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DNA microextraction from dried blood spots on filter paper blotters: potential applications to newborn screening

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Summary. Microextraction of DNA from dried blood specimens would ease specimen transport to centralized laboratory facilities for recombinant DNA diagnosis in the same manner as use of dried blood spots allowed the broad application of screening tests to newborn populations. A method is described which reproducibly yields 0.5 µg DNA from the dried equivalent of 50 µl whole blood. Though DNA yields decreased with storage of dried specimens at room temperature, good-quality DNA was still obtained. Sufficient DNA was routinely obtained for Southern blot analysis using repetitive and unique sequences. This microextraction procedure will allow immediate application of molecular genetic technology to direct newborn screening follow-up of disorders amenable to DNA diagnosis, such as sickle cell anemia, and may eventually permit primary DNA screening for specific mutations.

Introduction

Newborn screening efforts were facilitated by the use of dried blood spots on filter paper blotters, since these blotters simplified shipment of specimens and allowed centralization of laboratory facilities (Guthrie 1980). These dried blood samples have been used for various tests including those based on screening for red blood cell proteins, as in galactosemia or the hemoglobinopathies, for hormonal measurements, as in hypothyroidism, and for metabolite accumulation, as in phenylketonuria, homocystinuria, or maple syrup urine disease (Guthrie 1980; McCabe and McCabe 1983).

We have been interested in applying molecular genetics techniques to newborn screening. The development of a routine procedure for microextraction of DNA from dried blood spots would simplify specimen transport, would allow direct follow-up of positive screens, and eventually might permit primary screening at the DNA level. The purpose of the current investigation was to determine whether DNA was present in dried whole blood spots on filter paper blotters in a form that could be extracted and isolated and in sufficient quantities for use in DNA analyses.

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Methods

EDTA-anticoagulated fresh whole blood specimens were drawn, white blood cells prepared, and DNA was extracted using modifications of previously described methods (Kan et al. 1977; Poncz et al. 1982). Dried blood specimens were prepared on Schleicher and Schuell newborn screening blotters or Whatman 3 MM filter paper. Measured aliquots of whole blood were spotted directly or anticoagulated with EDTA before spotting. Dried blood specimens were stored at room temperature.

After several methods of extraction had been investigated, including direct total proteolysis of the dried blood specimen similar to that described for specimens dried on cotton cloth (Gill et al. 1985), we settled on the following procedure involving white blood cell rehydration, since it gave the highest reproducible yields of DNA. A dried blood spot on filter paper equivalent to 50 µl whole blood was shredded or minced and placed in a 10-ml plastic tube. Three milliliters 0.85% NaCl were added and allowed to stand at room temperature for 1 h with occasional gentle shaking. The 3-ml fluid layer was then pipetted off, leaving the paper behind. A 1.5-ml aliquot was placed into a 1.5-ml Eppendorf tube and centrifuged, the white blood cell pellet retained, and the supernatant discarded. This procedure was repeated with the remaining 1.5-ml aliquot in the same Eppendorf tube. The paper was washed with 1.5 ml 0.85% NaCl and mixed gently for 5 min. The liquid was added to the same Eppendorf tube containing the white blood cell pellet and centrifuged. The combined pellet was retained and then suspended in 0.5 ml STE (100 m*M* NaCl, 10 m*M* Tris, 1 m*M* EDTA, pH 8.0) to which was added 25 µl proteinase K (1 mg/ml) and 25 µl 10% sodium dodecyl sulfate; the suspension was incubated for 2 h at 37°C. This was followed by three phenol (saturated with 0.1 *M* Tris, 0.3 *M* NaCl, pH 8.0) extractions (500, 250, and 250 µl) and three ether (saturated with water) extractions (500, 250, and 250 µl). The last trace of ether was removed by blowing 100% nitrogen over the surface or by placing the tube in a 68°C water bath until the odor of ether was no longer present. Then 25 µl 4 *M* sodium acetate was added and mixed, and the DNA was precipitated with 1 ml 100% ethanol at −20°C overnight. After centrifugation, the precipitate was washed with 1 ml 70% ethanol, and the pellet was air-dried. TE 10 µl (10 m*M* Tris HCl and 1 m*M* EDTA, pH 8.0) was added to the pellet to resuspend the DNA (approximately 0.5 µg) for restriction en-

donuclease digestion. After digestion, electrophoresis of the DNA was carried out on a 6.5 × 9 cm 1% agarose gel in TBE (prepared from 5× concentrate containing 54 g Tris base, 27.5 g boric acid, and 20 ml 0.05 *M* pH 8.0 EDTA per 1 at 100 V for 50 min. The DNA was denatured, neutralized, transferred to a nitrocellulose filter, hybridized with a nick translated, phosphorus-32 labeled probe, and autoradiographed (Maniatis et al. 1982; Southern 1975; Huang and Zeng 1984). Hybridizations were carried out with a Y chromosome repetitive sequence (pY3.4) probe and an ε-globin probe.

Results

The method described above reproducibly gave yields of approximately 0.5 µg DNA from the dried equivalent of 50 µl whole blood and good-quality autoradiograms after electrophoresis, transfer, and hybridization (e.g., Fig. 1). Once we had shown that we could be consistently successful in obtaining sufficient quantities of DNA for analysis using this microextraction procedure, we investigated the quality of DNA obtained by this method, using larger amounts of starting material. Figure 2 shows an ethidium bromide-stained agarose gel after electrophoresis of undigested DNA from fresh whole blood, fresh dried blood, and 4.5-month-old dried blood and demonstrates that, although the yield of DNA may decrease somewhat with specimen age, there is no significant fragmentation of DNA after microextraction of blood dried and stored on filter paper. Figures 3a and 4a show ethidium bromide-stained gels of EcoRI- and HincII-digested DNA from the dried equivalent of 500 µl whole blood after 4.5 months storage and show that DNA undergoes complete digestion after microextraction. Figure 3b illustrates hybridization of this DNA with a repeat sequence probe, pY3.4. Since pY3.4 is Y chromosome-specific, the autoradiogram demonstrates the use of this technique for determining the presence of this particular chromosome in the individual whose dried blood is tested. Figure 4b shows hybridization of extracted DNA with a unique sequence probe, the ε-globin probe.

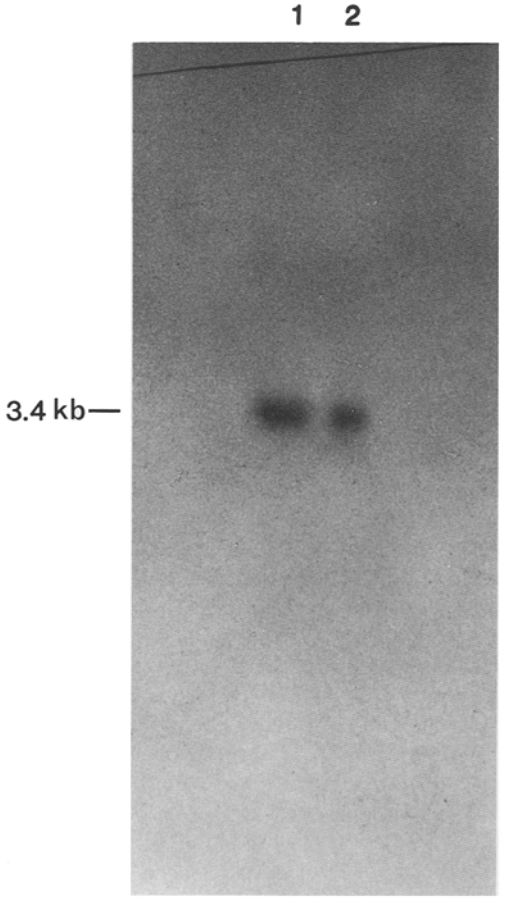

Fig. 1. Autoradiograms of DNA extracted from the dried equivalent of 50 µl whole blood after hybridization with the ^{32}P-labeled Y-chromosome repeat sequence, pY3.4. DNA was extracted from freshly prepared dried blood spots on filter paper blotters, digested with EcoRI, subjected to electrophoresis and transferred as described in the text. *Lanes 1* and *2* contain DNA from two male subjects

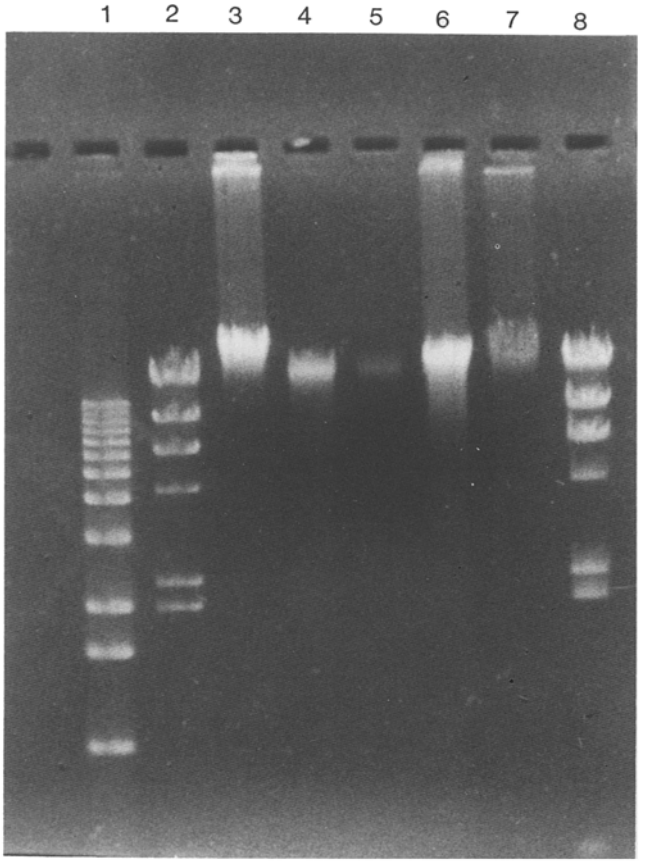

Fig. 2. Ethidium bromide-stained agarose gel after electrophoresis of undigested DNA. *Lane 1,* 1 kb DNA ladder; *lanes 2* and *8*, HindIII digest of phage DNA; *lanes 3* and *7*, DNA from 100 µl and 35 µl fresh whole blood, respectively; *lanes 4* and *5*, DNA from 200 µl and 50 µl fresh dried blood, respectively, used immediately after preparation; *lane 6,* DNA from dried equivalent of 500 µl whole blood used after 4.5 months storage at room temperature

Discussion

We have demonstrated that DNA can be obtained from dried blood spots on filter paper blotters in a form which can be extracted and isolated. The DNA is sufficient in quality and quantity for endonuclease digestion, electrophoresis, transfer, and hybridization. This microextraction procedure is based on

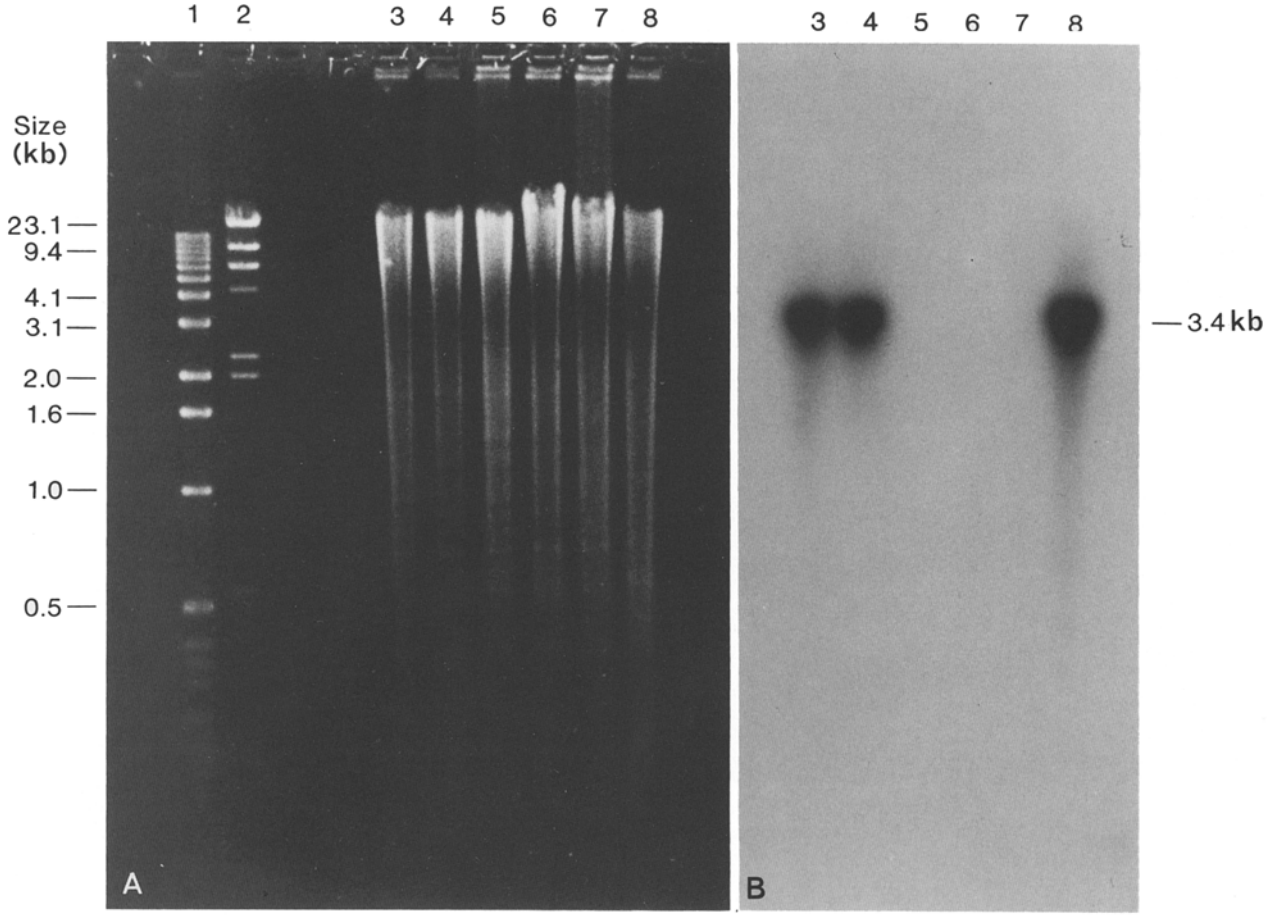

Fig. 3A, B. DNA extracted by a microprocedure from dried blood spots on filter paper blotters after digestion with EcoRI to show hybridization with a repeat sequence. **A** ethidium bromide-stained agarose gel; **B** autoradiogram of DNA shown in **A** after transfer and hybridization with ^{32}P-labeled pY3.4. *Lane 1,* 1 kb DNA ladder; *lane 2,* Hind-III digest of lambda phage DNA; *lanes 3, 4,* and *8,* DNA from three male subjects; *lanes 5, 6,* and *7,* DNA from three female subjects

rehydration of the white blood cells in the dried specimen and separation of these cells from the red cell hemolysate by centrifugation. In our hands this procedure is superior in DNA yield to a method involving incubation of the intact dried specimen with sodium dodecyl sulfate and proteinase K similar to that described by Gill et al. (1985). While the amount of DNA obtained from specimens at the lower limits of those investigated may allow only a single, direct analysis, the DNA could be amplified (Saiki et al. 1985) for more extensive investigation.

The ability to use dried blood specimens for DNA microextraction provides a new resource for population studies using molecular genetic techniques, since newborn screening programs store the used blotters for varying lengths of time. We have shown that DNA is stable in these specimens for over 4 months, and Gill et al. (1985) have shown stability of dried blood on cotton cloth for up to 4 years. In addition, the use of dried blood samples will facilitate specimen transport from individuals who are culturally or geographically isolated. Without the ability to use dried specimens, these individuals may not have access to recombinant DNA diagnostic technology because of limitations imposed by transit time and sample size using whole blood specimens and standard DNA extraction procedures.

The ability to extract DNA from very small dried blood specimens will have an impact on newborn screening programs. It will allow direct follow-up of positive newborn screens from the original blood blotter for disorders which are amenable to molecular genetic diagnosis, such as sickle cell anemia (Chang and Kan 1982; Orkin et al. 1982). This technique will permit positive identification of the source of blood on a screening blotter when concerns arise that specimens may have been switched or mislabeled. This will have important medicolegal application in cases involving a child who has a normal screen in the newborn period, but is subsequently diagnosed with a disease such as PKU. The original specimen can be retrieved, retested for the disease of interest, and identified by comparing DNA 'fingerprints' between the original specimen and a freshly obtained specimen from the child (Gill et al. 1985; Jeffreys et al. 1985a, b). Eventually, modification and automation may permit this procedure to be applied to the primary screening of specific human mutations using dot blots and single or mixed synthetic oligonucleotide probes (Conner et al. 1983; Kidd et al. 1983).

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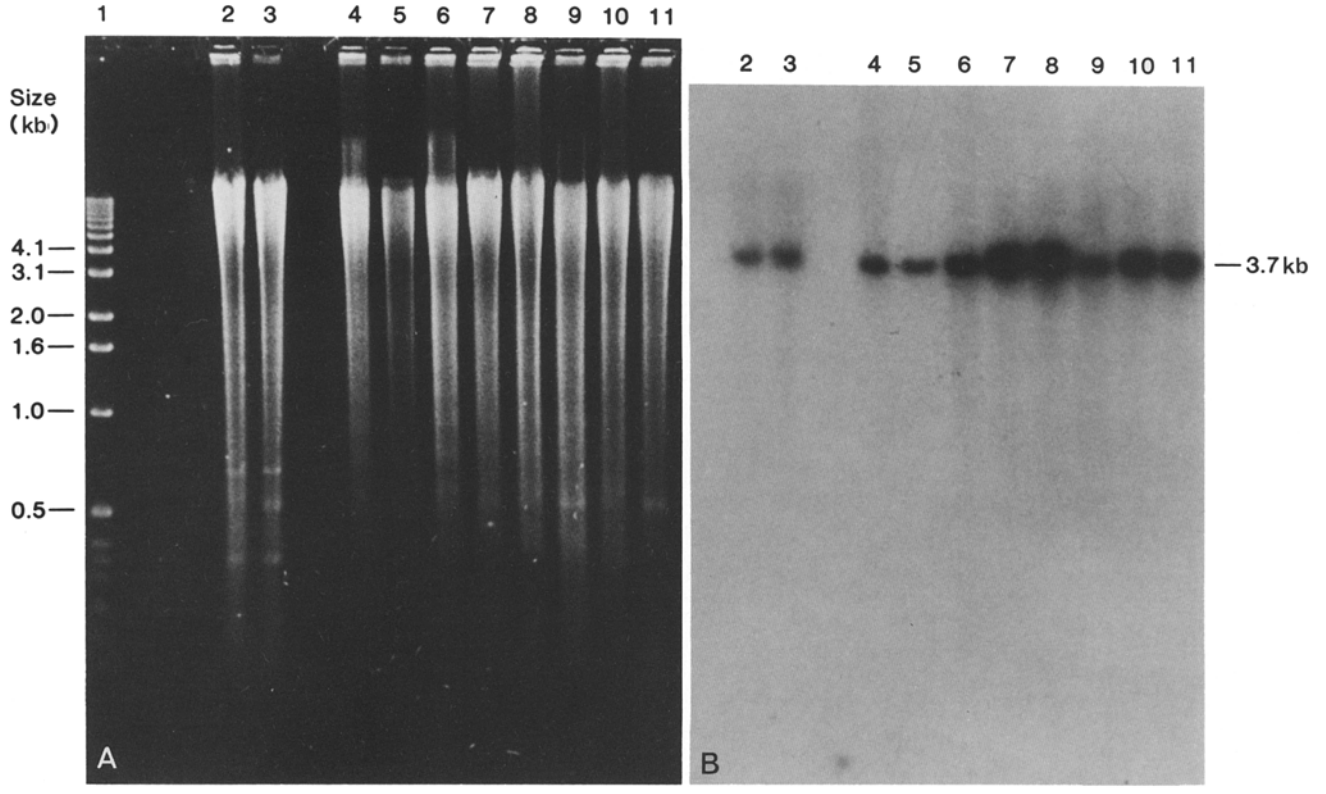

Fig. 4A, B. DNA extracted by a microprocedure from dried blood spots on filter paper blotters after digestion with EcoRI (*lanes 2* and *3*) and HincII (*lanes 4–11*) to show hybridization with a unique sequence probe. *Lane 1,* 1 kb DNA ladder. *Lanes 2–11* contain DNA from Caucasian subjects. **A** ethidium bromide-stained agarose gel; **B** autoradiogram of DNA shown in **A** after transfer and hybridization with ^{32}P-labeled ε-globin cDNA

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References

Chang JC, Kan YW (1982) A sensitive new prenatal test for sickle cell anemia. N Engl J Med 307:30–32

Conner BJ, Reyes AA, Morin C, Itakura K, Teplitz RL, Wallace RB (1983) Detection of sickle cell β^s-globin allele by hybridization with synthetic oligonucleotides. Proc Natl Acad Sci USA 80:278–282

Gill P, Jeffreys AJ, Werrett DJ (1985) Forensic application of DNA "fingerprints". Nature 318:577–579

Guthrie R (1980) Organization of a regional newborn screening laboratory. In: Bickel H, Guthrie R, Hammersen G (eds) Neonatal screening for inborn errors of metabolism. Springer, Berlin Heidelberg New York, pp 259–270

Huang S-Z, Zeng Y-T (1984) Prenatal diagnosis of α-thalassemia. Shanghai Med J 7:504–508

Jeffreys AJ, Wilson V, Thein SL (1985a) Individual-specific 'fingerprints' of human DNA. Nature 316:76–79

Jeffreys AJ, Brookfield JFY, Semeonoff R (1985b) Positive identification of an immigration test-case using human DNA fingerprints. Nature 317:818–819

Kan YW, Dozy AM, Trecartin R, Todd D (1977) Identification of a nondeletion defect in α-thalassemia. N Engl J Med 297:1081–1084

Kidd VJ, Wallace RB, Itakura K, Woo SLC (1983) α_1-Antitrypsin deficiency detection by direct analysis of the mutation in the gene. Nature 304:230–234

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

McCabe ERB, McCabe L (1983) Screening for PKU in sick or premature neonates. J Pediatr 103:502–503

Orkin SH, Little DFR, Kazazian HH, Boehm CD (1982) Improved identification of the sickle mutation by DNA analysis – application to prenatal diagnosis. N Engl J Med 307:32–36

Poncz M, Solowiejczyk D, Harpel B, Mory Y, Schwartz E, Surry S (1982) Construction of human gene libraries from small amounts of peripheral blood: analysis of β-like globin genes. Hemoglobin 6:27–36

Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N (1985) Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230:1350–1354

Southern E (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

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