysis of leukocytes with TKM2 buffer (10 m*M* Tris–HCl, pH 7.6, 10 m*M* KCl, 10 m*M* $MgCl_2$, 0.4 *M* NaCl, and 2 m*M* EDTA) and precipitation of denatured cellular proteins by saturated salts. There was no change in either yield or quality of DNA when NaCl was varied from 0 to 1 *M* in TKM2 buffer (data not shown). Thus the need for the inclusion of 0.4 *M* NaCl in TKM2 solution was abolished, and the simplified RM procedure replaced TKM2 with TKM. The step involving the addition of 1.2 *M* NaCl to precipitate the proteins out of DNA–protein complexes remained the same.

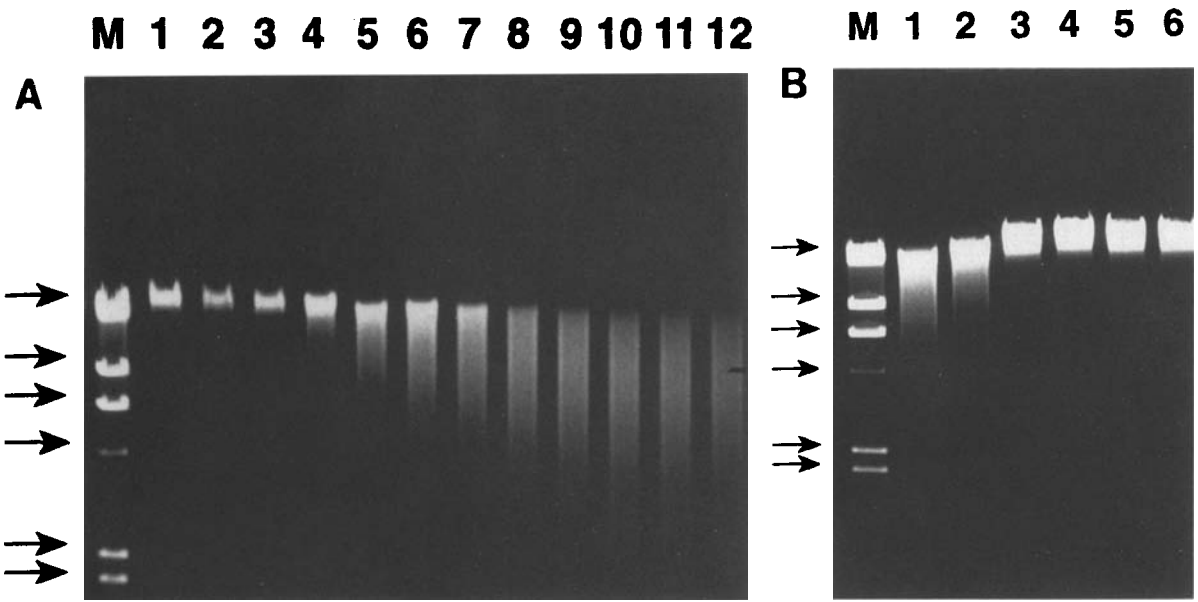

Fig. 1. Effect of different concentrations of $MgCl_2$ and EDTA in the extraction of DNA. (A) Leukocytes were isolated from 12 aliquots of 1 ml whole blood. Each was dissolved in TKE buffer containing 0.75% SDS and varying concentrations of $MgCl_2$. The rest of the procedures were as described under Materials and Methods. Agarose gel picture of DNA extracted from different samples that were processed in buffers differing only in $MgCl_2$: 0 m*M* (lane 1), 1.5 m*M* (lane 2), 3 m*M* (lane 3), 4 m*M* (lane 4), 5 m*M* (lane 5), 6 m*M* (lane 6), 8 m*M* (lane 7), 10 m*M* (lane 8), 12 m*M* (lane 9), 15 m*M* (lane 10), 20 m*M* (lane 11), and 25 m*M* (lane 12). Lane M represents the standard size markers for λ DNA + *Hin*dIII. Different arrows represent the size of fragments, from the top (kb): 23.1, 9.4, 6.6, 4.4, 2.3, and 2.0. (B) DNA was extracted from 1 ml of whole-blood samples under various concentrations of EDTA and Mg^{2+}: 10 m*M* $MgCl_2$ and 2 m*M* EDTA (lane 1), 10 m*M* $MgCl_2$ and 5 m*M* EDTA (lane 2), 10 m*M* $MgCl_2$ and 10 m*M* EDTA (lane 3), 0 m*M* $MgCl_2$ and 2 m*M* EDTA (lane 4), 0 m*M* $MgCl_2$ and 5 m*M* EDTA (lane 5), and 0 m*M* $MgCl_2$ and 10 m*M* EDTA (lane 6). Lane M represents the size markers as described in A.

Effect of Tris, KCl, and SDS in the Extraction of DNA

We varied the concentration of Tris in TKM buffer from 10 to 100 m*M*, keeping the same pH (7.6), and observed no significant change in either the yield or the quality of DNA (data not shown). KCl did not alter the yield of DNA significantly when tested between 5 and 20 m*M*. Above 25 m*M* KCl, the addition of SDS caused a milky white precipitate. The amount of SDS did not affect either the yield or the quality of DNA between 0.5 and 1.0% (data not shown). Below this, the yield of DNA decreased due perhaps to incomplete lysis of leukocyte nuclei. High concentrations caused a milky white precipitate to form along with the precipitation of DNA.

Effect of Storage Temperature and Freeze-Thawing in the Extraction of DNA

To determine the effect of temperature of the stored blood prior to the extraction of DNA, we incubated aliquots of the same blood at various temperatures for 24 hr in Parafilm-wrapped, capped microfuge tubes. When we extracted DNA from blood aliquots that were stored at 45, 37, 25 (RT), 4, −20, and −70°C, we observed no significant change in the yield or quality of DNA among those extractions. DNA preparations remained high molecular weight (Fig. 2, lanes 1–6). To determine the effect of freeze-thaw (FT) cycles, the following experiment was designed: one FT cycle consisted of rapidly freezing the sample by incubating for 10 min in a dry ice/ethanol mixture followed by a quick thawing at 37°C for 10 min. DNA extracted from samples that were passed through more than four FT cycles was found to be more degraded than corresponding frozen samples (lanes 7–9). We also extracted DNA from a dried blood sample (100 μl) by the simplified RM procedure. The quality of DNA was as good as from nondried samples (lane 10 vs lanes 1–6), and the yield was comparable to normal samples (2.6 μg DNA).

DNA was extracted successfully from whole blood of more than 100 individuals, and the DNA preparation was cleavable by restriction enzymes (data not shown). This extraction procedure may be scaled up or down safely. We could extract DNA from as little as 10 μl of blood (340 ng of DNA). DNA extracted by the RM can also be used in polymerase chain reaction-based assays (Saiki *et al.*, 1986).

Stability of DNA Samples in the Presence of Various Buffers

To determine the effect of different components of TE buffer on the quality of DNA prepared by the modified RM, the following "aging" experiment at various temperatures was performed. The same lot of stored blood was used

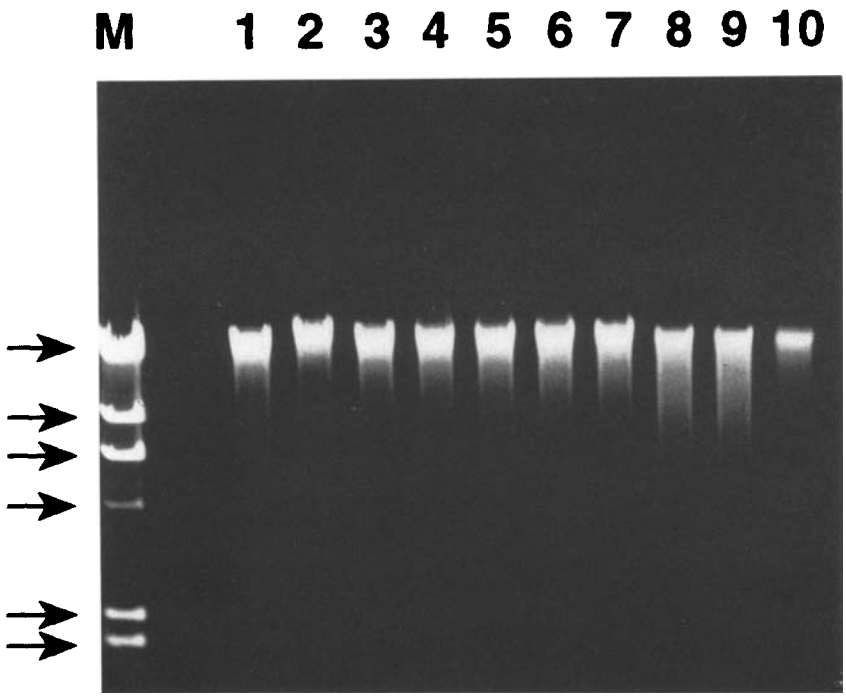

Fig. 2. Effect of different conditions of the stored blood in the extraction of DNA. Agarose gel picture of DNA from whole blood that was incubated for 24 hr at 45°C (lane 1), 37°C (lane 2), RT (lane 3), 4°C (lane 4), −20°C (lane 5), and −70°C (lane 6), blood that underwent FT cycle 2 (lane 7), FT cycle 4 (lane 8), and FT cycle 6 (lane 9) and was recovered from dried sample (lane 10). Lane M represents the size markers as described in the legend to Fig. 1A.

to extract DNA and 18 aliquots of equal amounts of DNA were made. These were divided into three batches of six tubes each. Each batch was dissolved (pH 8.0) in either 10 m*M* Tris–Cl and 2 m*M* EDTA (TE_2) or 10 m*M* Tris–Cl and 5 m*M* EDTA (TE_5) or 10 m*M* Tris–Cl and 10 m*M* EDTA (TE_{10}). Each tube within a batch was incubated for 40 days at one of six temperatures. The temperatures of incubation were −70, −20, 4, 25 (RT), 37, and 45°C (Fig. 3). At −70, −20, and 4°C, we observed no significant change in either the yield or the quality of DNA (lanes 1–9). However DNA samples in TE_2 buffer were degraded when incubated at higher temperatures (lanes 10, 13, and 16). This degradation could be slowed if the DNA was originally dissolved in TE_5 (lanes 11, 14, and 17) and it could be inhibited if DNA samples were dissolved in TE_{10} (lanes 12, 15, and 18). Thus degradation could be prevented in a higher concentration of EDTA. Similar results were obtained after 60 days of aging. There was no difference in the integrity of the DNA when Tris was varied in the buffer (data not shown).

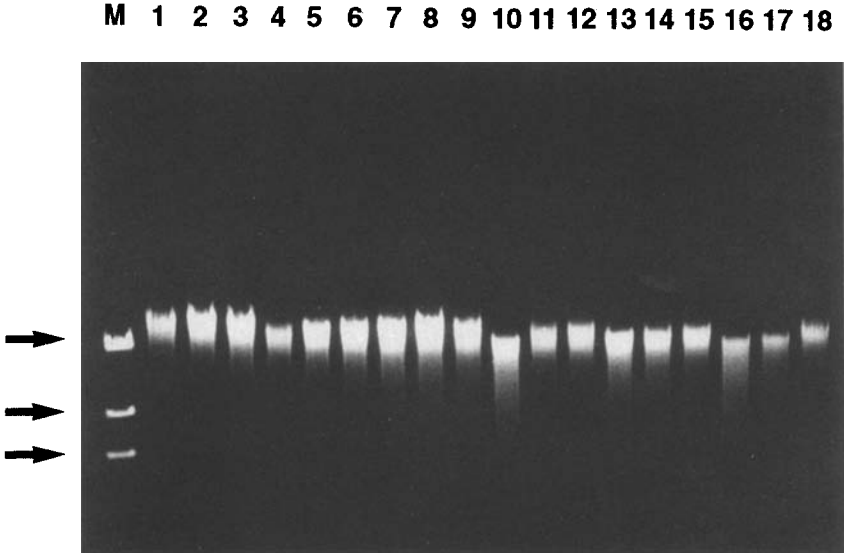

Fig. 3. Effect of different TE buffers on the stability of DNA. DNA as prepared by the RM was dissolved in TE_2 (lanes 1, 4, 7, 10, 13, 16), TE_5 (lanes 2, 5, 8, 11, 14, 17), and TE_{10} (lanes 3, 6, 9, 12, 15, 18). They were incubated for 40 days at −70°C (lanes 1–3), −20°C (lanes 4–6), 4°C (lanes 7–9), 25°C (lanes 10–12), 37°C (lanes 13–15), and 45°C (lanes 16–18).

DISCUSSION

Extraction of HMW DNA from human blood under various conditions and its recovery after long-term storage in an intact form are of great interest to those studying genetic diseases. Although many methods of isolating genomic DNA exist, most of them are inefficient to some degree (Miller *et al.,* 1988; Lahiri *et al.,* 1992; Douglas *et al.,* 1992). Cell lysis is often time-consuming, involving enzymes and organic solvents, and can result in damaged DNA. The "ideal method" should be simple, fast, safe, and economical and it should not involve enzymes or phenol/chloroform extractions. Our purpose here was to study yield and quality of DNA under various conditions that affect either the extraction or the storage of DNA.

Two detergents, NP-40 and Triton X-100, among others tested, gave the highest yield of DNA (average, 25–32 μg/ml blood). We extracted DNA in TKM buffers at various concentrations of $MgCl_2$. Using the twin criteria of yield and integrity of DNA, we selected 4 m*M* $MgCl_2$ for subsequent operations. This simplified RM can be applied successfully to a smaller amount of blood (10 μl) and to those samples that were stored at high temperatures. This is important for shipment of the blood samples at different seasons of the year. We also used this RM procedure to extract

DNA from dried blood specimens, which is useful for old blood specimens and forensic purpose (McCabe *et al.*, 1987).

We also examined the problem of long-term storage of purified DNA. We attempted to accelerate artificially the aging process (Madisen *et al.*, 1987). It was reasoned that the rupture of internucleotide bonds and hence the degradation of HMW DNA are more likely at higher temperature. The extent of degradation of aging DNA samples can be used as a test for the integrity of DNA samples extracted under the same condition but dissolved in different TE buffers. We have used this as a criterion for developing an ideal buffer to dissolve DNA for long-term storage or for DNA banking purposes. DNA prepared by the modified RM and dissolved in TE_{10} buffer remains intact under the conditions we used. Thus this method is well suited to extract DNA from smaller samples stored over a wide range of conditions. Preparation of intact DNA from whole blood is important because blood is a common source for genotype diagnostic services. At present, many laboratories receive blood samples by mail and/or store blood samples prior to DNA extraction. The modified RM method provides a fast, safe, and economical method in applications for genetic, clinical, and forensic studies.

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