A practical, low-cost, short-term storage method for genomic DNA

Demir Özdemir*,¹

¹Department of Agricultural Biotechnology, Faculty of Agriculture, Akdeniz University, 07070, Antalya, Turkey; *Author for correspondence: dozdemir@akdeniz.edu.tr

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ABSTRACT

The aim of this study was to assess the DNA preservation capability of cellulose paper towel and blotting paper as low-cost alternatives to commercial DNA preservation products. Chicken blood was applied as DNA source to each paper towel, blotting paper, FTA® cards and DNA/RNA Shield™. All samples were stored at room temperature for 130 days. DNA extraction from dried blood spots was performed after various time periods using Tris–EDTA and NaOH protocols. PCR activity and the mean amount of DNA isolated from paper towels were reliable. The results of this study demonstrated that cellulose-based blotting paper and especially paper towel had considerable DNA binding and preservation capacity for at least 130 days at room temperature without DNA degradation.

METHOD SUMMARY

Chicken blood was applied to each paper towel, blotting paper, FTA® cards and DNA/RNA Shield™. DNA extraction from dried blood spots was performed after various time periods using Tris–EDTA and NaOH protocols. The quality of the extracted DNA was determined by PCR amplification of chicken CHD1 gene fragment. The results of this study demonstrated that cellulose-based blotting paper and especially paper towel had considerable DNA binding and preservation capacity for at least 130 days at RT without degradation.

KEYWORDS:

cellulose • DNA preservation • dried blood spots • FTA • paper towel

The collection of animal blood samples for genomic DNA extraction is one of the essential parts of molecular studies on animals. The laboratory delivery and storage of blood samples obtained from animals without the degradation of DNA is a major obstacle for researchers during field studies, where samples often need to be preserved at the point of collection. DNA extraction cannot always be performed during sample collection, and samples must be stored intact for a period of time until analysis. Researchers are therefore interested in different preservation and storage methods of DNA in a wide range of fields and disciplines, such as biomedical research, diagnostics and forensics. Freezing is the most widely used preservation method for samples collected for DNA analyses for long-term storage [1]. However, it is also challenging to freeze samples instantly in field experiments and there is always a threat of thawing over long distances during shipment or long-term field studies [2]. Thus drying the blood samples is considered a cost-efficient method preferred in field studies, as its ease during transport allows it to protect DNA from degradation for a long time at room temperature.

The ability of cellulose to bind DNA has made it the main material used in dry preservation methods. In the case of adding blood or other hydrophilic solvents to the paper, produced by compressing pure cellulose fibers to varying degrees, cellulose causes the paper to swell due to the degradation of the hydrogen bonds between the polymers. Organic molecules in the blood can also interact with the hydrogen bonds of cellulose or fill in the voids and macromolecules, such as proteins and DNA, by entering the gaps between the fibrous layers. Therefore drying the paper allows it to revert to its corresponding state because molecules become trapped in the cellulose matrix. Molecules dried in the cellulosic paper are more stable because they are preserved from the environmental conditions and stabilized by the cellulose spine [3]. In a similar way, cellulose has also proven its ability to preserve DNA in studies in plants [4]. This feature makes cellulose an valuable material for DNA preservation.

Although the collection of blood samples on cellulose paper was first recognized by Ivar Christian Bang over a century ago [5,6], the widespread use of dried blood spots (DBSs) to test for phenylketonuria in newborns was only reported in the early 1960s [7]. The DBS process entails the of collection of blood spots directly onto a cellulose-based paper and then leaving them to dry at room temperature before shipping to laboratories for analysis [8]. In addition to their widespread use in human health, DBSs are also becoming increasingly common in animal research, such as veterinary diagnostics [9], animal molecular genetics [10,11], clinical pharmacokinetic studies [12,13] and toxicokinetics [14,15].

With recent developments in cellulosic paper modification and processing innovations, various commercial products based on cellulose are used for capturing and storing blood samples, such as FTA® (Fast Technology Analysis) cards that are chemically modified to lyse cells, denature proteins, prevent bacterial growth and protect nucleic acids from nucleases and oxidative and UV damage, accord-

ing to the manufacturer's instructions [16–18]. Although these commercial products are sensitive and have advantages for molecular assays, they still have a limitation for low-budget research due to the high costs per sample. Given the DNA binding ability of cellulose, this study aimed to test the DNA preservation capability of cellulose-based paper towel and blotting paper as low-cost alternatives to DNA preservation products.

Materials & methods

Source of DNA

In the experiment, fresh chicken blood, as a source of DNA, was applied to the 100% pure cellulose paper towel, cellulose blotting paper (60 g/m²), FTA® classic cards (Whatman, MA, USA) and DNA/RNA Shield™ (Zymo Research, CA, USA) to test the storage time and preservation ability of DNA. The blood samples used in this study were provided from the experimental material of project BAP-2019-4824 and all experimental protocols were approved by Akdeniz University Council for Ethical Licensing of Animal Experiments, ethical permit no 828/2019.01.01.

Sample preparation

The paper towel and blotting papers used in the experiment were cut into 4-cm² pieces and were then autoclaved to prepare the samples. Equal amounts of blood (50 µl each) were added on the paper towel, blotting paper and FTA® classic cards. All the samples were then left to dry for 6 h at room temperature to form DBSs, then the dried samples were stored in separate clean tubes at room temperature for 130 days until DNA isolation. The DNA/RNA Shield was prepared according to the manufacturer's instructions. Chicken blood (50 µl) was mixed with 100 µl DNA/RNA Shield for the sample preservation. The DNA/RNA Shield samples were stored at room temperature until DNA extraction. All samples were stored in an environmentally controlled room under conditions with 21 ± 1°C and 44 ± 5% relative humidity. Temperature and humidity were recorded using onset Hobo instrument (MA, USA).

DNA extraction methods

All samples were stored at room temperature for a short-term period of 130 days. DNA extraction was conducted at various time periods (days 1, 7, 30, 45, 90 and 130) to assess the efficacy of DNA preservation methods. The DNA extraction from DNA/RNA Shield was performed using the manufacturer's commercial DNA isolation kit (Zymo Research). According to the instructions, 200 µl BioFluid and Cell Buffer and 20 µl proteinase K were added to the DNA/RNA Shield sample. The mixture was vortexed for 15 s and then incubated at 55°C for 10 min. After incubation, 1 volume of genomic binding buffer was added to the digested sample and the mix was vortexed for 15 s. The mixture was transferred to a Zymo-Spin column in a collection tube and centrifuged at 12,000×g for 1 min. The collection tube was discarded after centrifugation and 400 µl DNA Pre-Wash buffer was added to the spin column in a new collection tube and centrifuged at 12,000×g for 1 min. Then the collection tube was emptied and 700 µl g-DNA wash buffer was added to the spin column and the tube was centrifuged at 12,000×g for 1 min. This step was repeated by adding 200 µl g-DNA wash buffer to the collection tube. After centrifugation, the spin column was transferred to a clean microcentrifuge tube and 50 µl DNA Elution buffer was added directly on the matrix. The microcentrifuge tube was incubated for 5 min at room temperature, then centrifuged at maximum speed for 1 min to elute the DNA. The eluted DNA was quantified by the BioDrop spectrophotometer, measuring absorbance at 260/280 nm (BioDrop, Cambridge, UK).

The DNA extraction from DBSs (paper towel, blotting paper and FTA classic cards) was performed by following Tris–EDTA (TE) and NaOH protocols.

TE protocol

From each cellulosic material (paper towel, blotting paper and FTA classic card), ten 1.5-mm diameter disks were punched and then the DBSs transferred into 1.5-ml microcentrifuge tubes. 500 µl dH₂O was applied to the disks and the tubes were shaken for 5 min at 37°C and 50×g. The wash procedure was repeated three more times, discarding the dH₂O for every interval. After the washing stage, 500 µl TE buffer (10 mM Tris, 10 mM EDTA, pH 8.0) and 10 µl proteinase K were added onto the discs and the samples were then vortexed for 15 s. After vortexing, the samples were incubated at 37°C overnight in the shaker (50×g). No red blood cell lysis buffer was needed in this study because chicken blood has nucleated erythrocytes. However, for non-nucleated erythrocyte blood samples, it is recommended that a red blood cell lysis buffer (0.32 M sucrose, 10 M EDTA, 5 mM MgCl₂) is used at this stage. After incubation, all samples were vortexed for 15 s. Then, to increase extraction efficiency, cell lysates were incubated at 95°C for 15 min and then cooled at room temperature for 10 min. A short vortex was performed after incubation. Then the samples were centrifuged at 13,000×g for 1 min and the DNA-containing supernatants were stored at -80°C until analysis. The DNA concentrations were determined by the BioDrop spectrophotometer measuring the absorbance at 260/280 nm.

NaOH protocol

The NaOH method is another method for isolating DNA from DBSs. We used the NaOH method to extract DNA from cellulosic materials (paper towel, blotting paper and classic FTA card). Ten 1.5-mm diameter disks were punched from each cellulosic material and transferred into 1.5-ml microcentrifuge tubes, then 200 µl NaOH (20mM) was added to each sample and agitated for 30 min at room

Figure 1. DNA amounts of samples isolated from different preservation methods using the Tris–EDTA protocol. Error bars denote one standard deviation around the mean and ^{a,b,c} letters on the bars of the same storage day indicate significant differences ($p < 0.05$). Each value represents the mean of all replicates ($n = 30$).

temperature on a horizontal shaker ($50 \times g$) [19]. The NaOH was then discarded and discs were washed by TE⁻¹ buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0) twice for 2 min. Discs were then dried at room temperature for 6 h.

Polymerase chain reaction

The quality of the extracted DNA was determined by PCR amplification of chicken *CHD1* gene fragment. The *CHD1* gene fragment was amplified using primers P2 forward (TCTGCATCGCTAAATCCTTT) and P8 reverse (CTCCCAAGGATGAGRAAYTG) (Sentebiolab, Ankara, Turkey). The amplification was performed in a total 20 μ l reaction volume containing 2 μ l extracted DNA (DNA samples extracted with TE protocol) or DNA on cellulosic discs (DNA samples extracted with NaOH method), 0.5 μ l of each primer, 4 μ l reaction buffer (Bioline, MA, USA), 0.2 μ l Taq polymerase (Bioline, MA, USA) and 12.8 μ l dH₂O. The amplification was executed in a thermal cycler (Thermo Fisher Scientific, Vantaa, Finland) and cycling conditions were as follows: an initial denaturation of 5 min at 95°C, followed by 35 cycles of 95°C for 30 s, 60°C for 1 min 30 s and 72°C for 45 s, concluding with 30 min at 60°C and ∞ min at 15°C. Amplimers (2 μ l) were analyzed by 1% agarose (Bioreagent) gel electrophoresis using 1 $\times$ TBE buffer (Grisp) containing 2 μ l ethidium bromide and then gels were visualized by transillumination (Ingenius Syngene, Cambridge, UK).

Band intensity quantification

The signal intensity of the PCR product bands in the gel was quantified by ImageJ software [20]. To optimize the image analysis, all gel images were converted to 8-bit and the background image was subtracted from the original 8-bit image in order to generate an image with detectable bands and a uniformly subtracted background. After calibration, the densitometric area of each PCR amplicon was calculated using the gel analyzer module of ImageJ. Logarithmic transformation ($\log_{10}$) was applied to densitometric values to normalize the data.

Statistical analysis

Data were analyzed statistically by IBM SPSS Statistics Base v23 (IBM Corp., NY, USA). The general linear model was used to test the DNA preservation ability of different DNA storage methods. Bonferroni and Duncan's *post hoc* tests were performed to compare the DNA amounts isolated from different DNA storage methods at different storage durations.

Results & discussion

DNA was fixed on cellulose discs in the NaOH method, and these discs were used as a direct DNA source in the PCR process. Thus samples of DNA extracted using the NaOH protocol could not be spectrophotometrically analyzed. The amount of DNA isolated from DBSs (paper towel, blotting paper and FTA classic card) by the TE method and the amount of DNA isolated from DNA/RNA Shield are shown in Figure 1, by type of DNA storage method and storage duration. Among the DBS samples, the minimum DNA amount was detected in

Figure 2. PCR amplification efficiency of DNA samples extracted using Tris-EDTA and NaOH methods from different DNA storage methods on different days.

*Blood samples stored in the DNA/RNA Shield were extracted using the specific DNA kit (Zymo Research).

SD: Storage days from 1st to 130th day.

FTA classic card samples at day 1 ($1.42 \text{ mg}/\mu\text{l}$) while the greatest amounts of DNA were detected in paper towel samples ($1.84 \text{ mg}/\mu\text{l}$) and blotting paper samples ($1.82 \text{ mg}/\mu\text{l}$) at day 1. The mean amounts of DNA extracted from FTA classic card, paper towel and blotting paper were $1.51 \pm 0.05 \text{ mg}/\mu\text{l}$, $1.56 \pm 0.04 \text{ mg}/\mu\text{l}$ and $1.54 \pm 0.04 \text{ mg}/\mu\text{l}$, respectively. The DNA/RNA Shield, performed with the manufacturer's DNA kit, had the highest mean value ($1.63 \text{ mg}/\mu\text{l}$) of DNA among the DNA preservation methods during the 130-day period. The difference among storage methods was significant ($p < 0.05$) in terms of the mean amount of DNA in the same storage period (Figure 1).

The *CHD1* gene has been amplified by PCR to determine the consistency of all extracted DNA samples. Amplification of the *CHD1* gene fragment from DNA samples extracted from DBSs with the TE method is shown in Figure 2. The 450-bp fragment of *CHD1* was successfully amplified from the fresh and the stored DNA samples extracted from DBSs (paper towel, blotting paper and FTA classic cards) with the TE method.

Although DNA quality was sufficient for all samples extracted from DBSs using the TE method, the samples extracted from FTA classic cards showed more intense bands than samples extracted from blotting paper and paper towel. As a commercial control, the bands were the most intense for DNA/RNA Shield samples which were isolated using the DNA kit without the TE or NaOH protocol. The PCR amplifications for DNA samples extracted with NaOH protocols were less dense than those obtained from DNA samples extracted with TE protocols (Figure 2). Compared with blotting paper and FTA cards, PCR activity of DNA samples isolated from paper towels with the NaOH protocol was the highest.

Digital analysis was performed with ImageJ to quantify the densitometric area of band intensities and to compare the PCR efficiency of DNA samples extracted from different storage methods through different extraction methods (Figures 3 & 4). Digital quantification of the PCR amplicons supported the qualitative gel image evaluation. According to the densitometric analysis of PCR amplicons from the TE method, FTA cards showed the highest mean optical density (3.92; range: 3.83–3.98), while the mean optical densities for paper

Figure 3. Densitometric analysis of PCR band signals of DNA samples extracted with the Tris-EDTA protocol in terms of DNA storage methods and storage duration.

*Blood samples stored in the DNA/RNA Shield were extracted using a specific DNA kit (Zymo Research) without the Tris-EDTA protocol.

towel and blotting paper were 3.68 and 3.66, respectively (Figure 3). The band densities of the samples extracted from the paper towel with the TE method were in the range 3.40–4.11 and showed the least variation among other DNA preservation methods (Figure 3). The densitometric quantification of PCR bands of DNA samples extracted by the NaOH method is shown in Figure 4. The paper towel samples showed the highest band intensities (mean: 3.60; range: 3.28–3.78). In contrast to the TE method, the lowest band densities in the NaOH method were seen in the FTA group, with an average of 3.08, while the average band density in the samples extracted from the blotting paper was 3.44. When the PCR band densities of DBS samples extracted with TE and NaOH methods were compared with those of the DNA/RNA Shield samples extracted with DNA kit, the DNA/RNA Shield samples were found to have a denser band, with a mean of 3.94.

Preservation and transportation of blood samples obtained from animals to the laboratory without the degradation of DNA is a major challenge for researchers during field studies. To find a solution to this problem, some products have been developed for the preservation of DNA from sample uptake to the analysis stage. Cellulose's ability to bind DNA molecules has made it a suitable material for products aimed at preserving DNA. Thus various commercial DNA preservation methods were produced by taking this property of cellulose into account and a large proportion of those products in the most common use are cellulose based. The cellulose-based FTA cards are one of the most effective and widely used DNA preservation and storage products that can preserve DNA in dry conditions at room temperature for long periods of time. With a similar approach using cellulose's DNA binding ability, cellulose-based paper towel and blotting paper were tested as low-cost alternatives to DNA preservation products.

Spectrophotometric DNA quantification results have shown that paper towel has a truly staggering level of DNA binding capacity. According to the results of the BioDrop analysis, the mean amount of DNA extracted from paper towel samples was the highest after

Figure 4. Densitometric analysis of PCR band signals of DNA samples extracted with the NaOH protocol in terms of DNA storage methods and storage duration.

*Blood samples stored in the DNA/RNA Shield were extracted using a specific DNA kit (Zymo Research) without the NaOH method.

the mean amount of DNA extracted from DNA/RNA Shield. DNA samples on paper towels showed better results for PCR performance with both TE and NaOH extraction methods than blotting paper samples, and paper towel samples even showed a more successful PCR performance in the NaOH method than FTA classic cards. This result may be related to the cellulose densities of the materials chosen as the preservation method. Moreover, the number of cellulose disks used in the DNA extraction could have an inverse effect on the amount of DNA amplified, which could result in greater sequestration of primers, dNTPs or other reagents in the amplification reaction [21]. Therefore paper towel samples, which are less dense in terms of cellulose content, had more successful amplification efficiency in the NaOH method, due to the fact that the cellulose density is higher in blotting paper and FTA classic cards.

In the current study, the FTA classic cards with successful results from the TE method did not show the same success with the NaOH extraction method. However, because the purification reagent specific to FTA classic cards was not used and DNA extraction was performed by TE and NaOH methods, it may not be the right approach to interpret the results for FTA classic cards. Another commercial DNA preservation product in the study, DNA/RNA Shield, showed highly successful results of the spectrophotometric analysis, gel electrophoresis images and digital quantification analysis across storage duration with the use of the product-specific extraction kit.

Although products such as FTA classic cards and DNA/RNA Shield allow DNA to be stored intact for long periods of time at room temperature, their costs limit the use of these products for low-budget research. Moreover, cooling or freezing samples immediately after sampling is a troublesome and stressful process for researchers in field studies. Although liquid nitrogen or dry ice may appear to be ideal methods for cooling or freezing samples until the laboratory stage, they are not very practical applications for long-term field studies in the preservation of molecules that are negatively affected by external conditions, such as DNA/RNA.

In conclusion, the results of this study demonstrated that cellulose-based blotting paper and especially paper towel had considerable DNA binding and preservation capacity for at least 130 days at room temperature without DNA degradation. The 130-day preservation period provided by the paper towel can be seen as a short period when compared with products such as FTA classic cards and DNA/RNA Shield that can store DNA for many years. However, the 130-day short-term preservation performance of the paper towel provides enough time to researchers for transporting the samples from the field to the laboratory without DNA degradation. According to the findings of this study, the paper towel has proven to be an adequate, low-cost and reliable short-term DNA preservation method that can be used for backing up samples or as an alternative to high-cost DNA preservation products.

Future perspective

The processing of animal blood samples for genomic DNA extraction is one of the important aspects of molecular studies on animals, and the preservation and transport of blood samples collected from animals to the laboratory without the degradation of DNA is a significant challenge for researchers during field experiments. This simple and cost-effective short-term DNA storage method is expected to save researchers from the cost of cold-chain applications and high-priced DNA preservation products in future field studies. Although the DNA preservation ability of paper towels seems to be successful in the short term, its long-term success in safeguarding DNA also needs to be tested. Therefore we would like to test the long-term preservation success of paper towels, as well as the potential degradation levels for short and long DNA fragments by performing real-time PCR in the future.

Executive summary

- This study showed that cellulose-based paper towel can preserve genomic DNA under room temperature conditions without degradation.
- This cost-effective, practical and reliable technique can be an inexpensive alternative to high-cost DNA preservation products.

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Ethical conduct of research

The blood samples used in this study were provided from the experiment material of project BAP-2019-4824, all experimental protocols were approved by Akdeniz University Council for Ethical Licensing of Animal Experiments, ethical permit no. 828/2019.01.01.

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