Biochemical Genetics
https://doi.org/10.1007/s10528-024-10882-7

METHODOLOGY ARTICLE

Optimization of Total DNA Extraction from Dried Blood Samples

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Received: 5 January 2024 / Accepted: 1 July 2024

Abstract

While dried blood spots are a convenient source of genetic material, they are usually associated with a lower DNA yield than from a native sample. The study evaluated the DNA yield from dried blood samples prepared on glass fibre and cellulose membranes and investigated the reasons for the yield reduction. The extraction of total DNA from membrane-dried blood samples was optimized by spin-column extraction method. It was shown that preliminary short-term (20 min) solubilization of a dried matrix in an aqueous medium, followed by standard extraction protocols for the mixture of the eluate with membranes, provides the highest DNA yield. The yield of DNA from a glass fibre membrane was 40–50% lower compared to a native sample, but on average, two times higher than from a conventional cellulose membrane (filter paper). The reduction of DNA yield when using a dried sample was found to be due to partial retention of nucleic acids by the membrane material during the lysis stage.

Keywords Dried blood samples · DNA · Extraction · Membranes · Cellulose · Glass fibre

Introduction

Biological samples in the form of Dried Matrix Spots in general and Dried Blood Spots (DBS) in particular have found application in diverse fields of human medicine, veterinary science, and biology due to the ease of their preparation, small volume of biomaterial required, convenience of transportation and storage, as well as the lower cost of these processes compared to standard native samples

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Published online: 12 July 2024

(McClendon-Weary et al. 2020; Jacques et al. 2022; Samsonova et al. 2022). DBS as a convenient source of genetic material is used to isolate DNA and RNA of pathogens, as well as for a variety of genetic studies (Hendrix et al. 2020; Bezerra et al. 2021; Mahittikorn et al. 2021; Samsonova et al. 2022). The choice of nucleic acids extraction method and its optimization is one of the fundamental issues (Love Stowell et al. 2018; Panda et al. 2019b). With all the variety of nucleic acids isolation methods, the researcher's task is to choose a procedure and optimize the protocol in accordance with the aim of investigation imposing certain requirements for the quantity, purity, and storage stability of genetic material. Several works have been published that focus on optimizing the extraction of DNA and RNA from DBS on filter paper (cellulose membrane) (Molteni et al. 2013; Kumar et al. 2019; Panda et al. 2019a; Gulas-Wroblewski et al. 2021; Lee and Tripathi 2023). Typically, researchers compare different nucleic acid extraction methods empirically without regard to yield estimates and look for the ways to improve extraction efficiency. It has been reported that only about 15–25% of the total DNA from DBS on cellulose cards can be recovered (Sjöholm et al. 2007; Choi et al. 2014).

The objective of this study was to optimize the extraction of total DNA from membrane-dried whole blood. Various methodological aspects of increasing the nucleic acids yield from dried blood, as well as different types of membrane material (glass fibre and cellulose) for dried blood sampling were considered. A few DNA extraction techniques for whole blood dried samples were examined as well. The study evaluated DNA yield from dried samples and investigated the reasons for the yield reduction.

Materials and Methods

All solutions were prepared with deionized water obtained on a Milli-Q unit (Merck Millipore, Germany). The following buffers were used: 0.1 M Tris buffer (pH 7.4), 0.1 M Tris–EDTA buffer (TE), Tris–borate-EDTA buffer (pH 8.3) (TBE), and physiological saline (0.9% NaCl).

The following commercial DNA extraction kits were used: D-blood (Biolabmix, Russia)—silica spin-column based method, sample lysis is carried out in the presence of proteinase K, Method 1, elution volume 100 µL; Diatom™ DNA Prep 100 (Galart Diagnosticum, Russia)—glass particles method, Method 2, elution volume 50 µL; PROBA-GS-GENETIKA (DNA technology, Russia)—a sorbent method, Method 3, elution volume 100 µL (all three solid-phase extraction methods), and Extra-DNA-Bio (ALKOR bio, Russia)—alcohol precipitation method, Method 4, sample volume 30 µL and elution volume 60 µL. DNA elution was performed by deionized water. DNA concentration was assessed using a NanoPhotometer NP60 (Implen Gmbh, Germany); sample concentration was averaged of three replicates at least.

DNA was isolated from the whole blood of domestic goats (*Capra hircus*) kept at the Shakhovskaya biostation (VIGG RAS OBFS no.945). Blood was collected from the jugular vein into EDTA tubes. Whole blood was stored up to a week at + 4 °C; for longer storage, blood was aliquoted (50–100 µL) and stored at − 20 °C. To obtain

dried samples, 50–100 µL blood was spotted onto a cellulose membrane (TE46, Hoefer scientific Instruments, USA) or applied onto a narrow 0.5×6 cm strip of glass fibre membrane (8964, Ahlstrom, Finland). The samples were dried for 1–2 h at room temperature and stored at + 4 °C in zip-lock plastic bags with a desiccant. Optimization of total DNA extraction from strip-dried blood samples was performed with silica spin-column-based method, Method 1. A few modifications were made to the kit procedure regarding the pretreatment of dried samples (the direct lysis or the lysis of preliminary eluted dried blood) and the composition of the lysate mixture at the spinning stage (Fig. 1, procedures 1–4, Table 1, rows 1–4). In procedures 2–4, DBS was preliminary eluted into deionized water within 20 min and interval stirring. After spinning stage, further steps were performed according to the kit instruction, and DNA was eluted by deionized water. For DNA extraction, the required number of 0.5×0.5 cm sections of a glass fibre strip (one section absorbs 10 µL of liquid) or one blood spot (cellulose membrane) cut into pieces was used. In each experiment, dried blood samples were run in parallel with paired liquid blood samples; the liquid blood DNA yield was assumed to be 100% as the maximum amount of DNA to be expected. The expected DNA yield of a sample was calculated as follows:

$$\text{The expected DNA yield} = \frac{\text{The total liquid volume} - \text{The liquid volume retained by the membrane material}}{\text{The total liquid volume}} \times 100\%$$

where "The total liquid volume" is the sum of all liquids volume that undergoes the lysis stage. "The liquid volume retained by the membrane material" is calculated as a number of 0.5×0.5 cm sections of a glass fibre strip multiplied by 10 µL (membrane absorption capacity). If membrane pieces were not eliminated from a mixture before column spinning stage (Table 1, rows 4 and 5), the retained volume was equal to 0 and expected DNA yield was taken as 100%.

The actual DNA yield of a sample was calculated as follows:

$$\text{The actual DNA yield} = \frac{\text{the sample DNA yield}}{\text{the DNA yield from the paired liquid blood sample}} \times 100\%$$

A glass fibre membrane MAPDS-0300 (Arista Biologicals, USA), a polymer membrane MA0120 (Joey Biotech Ltd, China) and a cellulose membrane TNF (Munktel, Germany) were examined in order to evaluate the absorption of DNA by different membrane materials. For this, 100 µL of whole blood was mixed with ten 0.5×0.5 cm sections of a membrane and then treated as described in Table 1, row 5.

Electrophoretic separation of the extracted DNA 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 min using a horizontal electrophoresis chamber. For electrophoretic analysis, 5–10 µL of DNA extract 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.

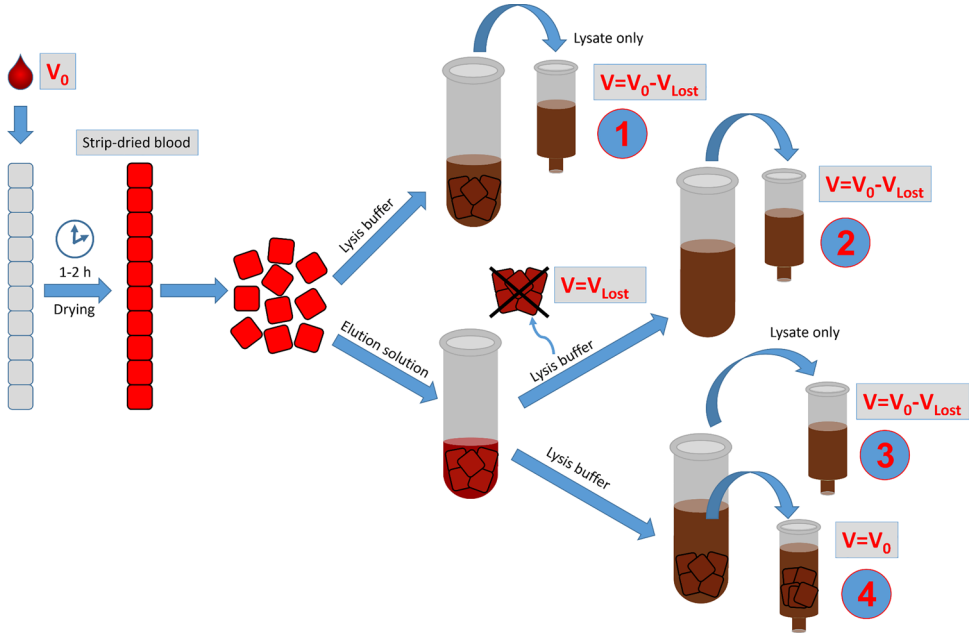

Fig. 1 The general scheme of strip-dried blood samples pretreatment and procedures of total DNA extraction by spin-column method (Method 1). V_0—volume of whole blood sample applied onto the glass fibre strip; V_{lost}—volume of liquid retained by the membrane material. Procedure 1: dried sample (no preliminary elution), direct lysis of a dried sample, transfer lysate (only) into the column (no membranes); Procedure 2: dried sample preliminary elution, take the eluate (no membranes), lysis of the eluate (no membranes), transfer lysate into the column; Procedure 3: dried sample preliminary elution, lysis of the eluate with membranes, transfer lysate (only) into the column; Procedure 4: dried sample preliminary elution, lysis of the eluate with membranes, transfer lysate with membranes into the column

Table 1 Total DNA extraction procedures from native and strip-dried blood samples (glass fibre membrane) using silica spin-column DNA extraction kit (Method 1)*

No	Dried/native whole blood sample pretreatment and total DNA extraction stages				The ratio "sample:solution volume"	The expected DNA yield, %	The actual DNA yield, %	The ratio "the actual DNA yield/the expected DNA yield"
Dried samples								
1	Dried sample (no preliminary elution)		Direct lysis of a dried sample	Transfer lysate (only) into the column (no membranes)	Ten (0.5 × 0.5 cm) pieces:lysis buffer (200 μL)	50	39 ± 2 (n = 2)	0.78 ± 0.04 (n = 2)
					Ten (0.5 × 0.5 cm) pieces:lysis buffer (400 μL)	75	43 ± 4 (n = 2)	0.57 ± 0.05 (n = 2)
					Nine (0.5 × 0.5 cm) pieces:lysis buffer (400 μL)	78	52 ± 10 (n = 3)	0.67 ± 0.13 (n = 3)
					Five (0.5 × 0.5 cm) pieces:lysis buffer (400 μL)	88	59 ± 15 (n = 3)	0.67 ± 0.17 (n = 3)
							Average: 49 ± 9 (n = 10)	
2	Dried sample elution	Take the eluate (no membranes)	Lysis of the eluate (no membranes) (400 μL lysis buffer)	Transfer lysate into the column	Ten (0.5 × 0.5 cm) pieces:elution solution (200 μL); take the eluate (~ 100 μL)	50	49 ± 17 (n = 6)	0.98 ± 0.34 (n = 6)
					Ten (0.5 × 0.5 cm) pieces:elution solution (300 μL); take the eluate (~ 200 μL)	67	37 ± 17 (n = 9)	0.55 ± 0.25 (n = 9)
					Five (0.5 × 0.5 cm) pieces:elution solution (200 μL); take the eluate (~ 150 μL)	75	56 ± 12 (n = 5)	0.75 ± 0.16 (n = 5)
					Three (0.5 × 0.5 cm) pieces:elution solution (200 μL); take the eluate (~ 170 μL)	85	57 ± 22 (n = 6)	0.67 ± 0.26 (n = 6)
							Average: 48 ± 19 (n = 26)	

Table 1 (continued)

No	Dried/native whole blood sample pretreatment and total DNA extraction stages				The ratio "sample:solution volume"	The expected DNA yield, %	The actual DNA yield, %	The ratio "the actual DNA yield/the expected DNA yield"
3	Dried sample elution	Lysis of the eluate with membranes	Transfer lysate (only) into the column		Ten (0.5 × 0.5 cm) pieces:(elution solution (200 μL) + lysis buffer (200 μL))	75	35 ± 12 (n = 4)	0.47 ± 0.16 (n = 4)
					Ten (0.5 × 0.5 cm) pieces:elution solution (200 μL) + lysis buffer (400 μL))	83	44 ± 20 (n = 5)	0.53 ± 0.24 (n = 5)
					Ten (0.5 × 0.5 cm) pieces:(elution solution (300 μL) + lysis buffer (400 μL))	86	35 ± 5 (n = 3)	0.40 ± 0.06 (n = 3)
							Average: 40 ± 14 (n = 12)	
4	Dried sample elution	Lysis of the eluate with membranes (400 μL lysis buffer)	Transfer lysate with membranes into the column	After spinning, remove membranes from the column	3–10 (0.5 × 0.5 cm) pieces:elution solution (200–300 μL)	100	59 ± 16 (n = 20)	0.59 ± 0.16 (n = 20)

Table 1 (continued)

No	Dried/native whole blood sample pretreatment and total DNA extraction stages				The ratio "sample:solution volume"	The expected DNA yield, %	The actual DNA yield, %	The ratio "the actual DNA yield/the expected DNA yield"
Native samples								
5	Native sample, 100 µL. Add 10 pieces (0.5×0.5 cm) of sample-free membranes, add 100 µL of deionized water		Lysis of whole blood with membranes (400 µL lysis buffer)	Transfer lysate with membranes into the column After spinning, remove membranes from the column		100	57 ± 9 (n = 6)	0.57 ± 0.09 (n = 6)
6			Lysis of whole blood with membranes (400 µL lysis buffer)	Transfer lysate (only) into the column (~ 500 µL)		83	55 ± 14 (n = 6)	0.66 ± 0.17(n = 6)
7		Take liquid (no membranes)	Lysis of whole blood (no membranes) (400 µL lysis buffer)	Transfer lysate to the column	Take the eluate (100 µL); 500 µL lysate to the column	50	50 ± 10 (n = 4)	1.0 ± 0.2 (n = 4)
					Take the eluate (140 µL); 540 µL lysate to the column	70	66 ± 1 (n = 2)	0.94 ± 0.01 (n = 2)
							Average: 56 ± 11 (n = 6)	

*The table summarizes the results of numeral experiments when conditions are changed (number of 0.5 × 0.5 cm pieces of membrane were varied from 3 to 10; volume of elution solution—from 200 to 300 µl)

Results and Discussion

Optimization of DNA extraction from strip-dried samples was carried out using a kit based on selective DNA adsorption to the silica (spin-column method, Method 1). The method is regarded as providing pure genetic material in comparison to other DNA extraction methods (Ali et al. 2017; Bukyya et al. 2021; Li et al. 2022). DNA extraction kits intended for DBS use usually prescribe the direct lysis from filter paper DBS (dried filter paper pieces mixed with lysis buffer) followed by further DNA extraction/purification stages. When isolating total DNA, a strip-dried sample (glass fibre membrane) was placed directly into the lysis buffer (Table 1, row 1) or pre-incubated in an elution solution and then lysed with or without membranes (Table 1, rows 2–4). In this work, all modifications of strip-dried blood pretreatment were done in order to demonstrate the difference in DNA extraction yield on each step modification (Fig. 1, Table 1). Due to a variety of experiments performed with modified parameters, the final results were assessed by the value of the actual dried sample DNA yield related to the DNA yield of paired liquid sample in the same experiment (the latter was considered as 100%). The total DNA amount extracted from goat blood samples on average was 16.1 ± 5.2 ng DNA per one μL of whole blood ($n = 40$). Under direct lysis, the process of release of the dried matrix into the lysis solution was less effective than for the two-stage procedure (sample elution—lysis) which provides homogeneous mixture of lysis buffer and eluted sample (Online Resource S1). For direct lysis (Fig. 1, procedure 1), the actual yield was quite high ($49 \pm 9\%$, $n = 10$) relative to a paired liquid sample. However, this procedure resulted in low purity DNA extract with extremely high A_{230} value when the membranes were also loaded to a column after direct lysis. Hence, the lysate only should be spinned through a column in this procedure to address this challenge. It can be assumed that this is a result of an unlysed material left on a membrane. It was also observed that for the pretreatment procedures associated with the separation of the eluate and membrane pieces at a specific stage (Fig. 1, procedures 1–3), the variability of the transferred volume has an impact on the final results (Table 1, rows 1–3). The greater the number of single square (0.5×0.5 cm) pieces per unit of total solution (eluate or elution solution together with lysis buffer), the greater the loss of original biomaterial when removing the membranes from the solution. Thus, it is necessary to strive to ensure that the loss of material removed with membranes is less, but the total volume of the solution is sufficient to conveniently carry out all manipulations during DNA isolation process. To sum up among all procedures, the maximum amount of DNA was extracted when both the eluate and membrane pieces were loaded onto silica-based columns after preliminary 20 min elution of dried sample followed by lysis that provided the actual DNA yield of $59 \pm 16\%$ ($n = 20$) (Fig. 1, procedure 4, Table 1, row 4). Furthermore, this procedure provided the most reproducible results. The incubation time of a strip-dried sample with an elution solution ranged from 15 to 60 min did not affect much the final yield of total DNA (the variation of the actual DNA yield did not exceed $\pm 20\%$), so, relatively short incubation (15–20 min) with intervals' stirring was sufficient

to elute the sample matrix into aqueous media. When using deionized water, physiological saline, or Tris/TE buffer as an elution solution, almost identical amounts of DNA were isolated with a slightly higher (15%) yield for water. The ratio between the dried blood and the volume of elution solution was also varied to investigate DNA extraction performance (number of 0.5×0.5 cm pieces of membrane were varied from 3 to 20; volume of elution solution—from 200 to 500 µL). According to the experimental data obtained, the elution solution:dried sample ratio should be at least 2:1–3:1 (Table 2).

A dried sample DNA yield is supposed to be lower than from an equivalent volume of native sample due to the fact that the membrane material retains part of the eluate in accordance with its absorption capacity (Fig. 1). As membrane is eliminated from a solution, a portion of the absorbed sample volume is lost, resulting in a lower DNA yield. Potentially, the maximum DNA yield can be achieved by lysing the mixture of the eluate together with the membranes and then passing both the lysate and the membranes through DNA-absorbing column. It is worth noting that the larger the total volume of the mixture, the less biomaterial is eliminated with membranes. But one should take into account the dilution factor of lysis buffer and volume capacity of a spin-column to optimize the extraction procedure. According to the experimental results with different extraction procedures, the actual yield of total DNA from a dried blood sample was always lower than for paired liquid sample, on average by 41–62% (Fig. 2, Table 1, rows 1–4). The reduced dried blood DNA yield is apparently associated with partial DNA absorption on the membrane material at the lysis stage. Silica spin-column DNA purification method is based on a principle of DNA binding to silica matrix in the presence of chaotropic agents of lysis solution. It can be considered that the same could be occurred at some extent towards glass fibre membranes at the lysis buffer because the membranes are fabricated of borosilicate glass. Indeed, it was experimentally shown that the presence of sample-free glass fibre membranes at the lysis stage reduces the amount of DNA yield to an average of 57% (Table 1, rows 5 and 6), while the removal of sample-free membranes from whole blood before lysis has virtually no effect on the actual DNA yield (Table 1, row 7). Thus, the DNA absorption by the glass fibre material occurs precisely at lysis stage. It was reported that DNA is absorbed by different solid materials (Ali et al. 2017; Ye and Lei 2023). In the presence of sample-free membranes of different chemical composition, the actual DNA yield of liquid blood was 31% ($n=2$) for cellulose membranes, 66% ($n=6$) for three types of glass fibre membranes, and 74% ($n=2$) for polymer membranes indicating partial absorption of the released DNA onto the membrane material during the lysis. This suggests that for the main membrane materials used for blood sampling and bioanalytical applications, DNA absorption is a common event. In this case, methodologically, the loss of DNA due to the removal of membranes with part of the material (after the elution stage) or due to the absorption of DNA on the carrier was quite similar (Table 1). Thus, for effective and uniform lysis of a dried blood sample, it is preferable to first elute the dried biomaterial into a solution (in the simplest case, into deionized water), with a subsequent lysis of the eluate together with the membranes and followed by passing both the eluate and membrane pieces through spin columns.

Table 2 Results of total DNA extraction from strip-dried blood sample (fibre glass) at different ratios sample volume:elution solution volume*

No	Number of 0.5 × 0.5 cm membrane pieces with dried blood (equivalent of native sample, µL)	Volume of the elution solution, µL	The ratio sample volume:elution solution volume	The expected DNA yield, %	The actual DNA yield ($n=2$), %	The ratio "the actual DNA yield/the expected DNA yield"	Concentration of the extracted DNA, ng/µL
1	3 (30 µL)	200	1:6.7	85	80 ± 35	0.94 ± 0.41	3.4 ± 1.4
2	5 (50 µL)	200	1:4	75	69 ± 15	0.91 ± 0.20	4.8 ± 1.0
3	10 (100 µL)	200	1:2	50	32 ± 7	0.64 ± 0.14	4.4 ± 0.7
4	10 (100 µL)	300	1:3	67	44 ± 20	0.65 ± 0.30	6.1 ± 2.3
5	10 (100 µL)	500	1:5	80	49 ± 18	0.61 ± 0.22	6.8 ± 2.0
6	20 (200 µL)	500	1:2.5	60	30 ± 2	0.49 ± 0.04	8.4 ± 1.2

*Strip-dried blood was incubated in the elution solution (deionized water) for 20 min, and then the eluate (only) was lysed (Table 1, row 2) and all other steps were performed in accordance to the kit instruction (spin-column method, Method 1). Column elution volume was 100 µL

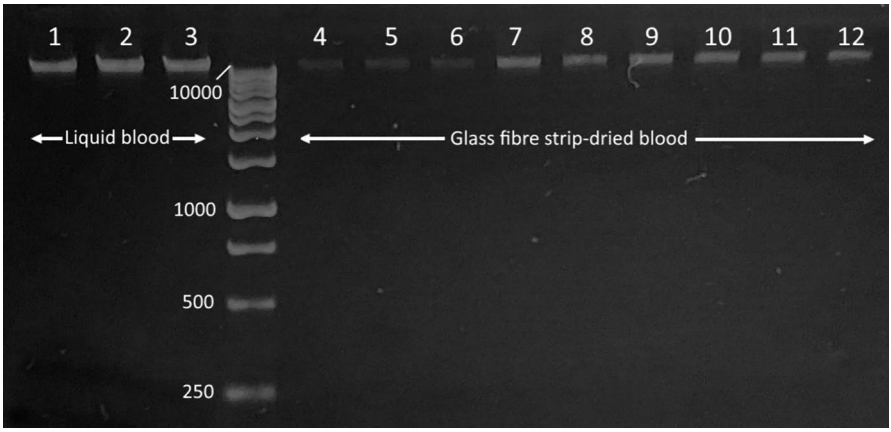

Fig. 2 Result of electrophoretic separation of total DNA extracted from pared native and strip-dried blood samples of domestic goats with the use of silica spin columns (Method 1, sample volume 100 µL, column elution volume 100 µL). Native blood samples: lanes 1–3, DNA concentration: 1—12.87 ng/µL; 2—17.40 ng/µL; 3—14.10 ng/µL. Strip-dried blood samples (glass fibre membrane): lanes 4–6—preliminary elution of dried sample, lysis of the eluate (no membranes); 4—2.80 ng/µL; 5- 2.57 ng/µL; 6—2.65 ng/µL; lanes 7–9—preliminary elution of dried sample, lysis of the eluate with membranes, lysate through the column; 7—4.48 ng/µL; 8—5.10 ng/µL; 9—5.61 ng/µL; lanes 10,11,12—preliminary elution of dried sample, lysis of the eluate with membranes, lysate with membranes through the column); 10—6.65 ng/µL; 11—5.91 ng/µL; 13—6.50 ng/µL

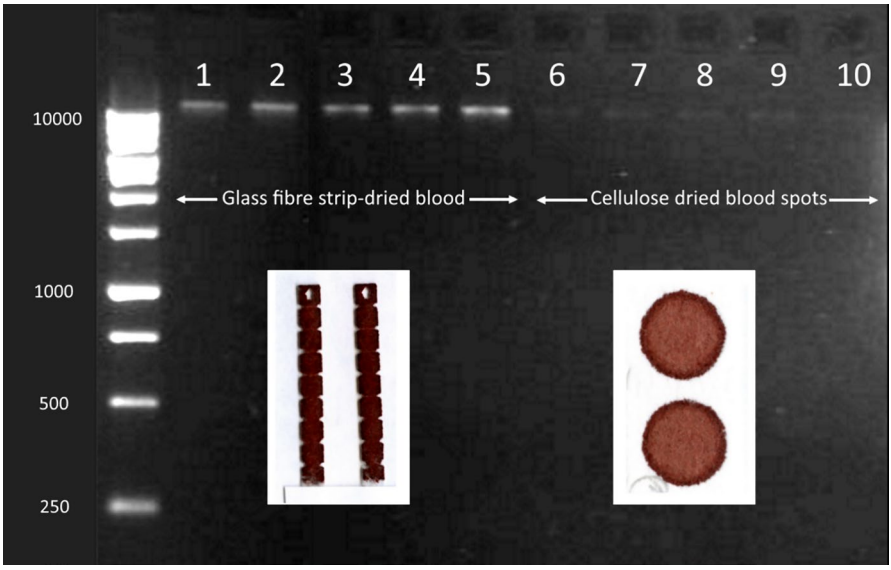

Fig. 3 Result of electrophoretic separation of total DNA extracted from paired dried blood samples (100 µL) of domestic goats. Lanes 1–5—glass fibre membrane (strip-dried samples), lanes 6–10—cellulose membrane (DBS). DNA was extracted on silica spin columns, Method 1. Sample volume was 100 µL, and column elution volume was 100 µL. Dried samples were eluted into 200 µL of deionized water for 20 min and then spinned through the column. DNA concentration was as follows: strip-dried samples—8.14 ± 1.33 ng/µL, $n = 5$; cellulose DBS—2.89 ± 0.37 ng/µL, $n = 5$

Cellulose DBS can provide a half lower DNA amount ($42 \pm 10\%$ ($n = 7$)) compared to the same volume of strip-dried sample (Fig. 3) and that corresponds to about 25% of a native sample which is in agreement with previously published data (Sjöholm et al. 2007; Choi et al. 2014). This result is probably caused by less efficient elution of the biomaterial from the cellulose during 20 min incubation. However, it was reported that overnight or even longer incubation of DBS in an elution solution resulted in similar results (Choi et al. 2014). Cellulose as a hydroxylated polymer possesses high binding affinity for DNA (Bukyya et al. 2021). Moreover, cellulose membrane is composed of porous fibres, so biological fluid is distributed both between and inside the fibres, which results in slower release of biological material into elution solution. It was also noticed that after spinning through a silica column, cellulose membranes are slightly coloured, indicating that some biomaterial is still trapped inside (Online Recourses S2). In a membrane made of glass or synthetic polymer fibres, liquid occupies the space between the solid fibres, thereby facilitating the release of biomaterial (Saushkin et al. 2016; Samsonova et al. 2016). Actually, only about 25% of the total DNA can be isolated from cellulose DBS, according the data obtained. On the other hand, glass fibre-dried samples can provide 50–60% of DNA compared to a native sample.

It should be noted that methods based on the absorption of DNA on different solid matrices such as spin columns or absorbing particles (Methods 1 and 2) make it possible to obtain fairly pure samples from strip-dried blood ($A_{260/280} > 1.7$) comparing to other reagent kits applied in this work (Methods 3 and 4). The quality of the extracted DNA was sufficient for the amplification of SNP loci and mtDNA D-loop (data are not presented in this manuscript and will be published in a forthcoming paper). DNA samples extracted by a sorbent method, Method 3, were characterized by elevated A_{230} value that makes it is difficult to evaluate DNA concentration based on A_{260} value. The use of an alcohol precipitation kit (Method 4) led to contaminated DNA samples in particular due to the presence of haemoglobin (Online Resources S3). Compared to the spin-column method, particles based DNA extraction kits are characterized by numerous washing stages, which can be attributed to the disadvantage of this isolation technique. In addition, when using DNA-absorbing particles, the membranes should be definitely removed from the eluate before the adding of the sorbent to avoid its loss.

Conclusion

Thus, the data obtained demonstrate that glass fibre material is more preferable for total DNA isolation due to higher yield of genetic material compared to cellulose membrane. Almost twofold fall in DNA yield compared to liquid blood was observed for glass fibre strip-dried samples; however, elution efficiency was high enough to get sufficient amount of DNA for further manipulations. In order to isolate total DNA, one can use methods based on the principle of nucleic acids absorption provided by silica columns, which require less stages of sample preparation

and allow membrane presence at the lysis stage without significantly affecting DNA yield.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10528-024-10882-7.

Author Contributions All authors contributed to the study conception and design. Experiment, data collection and analysis were performed by J.V.S., N.Yu.S., V.N.V. and A.K.P. Yu.A.S. and A.K.P. critically reviewed the manuscript. The first draft of the manuscript was written by J.V.S. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by the Russian Science Foundation (Project No. 22-76-10053).

Data Availability Data is provided within the manuscript.

Declarations

Competing Interests None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Ethical Approval All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

References

Ali N, Rampazzo RCP, Costa ADT, Krieger MA (2017) Current nucleic acid extraction methods and their implications to point-of-care diagnostics. Biomed Res Int. https://doi.org/10.1155/2017/9306564

Bezerra CS, Portilho MM, Frota CC, Villar LM (2021) Comparison of four extraction methods for the detection of hepatitis B virus DNA in dried blood spot samples. MicrobiologyOpen 10:e1161. https://doi.org/10.1002/mbo3.1161

Bukyya JL, Tejasvi ML, Avinash A, Chanchala HP, Talwade P, Afroz MM, Pokala A, Neela PK, Shyamilee TK, Srisha V (2021) DNA profiling in forensic science: a review. Global Med Genet 8(04):135–143

Choi EH, Lee SK, Ihm C, Sohn YH (2014) Rapid DNA extraction from dried blood spots on filter paper: potential applications in biobanking. Osong Publ Health Res Perspect 5:351–357. https://doi.org/10.1016/j.phrp.2014.09.005

Gulas-Wroblewski BE, Kairis RB, Gorchakov R, Wheless A, Murray KO (2021) Optimization of DNA extraction from field-collected mammalian whole blood on filter paper for *Trypanosoma cruzi* (Chagas disease) detection. Pathog 10:1040. https://doi.org/10.3390/pathogens10081040

Hendrix MM, Cuthbert CD, Cordovado SK (2020) Assessing the performance of Dried-Blood-Spot DNA extraction methods in next generation gequencing. Int J Neonatal Screen 6:36. https://doi.org/10.3390/ijns6020036

Jacques ALB, Santos MK, Gorziza RP, Limberger RP (2022) Dried matrix spots: an evolving trend in the toxicological field. Forensic Sci Med Pathol 18:86–102. https://doi.org/10.1007/s12024-021-00434-5

Kumar A, Mhatre S, Godbole S, Jha P, Dikshit R (2019) Optimization of extraction of genomic DNA from archived dried blood spot (DBS): potential application in epidemiological research and bio banking. Gates Open Res 2:57

Lee K, Tripathi A (2023) Insight into increased recovery and simplification of genomic DNA extraction methods from dried blood spots. Biopreserv Biobank. https://doi.org/10.1089/bio.2022.0181

Li P, Li M, Yue D, Chen H (2022) Solid-phase extraction methods for nucleic acid separation. A Rev J Sep Sci 45:172–184. https://doi.org/10.1002/jssc.202100295

Love Stowell S, Bentley E, Gagne R, Gustafson K, Rutledge L, Ernest H (2018) Optimal DNA extractions from blood on preservation paper limits conservation genomic but not conservation genetic applications. J Nat Conserv 46:89–96. https://doi.org/10.1016/j.jnc.2018.09.004

Mahittikorn A, Masangkay FR, Kotepui KU, De Jesus MG, Kotepui M (2021) Comparative performance of PCR using DNA extracted from Dried Blood Spots and whole blood samples for malaria diagnosis: a meta-analysis. Sci Rep 11:4845. https://doi.org/10.1038/s41598-021-83977-5

McClendon-Weary B, Putnick DL, Robinson S, Yeung E (2020) Little to give, much to gain-what can you do with a dried blood spot? Curr Environ Heal Reports 7:211–221. https://doi.org/10.1007/s40572-020-00289-y

Molteni CG, Terranova L, Zampiero A, Galeone C, Principi N, Esposito S (2013) Comparison of manual methods of extracting genomic DNA from Dried Blood Spots collected on different cards: implications for clinical practice. Int J Immunopath Pharmacol 26:779–783. https://doi.org/10.1177/039463201302600324

Panda BB, Meher AS, Hazra RK (2019a) Comparison between different methods of DNA isolation from dried blood spots for determination of malaria to determine specificity and cost effectiveness. J Parasit Dis 43:337–342. https://doi.org/10.1007/s12639-019-01136-0

Panda BB, Pradhan N, Hazra RK (2019b) Comparative analysis of three methods from dried blood spots for expeditious DNA extraction from mosquitoes; suitable for PCR based techniques. Mol Biol Rep 46:151–160. https://doi.org/10.1007/s11033-018-4456-5

Samsonova JV, Chadina AS, Osipov AP, Kondakov CE (2016) Porous membrane strip microsampling: a dried biofluid collection format and application for quantitative enzyme immunoassay. Anal Meth 24:4835–4843. https://doi.org/10.1039/C6AY00724D

Samsonova JV, Saushkin NY, Osipov AP (2022) Dried Blood Spots technology for veterinary applications and biological investigations: technical aspects, retrospective analysis, ongoing status and future perspectives. Vet Res Commun 46:655–698. https://doi.org/10.1007/s11259-022-09957-w

Saushkin NY, Samsonova JV, Osipov AP, Kondakov CE, Efimova MA, Chernov AN (2016) A new sampling format for the diagnostics of bovine infectious diseases in dried blood spots by ELISA. Moscow Univ Chem Bul 71:253–257. https://doi.org/10.3103/S0027131416040088

Sjöholm MI, Dillner J, Carlson J (2007) Assessing quality and functionality of DNA from fresh and archival dried blood spots and recommendations for quality control guidelines. Clin Chem 53:1401–1407. https://doi.org/10.1373/clinchem.2007.087510

Ye X, Lei B (2023) The current status and trends of DNA extraction. BioEssays 45:e2200242. https://doi.org/10.1002/bies.202200242