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Original Article

The Storage Stability of Total DNA in Dried Blood Samples under Different Temperatures and Sunlight Exposure

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Abstract

Introduction: The collection of dried blood may be linked to certain problems during storage and transportation. The stability of DNA on supports other than traditional cellulose is not well known. This study aimed to directly compare natural (cellulose) and non-natural (glass fiber) membrane supports for short-term and long-term storage of dried blood samples under various temperature and sunlight conditions.

Materials and Methods: Dried blood samples on two membrane supports (cellulose and glass fiber) were examined for both short-term and long-term storage stability across temperatures from –78 °C to +60 °C. The total DNA yield was measured, and the quality of extracted DNA was verified by PCR of mtDNA D-loop. The color change of dried blood was evaluated using RGB analysis.

Results: 53-68% of total DNA was lost in frozen whole blood or dried blood stored at -20 °C and -78 °C for one year. For dried samples stored at room temperature on a shelf exposed to sunlight, the greatest total DNA degradation occurred. RGB profile analysis of dried blood showed an increase in the green component over one year. A brownish or greenish color indicates significant DNA loss. After 1.5 years, PCR for the mtDNA D-loop was successful for all samples stored at +4 °C or lower.

Conclusions: The glass fiber membrane showed advantages over cellulose regarding DNA yield and storage stability. Sunlight and high temperatures are key factors to consider when collecting and transporting dried blood samples. The blood's color can serve as an indicator of possible DNA yield: the darker the blood, the lower the DNA yield.

Keywords: Dried Blood Spots, DNA, Membranes, Cellulose, Glass Fiber, Storage

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Introduction

Nucleic acids stability is a critical issue in epidemiological and biological investigations, biobanking, biomonitoring and many other fields.[1-5] The collection of genetic material from wild or domestic animals in remote areas often poses challenges related to storage and transportation. In this context, the collection of biological fluids and tissues in dried form on an absorbing support (porous membrane material) offers a convenient solution for sample transportation, storage, and subsequent analysis using a variety of methods.[6-8] The Dried Blood Spots (DBS) technology has numerous advantages over other blood storage methods, primarily due to the minimal equipment and time required for sample collection, as well as ease of processing, storage and shipping[8]. In addition, the small sample volume collected makes this method particularly attractive in cases when it is limited, such as in newborns or small animals. The use of filter paper allows the collection of blood without venipuncture, enables rapid drying of samples and facilitates their compact storage at different temperatures. This method is widely used in medical research and diagnostics, and has also found its place in veterinary and biology.[6-9]

Biofluids prepared in the form of dried blood spots (DBS) or dried matrix spots (DMS) are often used for genetic research and the detection of viral and other pathogens in human and animal samples.[10-16] Positive results have been reported in isolating animal DNA from dried blood spots,[2] milk,[10] and other samples[15] with successful genetic analyses performed after several years of storage on cellulose cards at ambient temperature. Moreover, DNA samples collected, for instance, during epidemiological studies, can remain stable on filter paper for many years or even decades when stored under dry conditions.[17] DNA has been successfully isolated from dried samples stored for 27 years at -20 °C, followed by whole-genome amplification and analysis of single-nucleotide polymorphisms (SNPs).[18] In some cases, membrane material is pre-treated with nuclease inhibitors and other preservatives. For example,

commercial FTA cards (Flinders Technology Association) contain reagents for cell lysis, protein denaturation, and nucleic acid protection.

The preservation and quality of samples can be affected during collection in the field and shipping to a laboratory due to changes in temperature, humidity, and other environmental parameters. According to published studies, in most cases, dried samples of genetic material can be transported to the laboratory under ambient conditions or even at elevated temperatures (37 °C) without significant degradation of DNA or RNA for at least 7 days.[6] For example, the shipment of dried spots of tongue and foot epithelium samples from cattle between Indian cities for 22-56 days at +21-45 °C and relative humidity 20-100% did not affect the detection of foot-and-mouth disease virus (RNA) genome or serotype across all samples.[19] However, some authors noted that humidity is likely the most critical parameter during the collection and storage of dried samples, including those intended for genetic studies.[20] In any case, dry storage is essential to avoid mold growth and possible membrane/sample contamination as well as to maintain the stability of the target analyte or genetic material in the dried sample. Cellulose being a natural and biodegradable material is susceptible to mold growth during storage and transportation due to humidity and temperature changes. In this regard, the use of bioassay support made of non-natural materials, such as polymer or glass fibers that are less susceptible to biodegradation, is promising.[6] DNA stability on alternative sampling materials has been the subject of few studies, and many questions in this area remain unanswered.

The aim of this study was to directly compare natural (cellulose) and non-natural (glass fiber) membrane supports for short-term and long-term storage of dried blood samples under various temperature and sunlight conditions. Special focus was placed on the RGB-based color assessment of the dried blood. The factors that limit the long-term storage stability of whole blood dried on a membrane support as a source of genetic material from domestic goats (*Capra hircus*) were considered in relation to the total DNA yield. DNA quality was assessed by PCR amplification of mtDNA D-loop after 1.5 years of storage.

Materials and Methods

Whole Blood Sampling

Whole blood from three domestic goats (*Capra hircus*) was collected from the jugular vein as described elsewhere.[21] Blood samples were collected into six EDTA tubes (40-45 ml whole blood per animal) in July 2023. Shortly after sampling, the whole blood was spotted onto a cellulose membrane (TE46, Hoefer Scientific Instruments, USA) (100 µl/spot) or applied onto narrow 0.5 × 6 cm strips of glass fiber membrane (8964, Ahlstrom, Finland) (100 µl/strip) (Figure 1). The samples were dried for 1-2 hours at ambient temperature and then put into zip-lock plastic bags with a desiccant. Additionally, whole blood was aliquoted (100 µl per 1.5 ml Eppendorf tube) and stored at +4 °C. On the same day, all samples were transported from the sampling site to the laboratory (150 km away) and were kept at +4 °C.

Figure 1. The Drying of Blood Spots (Cellulose) and Strip Samples of Whole Blood at the Collection Site.

Blood Samples Storage Experiments

On the next day, dried blood samples (on cellulose and glass fiber membranes) were sorted into tightly closed zip-lock plastic bags and placed in different storage conditions across temperatures ranging from –78 °C to +60 °C, as summarized in Table 1. On this day (day 0), DNA was extracted from two 100 µl aliquots of whole blood, two dried blood spots (cellulose), and two dried blood strips (glass fiber) for each of three goats. For DNA extraction, the D-Blood kit based on silica spin-columns was used (Biolabmix, Russia). At each control point (seven days, one month, two months, three months, six months, twelve months) of particular storage conditions, total DNA extraction was performed from two aliquots of 100 µl of frozen whole blood, two dried blood spots (cellulose), and two dried blood strips (glass fiber) for each of three goats.

Table 1. Storage Conditions of Liquid and Dried Samples of Whole Blood

Sample	Storage temperature	Storage time	Storage conditions
Liquid samples	-78 °C	Twelve months Control points: seven days, one month, two months, three months, six months, twelve months	100 µl/Eppendorf tube (1.5 ml capacity)
	-20 °C	-«-	-«-
Dried samples	-78 °C	-«-	100 µl/strip, 100 µl/spot (cellulose) in zip-lock bag with desiccant
	-20 °C	-«-	-«-
	+4 °C	-«-	-«-
	Ambient temperature (July 2023 – December 2023), varied	-«-	-«- A) in zip-lock bag in the dark B) in zip-lock bag, sunlight exposed C) non-packed, on a shelf, sunlight exposed
	+37 °C	Seven days	-«-
	+60 °C	24 hours	-«-

Dried blood spots and strip-dried blood samples were scanned after three and twelve months of storage under different conditions using the Epson Perfection V700 Photo scanner (Seiko Epson, Japan). The color intensity in RGB mode was evaluated with ImageJ software (USA). A color reflection target for input scanner calibration (EZ Color Monaco T8.7/2 color target, X-Rite Inc., USA) was used.

DNA Extraction Procedure

DNA from liquid or frozen blood aliquots (100 µl) was extracted according to the DNA extraction D-blood kit instructions (Biolabmix, Russia). Dried blood spots (manually cut into segments) or strip-dried samples (separated into 0.5 x 0.5 cm square pieces with the help of tweezers) were first eluted into 200 µl of deionized water (20 min incubation with periodic shaking) and then put in contact with lysis buffer and proteinase according to the kit instructions. The DNA elution volume was 100 µl (deionized water). DNA concentration was assessed using a NanoPhotometer NP60 (Implen GmbH, Germany); sample concentration was the average of three to four replicate measurements. Extracted DNA was stored at -20 °C.

Amplification of the mtDNA D-Loop Hypervariable Region

For the amplification of the mtDNA D-loop hypervariable region, primers CAP-F (5′-CGTGTATGCAAGTACATTA C-3′) and CAP-R (5′-CTGATTAGTCATTAGTCCATC-3′) were used.[22] The expected amplicon size is 598 bp, corresponding to positions 15653–16250 in the reference goat (*Capra hircus*) mitochondrial genome. PCR conditions were optimized on a MiniAmp Plus thermal cycler (Thermo Fisher Scientific, USA) using its Veriflex zones, which allow for simultaneous amplification at three different temperatures to determine the optimal primer annealing temperature. Based on this optimization, 61 °C was selected as the annealing temperature, which is consistent with previously reported protocols.[23] PCR amplification was performed using the 5X MasDDTaqMIX-2025 kit (Dialat Ltd, Russia) containing the thermostable “hot-start” SmarTaq polymerase. The amplification program was as follows: an initial denaturation at 94 °C for 10 min; followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 61 °C for 30 s, and extension at 72 °C for 30 s; and a final extension at 72 °C for 5 min.

Electrophoretic Separation Procedure

Electrophoretic separation of the extracted DNA and PCR products was carried out in a 1% agarose gel with ethidium bromide (in TBE buffer) at a voltage of 10-12 V/cm for 50-60 minutes using a horizontal electrophoresis chamber. For electrophoretic analysis, 5 µl of DNA extract or 5 µl of PCR product and 5 µl of a 1 kb DNA length marker ("DNA Ladder 1 kb", Evrogen, Moscow) mixed with dye (4X Gel Loading Dye, Blue, Evrogen, Moscow) were used.

Statistical Analysis

All calculations were performed by Microsoft Excel 2016. Visualization of the results of the correlation analysis was performed in scatter plots by integrated regression analysis tool. Power function was the bestfit trendline for describing

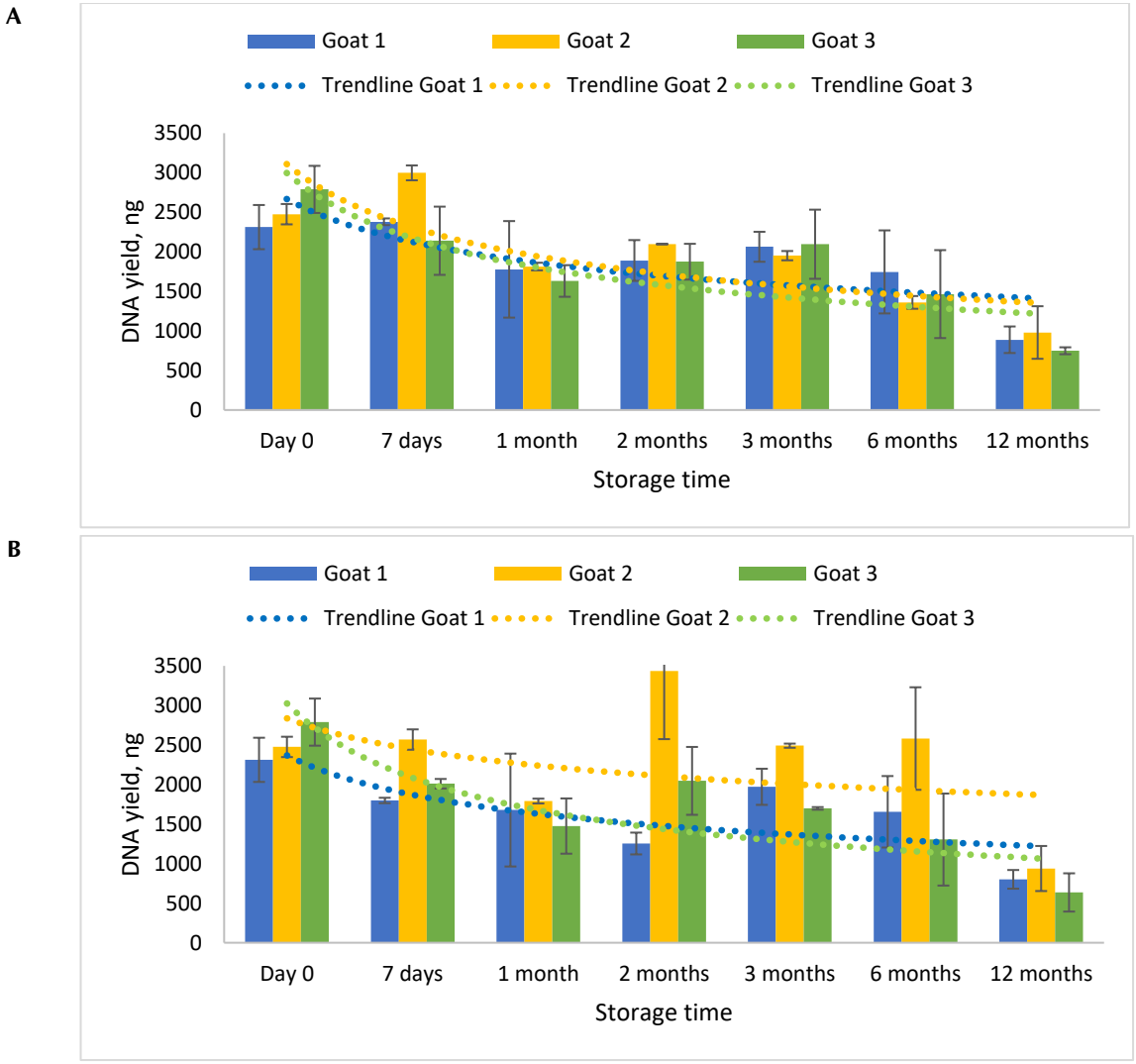

Figure 2. The Change in Total DNA Concentration Extracted from Frozen Whole Blood over Twelve Months of Storage at Different Temperatures: A) –78 °C, B) -20 °C. Blue – goat 1, yellow – goat 2, green – goat 3. Values represent mean ± SD, n = 2. Dashed lines – trendlines.

the decrease in DNA yield over time. Data was evaluated as mean ± SD.

Results and Discussion

When liquid blood samples were stored frozen (-20 °C or -78 °C), a gradual decrease in extracted DNA was observed in both cases (Figure 2). It is noteworthy that after one month of storage, the quantity of total DNA decreased by about 30%: the DNA yield was 68 ± 10% and 67 ± 21% of the initial amount on Day 0 for storage at -78 °C and -20 °C, respectively (n = 6). Subsequently, after twelve months of storage, the DNA yield was 37 ± 11% (n = 6, storage at -78 °C) and 32 ± 11% (n = 6, storage at -20 °C) of the initial amount. The whole blood stored at -20 °C for a period of twelve months had a darker color than the blood stored at -78 °C (Supplemental Information Figure S1). This demonstrated that oxidation processes were more active at -20 °C over time.

The influence of different storage conditions of goats' dried whole blood samples on total DNA yield was studied for two membranes that differed in chemical composition (cellulose and fiberglass) (Table 1). To simulate potential sampling and storage issues, as well as fluctuations in temperature and humidity, dried blood samples (on either fiberglass or cellulose) in a single bag with a desiccant were subjected to specific temperature regimes. Samples stored at room temperature were kept either in the dark or exposed to sunlight. When DNA extraction was required, the bag was opened and the required dried blood samples were removed. Subsequently, the bag was resealed and returned to its original storage conditions. When testing the storage stability of dried samples, the following patterns were observed. The short-term storage of dried samples at elevated temperatures, which simulated the potential mode of sample delivery to the laboratory (7 days at 37 °C and 24 hours at 60 °C) resulted in a decrease in total DNA for strip-dried samples stored on fiberglass (Figure 3). Even though the storage time was shorter, the DNA yield drop was more

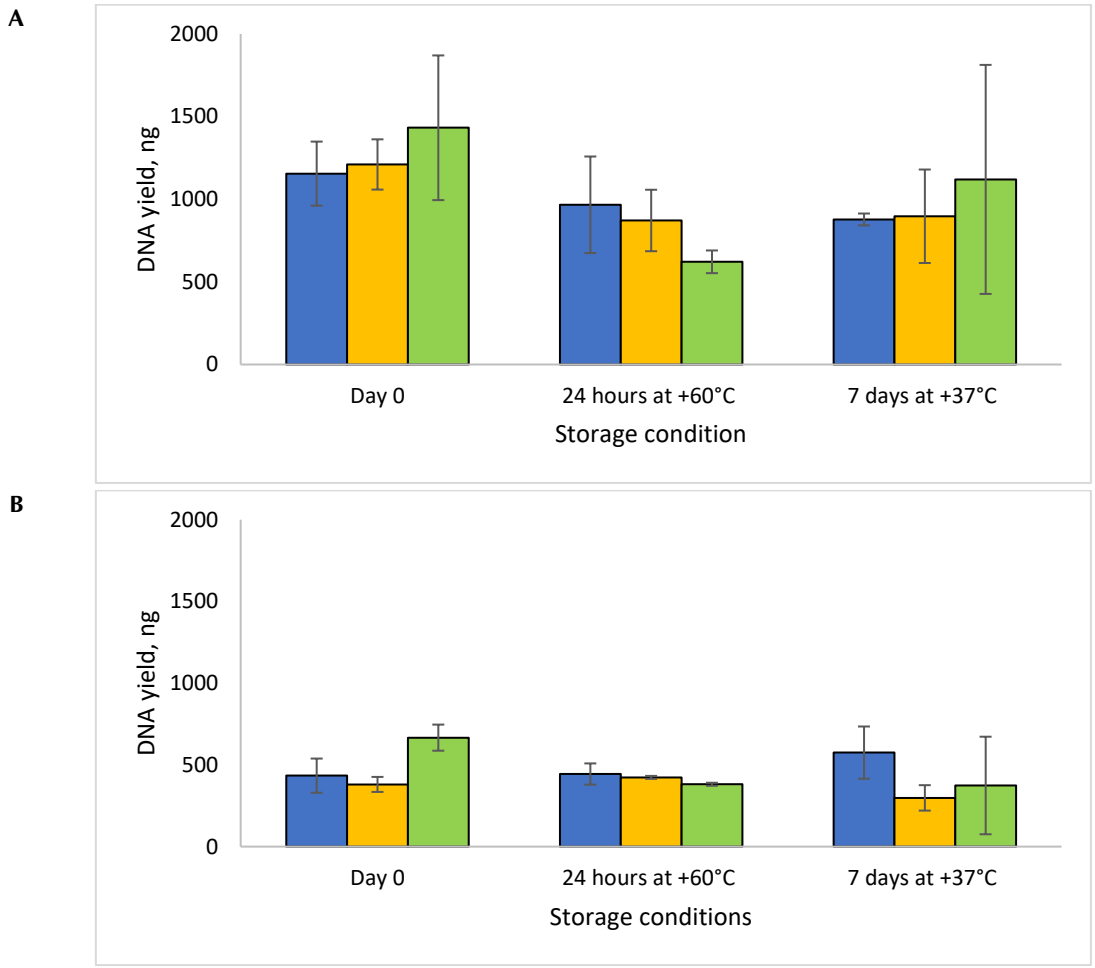

Figure 3. The Change in Total DNA Concentration in A) strip-dried whole blood samples (glass fiber membrane) and B) dried blood spots (cellulose membrane) stored at elevated temperatures. Blue – goat 1, yellow – goat 2, green – goat 3. Values represent mean ± SD, n = 2.

pronounced at higher storage temperatures, as expected: 66 ± 19% at 60 °C compared to 81 ± 38% at 37 °C (n = 6). Temperature is undoubtedly one of the primary factors affecting DNA stability because high temperatures cause DNA chain segments to move, which destabilises the molecule.[1,4] In some cases, high temperature (37 °C) and humidity can have a significant effect on the recovery of DNA from dried blood/plasma spots, as shown for HIV-1 investigations.[24,25] This fact underscores the need to transport specimens from the collection site to the laboratory as soon as possible. In this investigation, the DNA yield from the cellulose support was half that from an identical amount of dried blood on a glass fiber (Figure 3; Supplemental Information Figure S2 & S4), as was shown previously.[21] Consequently, it was difficult to ascertain the variation in DNA yield from cellulose for different storage modes over time, as the detected concentrations were close to the limit of DNA determination by a nanospectrophotometer (1 ng/µl) (Supplemental Information Figure S2 & S3). Our long-term storage studies have shown that the most critical factor when storing dried samples are temperature/humidity fluctuations when handling samples stored at low temperatures, as well as exposure to sunlight and air. Similar to frozen blood samples, for strip-dried blood samples stored at -78 °C, -20 °C or +4 °C for twelve months a gradual decrease in DNA yield was observed (Figure 4 A, B, & C; Supplemental Information Figure S4 A, B, & C). All these storage conditions resulted in a 53-68% reduction in the extracted DNA. It is noteworthy that dried blood stored under freezing conditions for twelve months did not show a significant advantage over storage at +4 °C. DNA yield was 37 ± 13% (n = 6) at –78 °C, 37 ± 14% (n = 6) at -20 °C, and 32 ± 14% (n = 6) at +4 °C. Therefore, a strip-dried sample could be stored in a fridge as effectively as in a freezer, which would save space in high energy-consuming equipment. Room temperature storage of dried blood led to the fastest reduction in DNA yield. For example, strip-dried samples stored on a shelf freely exposed to sunlight and air had their DNA yield drop to 62 ± 32% (n = 6) after seven days and to 21 ± 5% (n = 6) after one month (Figure 4F).

After one month of storage at room temperature, a decrease in DNA yield to 44 ± 16% (n = 6) and 39 ± 10% (n = 6)

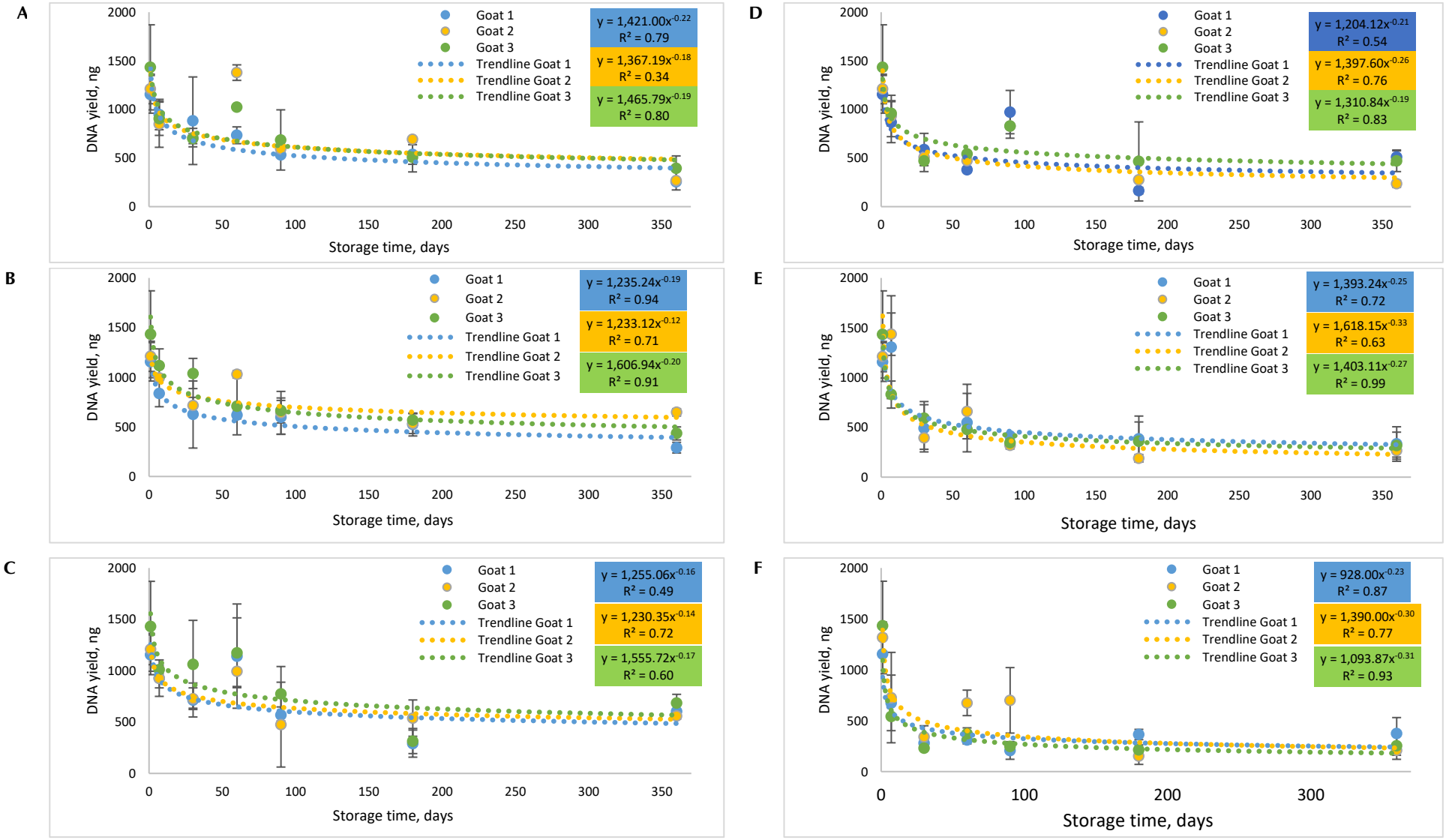

Figure 4. The Change in Total DNA Concentration Extracted from Strip-Dried Whole Blood (glass fiber membrane) over Twelve Months of Storage at Different Temperatures and Sunlight Conditions: A) –78 °C, B) –20 °C, C) +4 °C, D) RT (in zip-lock bag in the dark), E) RT (in zip-lock bag in the light), F) RT (non-packed, in the light). Blue – goat 1, yellow – goat 2, green – goat 3. Values represent mean ± SD, n = 2. Dashed lines – trendlines; power function equations and R2 values provided in colored text boxes.

was observed for packed dried blood samples stored in the dark and in the light, respectively (Figure 4D & E). After six months of storage, the DNA amount was only 22 ± 11% (n = 6) and 27 ± 18% (n = 6) of the original (Day 0) value for these samples (Figure 4D & E). The stability storage experiment began in July 2023 and continued until October 2023, when central heating was turned on. During this period, the day and night outside temperatures ranged from ±14 to 31 °C. During the heating season (up to May 2024), the room temperature was ±22-25 °C and the humidity level was low (around 20-30%). Thus, the ambient temperature was above ±20 °C for most of the storage time. This factor, in conjunction with UV irradiation, is essential for DNA damage caused by sunlight[1,4]. In general, for all storage conditions, a noticeable decrease in DNA yield was observed after one to three months of storage, including liquid samples stored frozen (Figure 2 & 4, Supplemental Information Figure S2-S4). A power function was the best-fit trendline for describing the decrease in DNA quantity over time (Figure 4).

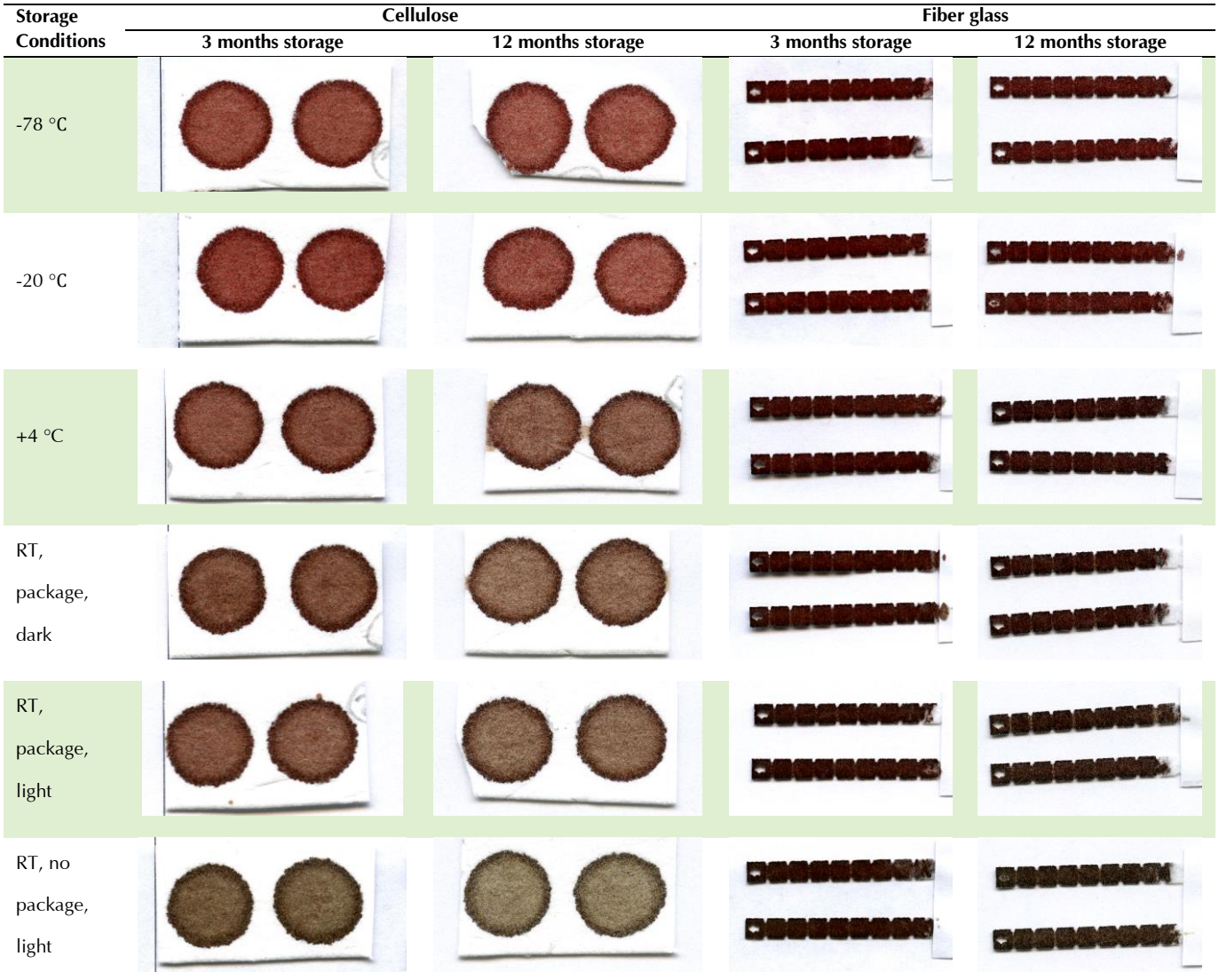

Figure 5. The Appearance of Dried Blood Spots and Strip-Dried Samples after Three and Twelve Months of Storage at Different Temperatures and Sunlight Conditions.

It should also be noted that the color of the dried blood changed during storage on both cellulose and fiberglass, which correlates with the amount of DNA extracted (Figure 4 & 5). After three months of storage, a significant visual color change of dried blood was observed. Specifically, analysis of the color spectrum of dried blood using the RGB model showed an increase in the intensity of the blue and green components, while the signal corresponding to the red component decreased (Figure 6). The most pronounced changes were recorded for dried blood spots on cellulose, likely due to the white color of the membrane material. Since glass fiberis highly porous and translucent, the observed color change was less noticeable. Visually, the spots acquired a brownish and even greenish hue, which appears

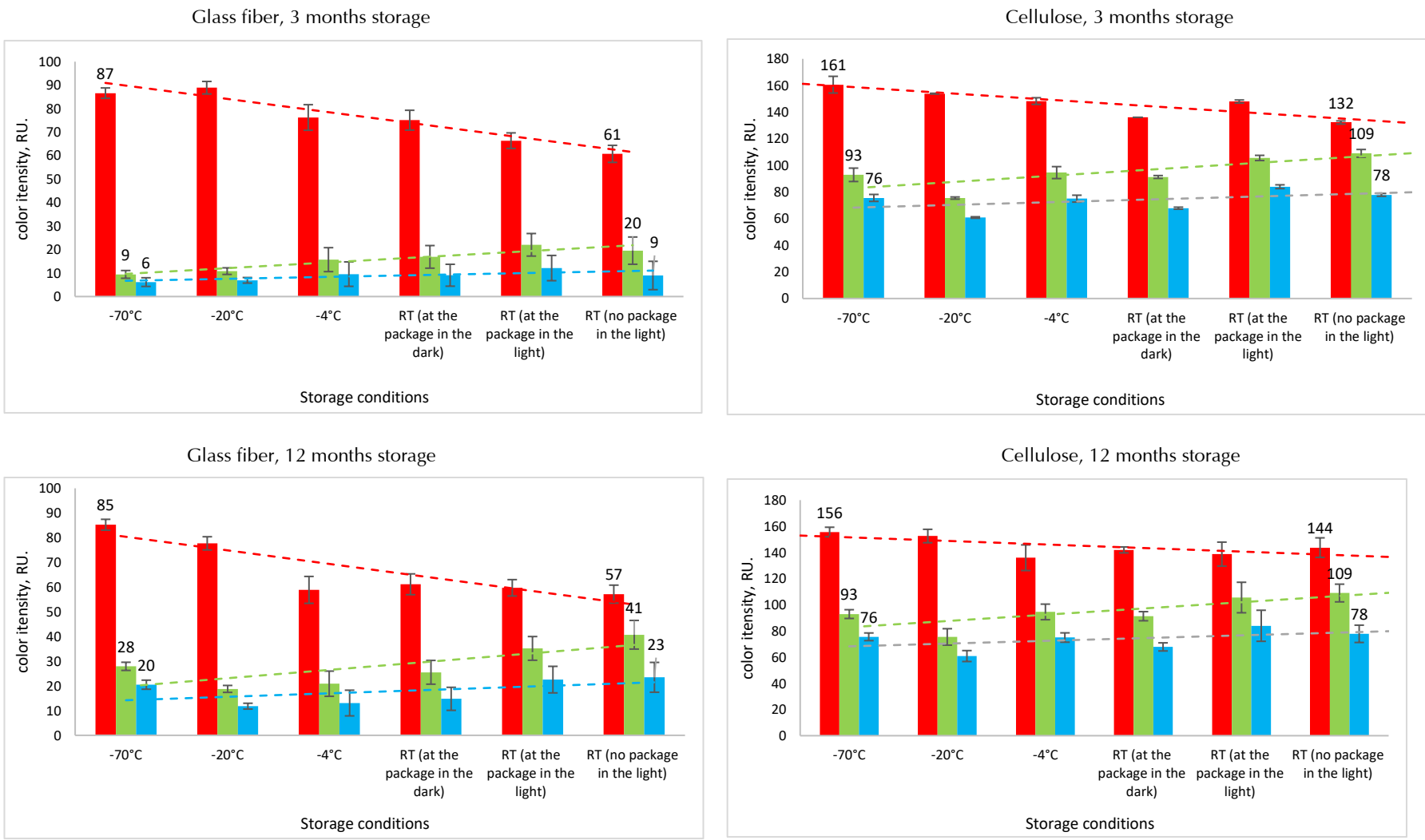

Figure 6. RGB Values of Dried Blood Spots and Strip-Dried Samples Color after Three and Twelve Months of Storage at Different Temperatures and Light Conditions. Values represent mean ± SD, n = 2. Dashed lines – trendlines.

to indicate the occurrence of oxidative processes. To our knowledge, this is the first study to evaluate the color change of dried blood using the RGB model in relation to DNA yield. Reflectance measurements of haemoglobin and its derivatives at specific wavelengths are used to determine the hematocrit value from dried blood spots.[26] This technique was later implemented in a fully automated system based on reflectance analysis.[27] It is known that upon the aging of dried blood spots/stains, hemoglobin oxidizes to three main derivatives - oxyhemoglobin, methemoglobin, and hemichrome.[28,29] Dried blood spots/stains color indicates the concentration ratios of hemoglobin and its derivatives, ranging from red to black. Bloodstain age estimation is a crucial problem in forensic science, and numerous UV/Vis spectroscopy methods have been developed to address this issue.[29-31] For instance, hyperspectral imaging of a dried blood spot allows for rapid analysis of its homogeneity and provides information on the dynamics of the processes taking place during drying.[28] However, the color of dried blood spots/stains depends not only on the storage time (exposure to atmospheric oxygen) but also on temperature, humidity, and sunlight intensity.[29] It was also found that different surface materials (cotton, glass, polyester) can influence the spectroscopic results of bloodstains.[30] Our investigations on dried blood stored under varying temperature and sunlight conditions clearly support these earlier results (Figure 5 & 6). Therefore, the color of a dried blood spot/stain might be associated with DNA content, as both are influenced by the same environmental factors that affect hemoglobin oxidation, which in turn influences DNA degradation in a dried sample. This is because the color of a dried blood spot/stain serves as an indicator of changes in the surrounding conditions.

As for the DNA extraction process, it was noted that the release of blood from the samples, which had visibly changed color, was incomplete (Supplemental Information Figure S5). For example, strip-dried samples (glass fiber) stored at room temperature in sunlight (in a bag or unpacked on a shelf) showed the most pronounced color change (Figure 6). Incomplete washing of the dried blood from the membrane was observed for these samples (Supplemental Information Figure S5). This highlights the importance of storing samples away from direct sunlight. Conversely, samples stored at room temperature in the dark showed complete washing of dried blood from glass fibers (Supplemental Information Figure S5). For dried blood spots on cellulose, incomplete sample washing was observed under all conditions, which is characteristic of cellulose due to its hollow fibers.[21] These observations can indicate lower DNA yield during the extraction of such samples. The most significant factors affecting the appearance of blood spots and, consequently, the DNA yield are likely a combination of sunlight, air, and elevated temperature.

DNA storage and handling can involve different degradation pathways and molecular mechanisms of damage, such as oxidation, hydrolysis, and UV- induced damage.[3,32] The extracted DNA was stored at -20 °C in 1.5 ml Eppendorf tubes (100 µl per tube) for eleven months. Periodically, the DNA was thawed, its concentration was checked, and electrophoretic separation was performed (Supplemental Information Figure S6). It was observed that DNA concentrations decreased during storage with a reduction of 40-50% for some samples (Supplemental Information Table S1). The decrease was observed after the second freeze-thaw cycle (two months of storage), but no further significant reduction in DNA concentration was noted. At the same time, the results of electrophoretic separation showed that the total DNA quality was good throughout the eleven-month storage period (Supplemental Information Figure S7). Recently, Cordsmeier and Hahn[25] showed that neither mechanical stress, nor pH 6 led to degradation during repeated freeze-thaw cycles of a 2686 bp long plasmid DNA. However, degradation of DNA was observed in the presence of metal ions (Fe^{2+}).

The quality of the extracted DNA was sufficient for the amplification of the mtDNA D-loop (Figure 7). It is worth noting that PCR amplification from dried blood samples stored for 1.5 years was successful for all samples stored at +4 °C and below. However, DNA yield was quite low for the majority of the dried samples (Supplemental Information Figure S7). The results demonstrated that amplification of the mtDNA D-loop for blood stored at room temperature could be challenging, regardless of sunlight exposure. However, blood stored on glass fiber had an advantage over blood stored on cellulose in yielding a PCR product. The fact is definitely associated with a lower DNA yield from the cellulose membrane, as reported earlier.[21] At the same time, recent study has demonstrated that the success of direct PCR from crude DNA is unaffected by the storage of chicken whole blood, dried blood spots, and feathers at room temperature up to 5 years.[15] This is likely related to the mild conditions used for DNA extraction by heating samples in a buffer. Li et al. (2024) found, that relatively large DNA fragments could still be preserved after almost 15 years of storage on simple filter paper without chemical protection, but the overall DNA quality of dried blood spots was much lower than that of cold-chain stored samples.[2]

Consequently, the following approach should be recognized as optimal for the preparation of dried whole blood samples: the use of a glass fiber membrane, as it provides a higher DNA yield than cellulose; drying samples away from direct light; storage in tightly sealed bags with a desiccant at +4 °C or lower temperatures, and preferably in individual packaging. When transporting samples from the field to the laboratory, it is important to avoid overheating to prevent DNA degradation.

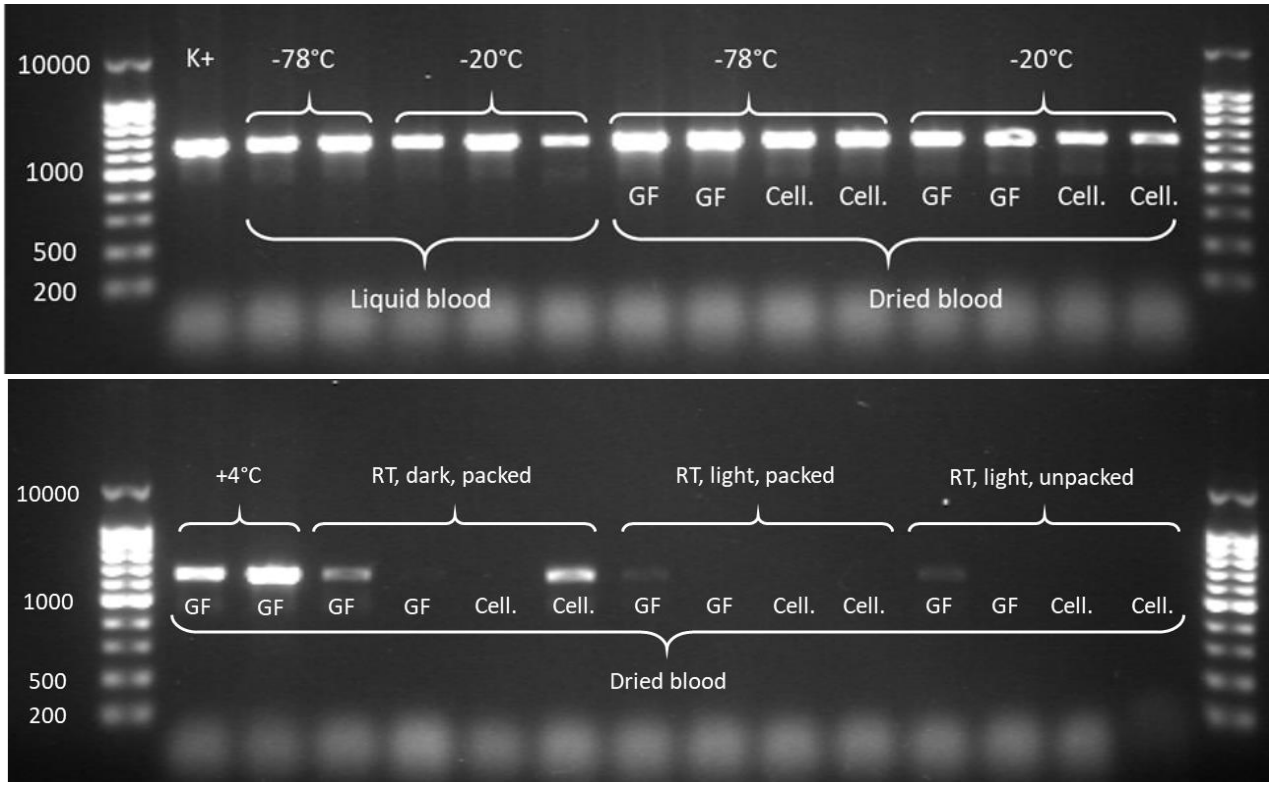

Figure 7. mtDNA D-loop PCR Results for DNA Extracted from Frozen Blood, Dried Blood Spots, and Strip-Dried Blood Samples Stored at Different Temperatures and Sunlight Conditions for 1.5 Years. K+ - positive control, GF – glass fiber membrane, Cell. – cellulose membrane.

Conclusion

The glass fiber membrane was found to have an advantage in terms of total DNA yield and the storage stability of dried blood when natural and non-natural membrane supports were directly compared under various storage conditions. The influence of sunlight and elevated temperatures on total DNA concentration highlights the key factors that should be considered when collecting dried blood samples in the field and transporting them. Long-term storage of dried blood spots on two types of membrane supports revealed that the most rapid reduction in total DNA was caused by temperatures above ambient and exposure to sunlight. Nevertheless, one year of storage resulted in a reduction of more than half the total DNA amount, even in frozen whole blood aliquots. Furthermore, the bright red color of dried blood can serve as an indicator of potential DNA yield: the darker the dried blood, the lower the DNA yield. The semi-quantitative evaluation of dried blood color change in relation to potential DNA yield, which can be visualized using specific color patterns, may be applicable to both scientific investigations and applied research (e.g. sample collection, preservation, and storage). Likewise, the preservation of whole blood in dried form can offer a cost-effective alternative for storing samples at non-freezing temperatures without compromising DNA quality.

Authors' Contributions

All authors contributed to the study conception and design. Experiment, data collection and analysis were performed by JVS, NYS, FRG, VNV, and AKP. YAS and AKP critically reviewed the manuscript. The first draft of the manuscript was written by JVS and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethical Approval

Animal-related experiments were conducted in accordance with the NIH Guidelines for the care and use of laboratory animals (http://oacu.od.nih.gov/regs/index.htm). Animal protocols were approved by the Ethics Committee of the Institute of General Genetics, Russian Academy of Sciences, protocol no. 3, dated December 14, 2020.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

Acknowledgment

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References

1. Muller R, Betsou F, Barnes MG, Harding K. Preservation of biospecimens at ambient temperature: special focus on nucleic acids and opportunities for the biobanking community. Biopreserv Biobank. 2016:14(2):89-98. doi:10.1089/bio.2015.0022

2. Li J, Ulloa GM, Mayor P, Santolalla Robles ML. Nucleic acid degradation after long-term dried blood spot storage. Mol Ecol Resour. 2024:24(6):e13979. doi:10.1111/1755-0998
3. Cordsmeier L, Hahn MB. DNA Stability in biodosimetry, pharmacy and DNA based data-storage: optimal storage and handling conditions. Chembiochem. 2022:23(20): e202200391. doi:10.1002/cbic.202200391
4. Tan X, Ge L, Zhang T, Lu Z. Preservation of DNA for data storage. Russ Chem Rev. 2021:90(2):280–291. doi:10.1070/RCR4994
5. Kumar A, Mhatre S, Godbole S, Jha P, Dikshit R. Optimization of extraction of genomic DNA from archived dried blood spot (DBS): potential application in epidemiological research & bio banking. Gates Open Res. 2019;2:57. doi:10.12688/gatesopenres.12855.3
6. Samsonova JV, Saushkin NY, Osipov AP. Dried Blood Spots technology for veterinary applications and biological investigations: technical aspects, retrospective analysis, ongoing status and future perspectives. Vet Res Commun. 2022:46:655-698. doi:10.1007/s11259-022-09957-w
7. McClendon-Weary B, Putnick DL, Robinson S, Yeung E. Little to give, much to gain-what can you do with a dried blood spot? Curr Environ Heal Rep. 2020:7:211-21. doi:10.1007/s40572-020-00289-y
8. Freeman JD, Rosman LM, Ratcliff JD, Strickland PT, Graham DR, Silbergeld EK. State of the science in Dried Blood Spots. Clin Chem. 2018:64(4):656-79. doi:10.1373/clinchem.2017.275966
9. Jacques ALB, Santos MK, Gorziza RP, Limberger LP. Dried matrix spots: an evolving trend in the toxicological field. Forensic Sci Med Pathol. 2022:18(1):86-102. doi:10.1007/s12024-021-00434-5
10. Venkatesh P, Gopal D. Rapid DNA extraction from dried milk spots: application in non-invasive detection of the A1/A2 variants of beta casein in cow. Proc Natl Acad Sci India Sect B Biol Sci. 2018;88:525-9. doi:10.1007/s40011-016-0781-4
11. Manswr B, Ball C, Forrester A, Chantrey J, Ganapathy K. Evaluation of full S1 gene sequencing of classical and variant infectious bronchitis viruses extracted from allantoic fluid and FTA cards. Avian Pathol. 2018;47(4): 418-26. doi:10.1080/03079457.2018.1471196
12. Rasolonjatovo FS, Guis H, Rajeev M, Dacheux L, Arivony Nomenjanahary L, Razafitrimo G, et al. Enabling animal rabies diagnostic in low-access areas: Sensitivity and specificity of a molecular diagnostic test from cerebral tissue dried on filter paper. PLoS neglected tropical diseases. 2020;14(3):e0008116. doi:10.1371/journal.pntd.0008116
13. El Daous H, Mitoma S, Elhanafy E, Thi Nguyen H, Thi Mai N, Hara A, et al. Establishment of a novel diagnostic test for *Bovine leukaemia virus* infection using direct filter PCR. Transbound Emerg Dis. 2020:67:1671-6. doi:10.1111/tbed.13506
14. Maniego J, Harding C, Habershon-Butcher J, Hincks P, Stewart G, Proudman C, et al. Detection of transgenes in equine dried blood spots using digital PCR and qPCR for gene doping control. Drug Test Anal. 2025:17(5):626-33. doi: 10.1002/dta.3755
15. Garg R, Ramachandran K, Jayashree S, Agarwal R et al. Evaluation of blood samples collected by dried blood spots (DBS) method for hepatitis B virus DNA quantitation and its stability under real life conditions. J Clin Virol Plus. 2022:2(4):100111. doi:10.1016/j.jcvp.2022.100111
16. Troyano-Hernáez P, Herrador P, Gea F, Romero-Hernández B, Reina G, Albillos A, et al. Impact of storage time in dried blood samples (DBS) and dried plasma samples (DPS) for point-of-care hepatitis C virus (HCV) RNA quantification and HCV core antigen detection. Microbiol Spectrum. 2023:11(5):e01748-23. doi:10.1128/spectrum.01748-23
17. Gauffin F, Nordgren A, Barbany G, Gustafsson B, Karlsson H. Quantitation of RNA decay in dried blood spots during 20 years of storage. Clin Chem Lab Med. 2009:47(12):1467-9. doi:10.1515/CCLM.2009.351
18. Hollegaard MV, Grauholm J, Nielsen R, Grove J. Archived neonatal dried blood spot samples can be used for accurate whole genome and exome-targeted next-generation sequencing. Mol Genet Metab. 2013:110(1-2):65-72. doi:10.1016/j.ymgme.2013.06.004
19. Madhanmohan M, Yuvaraj S, Manikumar K, Kumar R, Nagendrakumar SB, Rana SK, et al. Evaluation of the flinders technology associates cards for storage and temperature challenges in field conditions for foot-and-mouth disease virus surveillance. Transbound Emerg Dis. 2016:63:675-80. doi:10.1111/tbed.12316
20. Stowell SM, Bentley EG, Gagne RB, Gustafson KD, Rutledge LY, Ernest HB. Optimal DNA extractions from blood on preservation paper limits conservation genomic but not conservation genetic applications. J Nat Conserv. 2018:46:89-96. doi:10.1016/j.jnc.2018.09.004
21. Samsonova JV, Saushkin NY, Voronkova VN, Stolpovsky YA, Piskunov AK. Optimization of total DNA extraction from dried blood samples. Biochem Genet. 2024. doi:10.1007/s10528-024-10882-7
22. Soloshenkova EA, Piskunov AK, Voronkova VN, Konorov EA, Stolpovsky YA. A nuclear and mitochondrial marker based research of Russia's Ural and central regions gout population structure. Russ J Genet. 2025:61(3):296-307. doi:10.1134/S1022795424701680
23. Al-Araimi NA, Gaafar OM, Costa V, Neira AL, Al-Atiyat RM, Beja-Pereira A. Genetic origin of goat populations in Oman revealed by mitochondrial DNA analysis. PLoS One. 2017:12(12):e0190235. doi:10.1371/journal.pone.0190235
24. Mitchell C, Jennings C, Brambilla D, Aldrovandi G, Amedee AM, Beck I, et al. Diminished human immunodeficiency virus type 1 DNA yield from dried blood spots after storage in a humid incubator at 37 degrees C compared to -20 degrees C. J Clin Microbiol. 2008:46(9):2945-9. doi:10.1128/JCM.00359-08
25. García-Lerma JG, McNulty A, Jennings C, Huang D, Heneine W, Bremer JW. Rapid decline in the efficiency of HIV drug resistance genotyping from dried blood spots (DBS) and dried plasma spots (DPS) stored at 37 degrees C and high humidity. J Antimicrob Chemother. 2009:64(1):33-6. doi:10.1093/jac/dkp150
26. Capiau S, Wilk LS, De Kesel PM, Aalders MC, Stove CP. Correction for the hematocrit bias in dried blood spot analysis using a nondestructive, single-wavelength reflectance-based hematocrit prediction method. Anal Chem. 2018;90(3):1795-804. doi:10.1021/acs.analchem.7b03784
27. Luginbьhl M, Fischer Y, Gaugler S. Fully automated optical hematocrit measurement from Dried Blood Spots. J Anal Toxicol. 2022:46(2):187-93. doi:10.1093/jat/bkaa189
28. Majda A, Wietecha-Posłuszny R, Mendys A, Wójtowicz A, Łydżba-Kopczyńska B. Hyperspectral imaging and multivariate analysis in the dried blood spots investigations. Appl Phys A. 2018:124:312. doi:10.1007/s00339-018-1739-6
29. Bergmann T, Labudde D, Thomas A, Grabherr S. Chronology of forensic bloodstain age estimation using UV-Vis spectroscopy-A comprehensive review. Forensic

Sci Int. 2025:374:112530. doi:10.1016/j.forsciint.2025.112530
30. Bergmann T, Labudde D. Forensic Analysis of Bloodstain Color. In: Advances in Colorimetry. Kumar Samanta, A. (Ed.). IntechOpen. 2024. doi:10.5772/intechopen.108083
31. Marrone A, La Russa D, Montesanto A, Lagani V, La Russa MF, Pellegrino D. Short and long time bloodstains age determination by colorimetric analysis: a pilot study. Molecules. 2021:26(20):6272. doi:10.3390/molecules26206272
32. Matange K, Tuck JM, Keung AJ. DNA stability: a central design consideration for DNA data storage systems. Nat Commun. 2021:12(1):1358. doi:10.1038/s41467-021-21587-5