STABILITY OF GENOMIC DNA IN DRIED BLOOD SPOTS STORED ON FILTER PAPER

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Abstract. The stability of DNA in dried blood samples obtained from the neonatal screening program in Thailand was retrospectively studied in order to determine the conditions necessary for the long term storage of samples for DNA banking. Specimens from 1991 to 2001, which had been kept in the ambient conditions at the Department of Medical Science, Ministry of Public Health, Thailand, were randomly sampled and used for the study. Genomic DNA was extracted from the samples and DNA fragments of the PAX8 and β-globin genes were amplified by PCR to determine DNA stability. The study showed that 255-bp and 674-bp fragments of the PAX8 gene could be amplified from all the samples. The DNA fragment of 1,039 bp of the β-globin gene could be detected in all of the samples for the years 1993 to 2001, but only in seven and five out of the ten studied samples for each of the years 1991 and 1992, respectively. Our study shows that genomic DNA is stable in dried blood stored on filter paper at ambient tropical conditions for at least 11 years. However, DNA quality for amplification of larger DNA fragments decreased when the specimens were stored for longer than 10 years.

INTRODUCTION

Dried blood stored on filter paper has been used in newborn screening programs in many countries to identify inborn diseases in neonates since the early 1960s (Guthrie and Susi, 1963). Residual specimens, after screening analysis, have been retained mainly for quality control and public health purposes. The length of retention of the residual specimens, however, depends on the particular purposes and stability of analytes to be studied. For instance, the stability of genomic DNA stored in dried blood specimens is a major concern for genetic studies.

Some states in the USA save all residual dried blood specimens for extended periods and justify this on the basis of public health needs and concerns (Therrell *et al*, 1996). The Thailand National Neonatal Screening Program has also retained residual dried blood specimens after newborn screening analyses for public health purposes, such as genetic and epidemiologic studies, or for establishing DNA detection method(s) for the screening of inborn diseases commonly occurring in Thai populations. Dried blood samples have also been used for DNA detection analysis in newborn screening to identify genetic mutation diseases, such as cystic fibrosis (Seltzer *et al*, 1991; Audrezet *et al*, 1993; Farrell *et al*, 1994) and sickle cell disease (Jinks *et al*, 1989; Skogerboe *et al*, 1991).

The dried blood specimens from the National Neonatal Screening Program in Thailand have been retained for a long period based on the stalability of DNA is the most stable analyte in dried blood (Therrell *et al,* 1996) and an important tool for population-based genetic studies. To our knowledge, there have been no published data regarding the effects of long-term storage of dried blood specimens on the stability of genomic DNA. Casole and colleages (Cassol *et al*, 1992) showed that the maximum length of stability of HIV proviral DNA in dried blood spots kept at ambient temperature and dessicated at -20ºC was about 3.5 months. β-globin DNA has been detected in dried blood stored at ambient temperature for about 1 year (Rubin *et al*, 1989). However, most stability stud-

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ies on dried blood specimens have shown inconsistent result (Therrell *et al,* 1996).

We carried out this study to determine the stability of genomic DNA in dried blood specimens collected and retained for the National Neonatal Screening Program in Thailand from the years 1991 to 2001. The results should provide information regarding duration and conditions for storage of the specimens, which can be used for decision making regarding long term storage of dried blood specimens for a DNA bank in Thailand.

MATERIALS AND METHODS

Dried blood spot samples

Dried blood spot specimens retained for the National Neonatal Screening Program in Thailand during the years 1991 to 2001 were used for the study. Blood specimens were collected on filter paper (Schleicher and Schuell, Keene NH, USA) following National Committee on Clinical Laboratory Standards (NCCLS) specifications (Hannon *et al*, 1997). They were stored in sealed plastic bags at the Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand, at an average tmperature of 28±2ºC and 73±5% relative humidity. Ten blood specimens were randomly sampled from the stock of residual dried blood specimens for each year collection. All the samples were from the same province, participating in the program since 1991. Sample variation, occurring from variations in blood collection techniques was eliminated. The samples were kept at 4ºC during the study.

Extraction and purification of genomic DNA from dried blood on filter paper

Genomic DNA was extracted and purified from dried blood samples using the method previously described (Chaisomchit *et al*, 2003).

Extraction of genomic DNA from whole blood

Blood samples obtained from a volunteer with written consent were freshly collected in an EDTA-coated tube (Vacuette, Greiner Labortecnik, Austria). Lymphocytes were prepared from peripheral blood samples as previously described (Chaisomchit *et al,* 2003).

PCR analyses of the 255-bp and 674-bp fragment of the PAX8 gene

PCR amplification of the 255-bp fragment exon 5 of the PAX8 gene (11) was performed using the following primers 5′ TCTCCCTCTC CCCCACTG 3′ and 5′ GCAGAGCCCCTAC AAAGTCC 3′ for amplification of the 255-bp fragment and 5′ TCTCCCTCTCCCCCACTG 3′ and 5′ CACAGGCTCATTTGGAGAAT 3′ for amplification of the 674-bp fragment. PCR reactions were carried out in the Perkin Elmer 9600 Thermocycler machine using conditions described previously (Chaisomchit *et al*, 2003). Genomic DNA extracted from fresh blood was used as a positive control. The amplified products were analyzed by agarose gel-electrophoresis.

PCR analysis of the 1,039-bp fragment of the β-globin gene

To amplify the β-globin gene, the primers 5′ TGCCTATTGGTCTATTTTCC 3′ and 5′ AATCC AGCCTTATCCCAACC 3′ were used. The cycling profile consisted of one cycle of template denaturation at 94ºC for 2 minutes, 40 cycles of denaturation at 94ºC for 30 seconds, and annealing and extension at 60ºC for 1 minute 30 seconds, followed by one cycle at 70ºC for 5 minutes. The PCR product was analyzed by agarose gel-electrophoresis.

RESULTS

To determine the stability of the genomic DNA from the dried blood spots, residual dried blood samples kept from 1991 to 2001 were randomly sampled and used for the study. PCR analyses were performed on extracted genomic DNA to identify the 255-bp and 674-bp fragment of exon 5 of the PAX8 gene and the 1,039-bp fragment of the β-globin gene. Genomic DNA extracted from the fresh blood of a volunteer was used as a positive control. The results showed that the short DNA fragment, 255 bp, could be amplified from the selected samples. This indicates that the genomic DNA (Fig 1A) was still present in the specimens stored since 1991(data not shown). To further verify the stability and quality of the genomic DNA stored on the filter paper, larger DNA fragments were

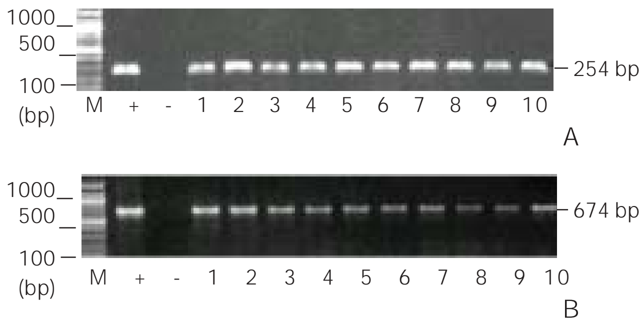

Fig 1–PCR amplification of the 255-bp(A) and 674-bp fragment (B) of the PAX8 gene in residual dried blood samples obtained in 1991. M=100 bp DNA ladder; +=positive control; -=negative control.

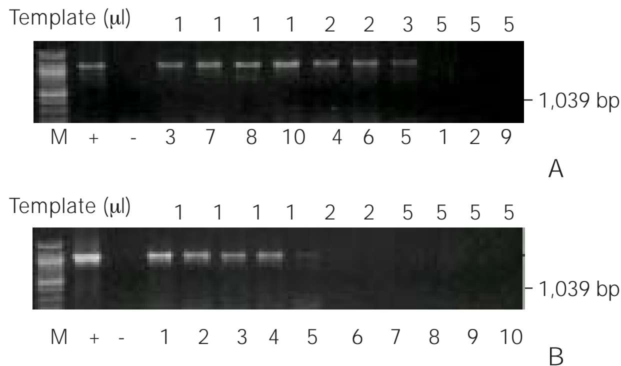

Fig 2–PCR amplification of the 1,039-bp fragment of the β-globin gene from residual dried blood samples obtained in 1991 (A), and retained in 1992 (B). M=100 bp DNA ladder; +=a positive control; -=a negative control.

generated by PCR amplification, 674-bp and 1,039-bp fragments of the PAX8 and β-globin sequences, respectively. Results showed that the 674-bp DNA fragment of the PAX8 gene could be detected in all the samples from 1991 to 2001(Fig 1B). The 1,039-bp DNA fragment of the β-globin gene was detected in all the studied samples obtained from 1993 to 2001 (data not shown). The large fragment of the β-globin gene was detected in 7 out of 10 samples from the year 1991 and 5 out of 10 samples from the year 1992 (Figs 2, A and B). This large fragment could not be amplified even after increasing the template amount five times.

DISCUSSION

For over a decade, dried blood spots collected on filter paper have been used in the National Neonatal Screening Program in Thailand to identify congenital diseases highly prevalent in the Thai population. As a result, residual dried blood specimens from the program have been kept in large repositories for population-based studies, tracibility and/or quality control purposes. Such population-based studies include genetic epidemiologic studies to assess genetic risk factors. The duration of sample retention, is dependent on the stability of the analyte of interest. To our knowledge, there is no data available regarding the stability of genomic DNA stored on filter paper, when stored in tropical conditions, such as Thailand. Ideally, dried blood specimens retained for DNA testing should be stored at 4°C in sealed plastic bags with low gas permeability, containing a desiccant for humidity control (Therrell *et al*, 1996). Such storage conditions are too expensive for developing countries. These specimens had been kept in ambient conditions at the Department of Medical Sciences since 1991. The criteria for how long the samples would be retained was dependent on the stability of the genomic DNA.

Our study clearly shows that genomic DNA in dried blood stored on filter paper is stable for at least 10 years under tropical conditions, with an average temperature of 28°C and 73% relative humidity, the average weather conditions at the Department of Medical Science, Ministry of Public Health, Nonthaburi, Thailand. The ability to detect short DNA fragment of the PAX8 gene from extracts of dried blood specimens kept for more than 10 years indicates that dried blood specimens stored for long periods can be use for epidemiologic and genetic studies, such as the prevalence of disease-associated polymorphism. We used the PAX8 gene (Strachan and Read, 1994) for this study because this gene has been reported to be involved in congenital hypothyroidism (Macchia, 2000), one of the highly prevalent congenital diseases found in the Thai population. This gene may be included in population-based genetic

studies in the future to determine factors involved in the development of this congenital disease in Thais.

The ambient conditions in Thailand may not be appropriate for the storage of dried blood on filter paper for more than ten years for the genetic study of long DNA sequences. Long term storage of dried blood specimens to be use in studies of large DNA fragments, may require the conditions suggested by Therrell and colleagues (1996) mentioned above.

To our knowledge this is the first evidence showing that genomic DNA in dried blood on filter paper is stable for more than 10 years. Our study is not only useful for the development of a DNA bank in Thailand but for other countries located in tropical or subtropical weather conditions as well.

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