Dry blood spots in filter paper: a valuable option for DNA extraction?

Ana Alves[1], Ana Xisto[1], Beatriz Tomaz[1], Miguel Brito[1,2], Edna Ribeiro[1,2]

1. Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa. Lisboa, Portugal.
2. H&TRC – Health & Technology Research Center, ESTeSL – Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa. Lisboa, Portugal. edna.ribeiro@estesl.ipl.pt

ABSTRACT: Dry blood spots, appear to be a valuable option for sample collection for DNA extraction, especially in poorer communities with less access to medical care. Dry blood spots can be easily transported and ensure a non-invasive method of rapid blood collection which can be coupled with DNA extraction methods. However, the storage and transportation conditions (time and temperature) as well as sample type and volumes may affect DNA extraction efficiency and become a challenge. In this context, we aim to assess the effects on DNA extraction efficiency of six storage conditions (24 hours at 4 °C, 24 hours at room temperature, 24 hours at 50 °C, seven days at 4 °C, seven days at room temperature, and seven days at 50 °C) for dry blood spots with venous blood and capillary blood samples. In order to evaluate DNA extraction proficiency, under each of these conditions, we performed DNA extraction with the QIAamp® Blood Mini Kit and assessed DNA final concentrations and purity ratios, through UV spectrophotometry, with the aid of the Nanodrop® OneC. Statistical analysis was performed using a T-test in Microsoft® Excel. Overall, our data demonstrated that it is possible to extract a greater DNA quantity from capillary blood samples in dry blood spots, although with less purity than venous blood samples. Also, our results show that the assessed temperature and storage time (seven days) do not affect DNA extraction results.

Keywords: DNA extraction; Dry blood spots; Storage conditions; Blood samples.

Manchas de sangue seco em papel filtro: uma opção válida para extração de DNA?

RESUMO: As manchas de sangue seco parecem ser uma opção válida para a recolha de amostras para extração de DNA, especialmente em comunidades mais pobres e com menos acesso a cuidados médicos. As manchas de sangue seco podem ser facilmente transportadas e garantem um método não invasivo de colheita rápida que pode ser acoplado a métodos de extração de DNA. No entanto, as condições de armazenamento e transporte (tempo e temperatura), bem como o tipo e volumes de amostra podem condicionar a eficiência da extração de DNA e tornar-se um desafio. Neste contexto avaliaram-se os efeitos na eficiência da extração de DNA de seis condições de armazenamento (24 horas a 4 °C, 24 horas à temperatura ambiente, 24 horas a 50 °C, sete dias a 4 °C, sete dias à temperatura ambiente temperatura e sete dias a 50 °C) para amostras de sangue seco com sangue venoso e amostras de sangue capilar. Para avaliar a proficiência na extração de DNA, em cada uma dessas condições, realizou-se a extração de DNA com o *QIAamp® Blood Mini Kit* e avaliaram-se as concentrações finais e taxas de pureza do DNA, por meio de espectrofotometria UV com auxílio do *Nanodrop® OneC*. A análise estatística foi realizada por meio do teste *T* no *Microsoft® Excel.* Os resultados obtidos demonstraram que é possível extrair uma quantidade maior de DNA de amostras de sangue capilar em amostras de sangue seco, embora com menor pureza do que amostras de sangue venoso. Além disso, os resultados mostram que a temperatura avaliada e o tempo de armazenamento (sete dias) não afetam os resultados da extração de DNA.

Palavras-chave: Extração de DNA; Manchas de sangue seco; Condições de armazenamento; Amostras de sangue.

Introduction

The study of genetics is recent, with its beginnings dating back to the mid-19th century by Gregor Mendel[1]. Genetics aims to study all conditions associated with genes, with the field having undergone great development in the first half of the 20th century[1]. This area has great impact in the way diagnosis and treatment of various diseases is approached. DNA extraction is the starting point for numerous practices; therefore, it is important that sufficient amounts of DNA can be extracted for analysis and that it is pure[2-4]. In recent years, DNA extraction has been optimized and there are several kits and protocols that facilitate this process[1,4]. Even so, as there are several tested and established methods for extraction through blood collected in Dry Blood Spots (DBS), it is not defined what the best storage conditions are[5].

DBS themselves are especially useful in the field of genetics as they are easy to harvest, store, and transport[2,5-6]. Since extraction is such a crucial step for performing various techniques, it is important to study and define the best possible method; given that one of the great disadvantages of using DBS is precisely the difficulty in generating quantitative results and additionally the amounts of extracted DNA are sometimes low and of inferior quality[7-11].

DBS blood sampling involves collecting lesser amounts of blood onto a cellulose or polymer paper, followed by drying the sample[5,12]. The collected blood can be directly deposited onto the filter paper after a capillary puncture or after a venous blood collection[5]. This collection method is on the rise for sampling outside healthcare facilities[5,13-14]. The ease of storage and transport for DBS enables exchanges of information between countries[5-6,14]. The use of DBS gains relevance in impoverished communities, without access to medical care and without the population being able to travel to screening and diagnosis centers[5]. This method of blood collection allows health professionals to travel to these communities, and the DBS to be taken to laboratories that are able to meet the needs of these populations, ensuring basic care to those most in need[6]. It is important to note that the method has disadvantages such as the impossibility of obtaining immediate results and requiring the person to return for a confirmatory test[14]. This collection method has grown so much in relevance that it is currently recognized and recommended by the World Health Organization (WHO), with the possibility of samples being sent to competent laboratories anywhere in the world[11,14-15].

The main goal of the study was to correlate the storage conditions of DBS in venous and capillary blood with the efficiency and purity of the DNA extraction, in order to evaluate the applicability potential of the method in communities without access to medical care.

Methods

Venous blood and capillary samples

Venous blood and capillary blood were collected from five healthy volunteers, one man and four women, between 18 and 25 years old, without known pathologies. Venous blood was collected into an EDTA tube (3mL) through a closed-system blood draw and immediately and gently mixed to avoid any clots. Then, 200µL were pipetted onto filter paper. Capillary blood was collected using a lancet and 20µL was pipetted onto filter paper. All samples were left to air dry[16]. The obtained samples were divided according to Figure 1. For the control group, the remainder whole blood contained in the EDTA tube was used as a fresh blood control. Here, we utilized venous whole blood with EDTA, stored for 24 at 4 °C as a control group, considering that it is the current standard method for DNA extraction from blood samples. DBS samples were stored in sealed plastic bags for 24 hours at 4 °C, 24 hours at room temperature (RT), 24 hours at 50 °C, seven days at 4 °C, seven days at RT, and seven days at 50 °C[5]. For 50 °C incubation samples were placed in a laboratory oven.

DNA Extraction

The DNA extractions were performed after 24 hours and after seven days. Punches of 6mm in diameter, corresponding to approximately 8µL of whole blood, were used for each sample[17]. For the control group, 8µL of whole blood was used per sample[17]. DNA was extracted using the QIAamp® Blood Mini Kit according to the manufacturer's instructions[18-19].

DNA quantification

In order to access DNA concentration and purity ratio, 2µL of each sample was used to quantify using NanoDrop™ OneC (Thermo Scientific) at 260nm and 280nm[20-22]. A ratio of ~1.8 was accepted as "pure" for DNA.

Statistical analysis

The average values along with the standard deviation (SD) for the concentration and purity ratios for both venous and capillary blood, were calculated for each sample storage condition. A T-test was then applied to those values[23].

Ethics

This study complies with the ESTeSL Ethics Commission.

Results

DNA extraction efficiency

Results were analyzed by sample type (venous and capillary blood), temperature, and time of storage. For each sample condition, DNA average concentration and purity ratio ABS 260/280 was calculated[22-23]. For the control sample, stored at 4 °C for 24 hours, the average for the DNA extraction was 3.20±1.90ng/µL with a purity ratio average of 2.22±0.40; for the venous blood samples stored at 4 °C for 24 hours, the average for the extraction was 1.91±0.55ng/µL with a purity ratio average of 1.71±0.64; for the capillary blood samples stored at 4 °C for 24 hours the average for the extraction was 6.56±1.74ng/µL with a purity ratio average of 1.37±0.09; for the venous blood samples stored at RT for 24 hours the average for the extraction was 2.37±0.81ng/µL with a purity ratio average of 1.73±0.64; for the capillary blood samples stored at RT for 24 hours the average for the extraction was

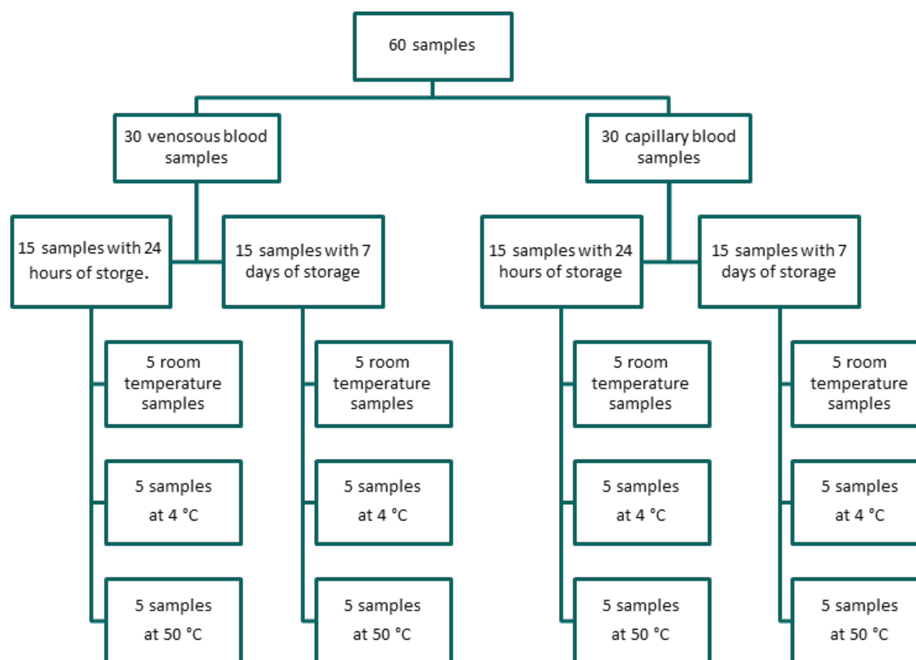

Figure 1. Scheme of the sample organization conditions for the optimization of DNA extraction in DBS from filter paper.

5.01±1.02ng/µL with a purity ratio average of 1.67±0.47; for the venous blood samples stored at 50 °C for 24 hours the average for the extraction was 0.93±0.52ng/µL with a purity ratio average of 1.35±1.69; for the capillary blood samples stored at 50 °C for 24 hours the average for the extraction was 4.53±2.04ng/µL with a purity ratio average of 1.52±0.62; for the venous blood samples stored at 4 °C for seven days the average for the extraction was 1.98±0.79ng/µL with a purity ratio average of 1.41±0.24; for the capillary blood samples stored at 4 °C for seven days the average for the extraction was 5.31±1.10 ng/µL with a purity ratio average of 1.35±0.09; for the venous blood samples stored at RT for seven days the average for the extraction was 1.25±0.92ng/µL with a purity ratio average of 1.13±0.91; for the capillary blood samples stored at RT for seven days the average for the extraction was 5.21±2.07ng/µL with a purity ratio average of 1.62±0.29; for the venous blood samples stored at 50 °C for seven days the average for the extraction was 1.31±0.96ng/µL with a purity ratio average of 2.37±2.38; for the capillary blood samples stored at 50 °C for seven days the average for the extraction was 5.55±3.72ng/µL with a purity ratio average of 1.71±0.74. These results are summarized in Table 1.

Table 1. Results of the average concentration of DNA extraction and average purity ratio (ABS 260/280) for each of the studied conditions

Experiment Conditions	Nucleic Acid (ng/µL) (Average ± SD)	ABS260/280 (Average ± SD)
24H_4 °C_CTRL	3.20±1.90	2.22±0.40
24H_4 °C_VB	1.91±0.55	1.71±0.64
24H_4 °C_CB	6.56±1.74	1.37±0.09
24H_RT_VB	2.37±0.81	1.73±0.64
24H_RT_CB	5.01±1.02	1.67±0.47
24H_50 °C_VB	0.93±0.52	1.35±1.69
24H_50 °C_CB	4.53±0.99	1.52±0.09
7D_4 °C_VB	1.98±0.79	1.41±0.24
7D_4 °C_CB	5.31±1.10	1.35±0.09
7D_RT_VB	1.25±0.92	1.13±0.91
7D_RT_CB	5.21±2.07	1.62±0.29
7D_50 °C_VB	1.31±1.01	2.37±2.21
7D_50 °C_CB	5.55±1.64	1.71±0.32

DNA concentrations and purity divergence among the analyzed samples and storage conditions

In order to compare the results of DNA extraction and DNA purity between the control group and the test groups, a T-test was applied. We analyzed the extraction efficiency between the capillary and venous blood samples; the time between collection and extraction (24 hours and seven days) and the different temperatures of storage (4 °C, RT, and 50 °C)[24]. The results obtained were as follows: the *p*-value obtained between the comparison of the control and all the samples for the DNA extraction was 0.628 and for purity, the ratio was 3.585×10^{-7}; the *p*-value obtained for the comparison between the capillary blood and the venous blood for DNA extraction was 1.005×10^{-6} and for purity, the ratio was 0.689; *p*-value obtained for the comparison between the time of collection and DNA extraction was 0.924 and for purity, the ratio was 0.830; the *p*-value obtained between the comparison of 4 °C and RT for DNA extraction was 0.764 and for purity, the ratio was 0.664; the *p*-value obtained between the comparison of 4 °C and 50 °C for DNA extraction was 0.116 and for purity, the ratio was 0.298 and lastly the *p*-value obtained between the comparison of RT and 50 °C for DNA extraction was 0.137 and for purity, the ratio was 0.478[24-25]. The results can be observed in Tables 2 and 3.

Table 2. Results of the *p*-value for the T-test comparison of the control group *vs* test group, capillary blood *vs* venous blood, 24 hours *vs* 7 days, 4 °C *vs* RT, 4 °C *vs* 50 °C and RT *vs* 50 °C, regarding the concentration of DNA extracted

Conditions analysis	*p*-value
Control group *vs* Test group	0.628
Capillary blood *vs* Venous blood	1.005
24 hours *vs* 7 days	0.929
4 °C *vs* RT	0.764
4 °C *vs* 50 °C	0.116
RT *vs* 50 °C	0.137

Table 3. Results of the *p*-value for the T-test comparison of the control group *vs* test group, capillary blood *vs* venous blood, 24 hours *vs* 7 days, 4 °C *vs* RT, 4 °C *vs* 50 °C and RT *vs* 50 °C, regarding the purity ratio of DNA extracted

Conditions analysis	*p*-value
Control group *vs* Test group	3.585
Capillary blood *vs* Venous blood	0.689
24h *vs* 7 days	0.830
4 °C *vs* RT	0.664
4 °C *vs* 50 °C	0.298
RT *vs* 50 °C	0.478

Our data demonstrated that the only results with a statistically significant difference ($p<0.05$) are the ones observed for the concentration of extracted DNA between capillary and venous blood samples and for the purity ratio between the control group and test group[25]. For all the other comparisons the *p*-value was higher than or equal to 0.05, meaning there was no statistically significant difference between the results of the various groups[25].

Discussion

DBS has been described as a valuable alternative for sample collection for DNA extraction, however, the storage and transportation conditions (time and temperature) as well as sample type and volumes may influence DNA extraction efficiency and become a challenge.

Here we quantitatively and qualitatively compare the extraction of DNA from venous and capillary blood samples after being stored in six different conditions. According to the data obtained, it is possible to observe that there is not a statistically significant difference in the concentration and purity of DNA extracted related to the temperature or time of storage. According to the 2018, Badu-Boateng *et al.* study – that compared storage conditions (at 4 °C, at RT, and at -20 °C) for blood samples and its effect on DNA extraction – after less than two weeks the extracted DNA did not show significant signs of degradation for all conditions[26]. The authors proposed that only after two weeks of DBS storage, a significant decrease in the concentration of DNA extracted relative to the control could be observed. This agrees with our data that highlights that within one week of storage, the DNA concentration remains unchanged[26].

The major difference observed in our study was between blood sample types (capillary and venous blood) regarding DNA concentration ($p=1.005\text{x}10^{-6}$). Capillary blood samples resulted in DNA extraction of higher concentration, but with lower purity rates than venous blood samples independently of storage conditions. Meanwhile, venous blood, despite the DNA extraction of lower concentration, allows us to obtain samples with greater purity ratios (despite the difference not being statistically significant). Kazachkova *et al.* compare the use of venous and capillary blood for early determination of fetal sex, also reaching the conclusion that capillary blood can be used as a viable alternative for venous blood[27]. Further corroborating the obtained results, Kumar *et al.* performed an optimization of DNA extraction from capillary blood in DBS, concluding that these can be used for genetic studies[10].

Overall, the concentration of DNA in all extraction conditions was low. According to the DNA extraction kit manufacturer, the concentration of DNA from blood obtained for a venous sample should be between 30 and 40ng/µl. Thus, for the 8ul used, the extracted DNA should be present at higher concentrations[28]. Despite this, Strøm *et al.* compared four methods of DNA extraction in DBS and reported poorer results in stored samples; confirming that there can be expected a decrease in the concentration of extracted DNA, as also verified in the present study[29]. Moreover, Tuaillon *et al.*

used DBS for diagnosis and monitoring of HIV and hepatitis B and C and reported that despite the performance of DBS not being optimal, it is high enough for DBS to be considered a useful tool for infectious despise diagnosis in hard-to-reach populations[14].

On the other hand, the purity of DNA in all assays was also lower than expected, when comparing the results of the purity ratios for the control group with the test group, as the values for the test group were statistically significantly lower (p=3.585x10^{-7}). Considering that all samples were properly conditioned and sealed there should not have been any contamination. The ideal ABS260/280 ratio is close to 1.8, although some sample values are considered sufficient (24H_4 °C_VB: 1.71±0.64; 24H_RT_VB: 1.73±0.64 and 7D_50 °C_CB: 1.71±0.74), most were either too high or too low[6,23]. These results may have been a consequence of the low concentrations obtained, which increased the error associated with the reading of the absorbance by the Nanodrop® OneC[20-21]. Furthermore, technical errors due to working with such small volumes might also have occurred. Hue *et al*. used 1cm punches of DBS from capillary blood and obtained an average DNA concentration of 7.7ng/µL, also using a commercial kit; they were able to obtain results with a purity of 1.8[30]. Proving that with only a slightly higher volume of blood, it would be possible to obtain a more accurate reading for the purity ratio[30]. Therefore, for future studies, it is recommended to collect a higher volume of blood, in order to reduce these errors.

Conclusion

Our data demonstrated that the assessed time and temperature of storage do not influence the concentration or purity of the DNA extracted from DBS. On the other hand, capillary blood samples allow for the extraction of higher concentrations of DNA, although less pure. Therefore, DBS using capillary blood samples can be applied in communities without access to medical care to perform DNA extraction, although, with some applicability limitations associated with the low concentration and purity of the DNA obtained.

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Conflicts of interest

The authors declare no conflict of interest.

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