https://doi.org/10.22491/2357-9730.114246

Standardization of an organic DNA extraction method from dried blood spots and its downstream molecular applications in neonatal screening and diagnostic confirmation of lysosomal disorders

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Clin Biomed Res. 2022;42(3):218-225

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ABSTRACT

Introduction: Dried blood spot (DBS) samples have been used for diagnostic purposes since their introduction in the neonatal screening of phenylketonuria almost 50 years ago. The range of its application has been extended to modern approaches, such as next-generation sequencing (NGS) for molecular genetic testing. This study aimed to evaluate the use of a standardized organic method for DNA extraction from DBS samples in the diagnostic setting.

Methods: The clinical applicability of the method was tested using 3 samples collected from a newborn screening project for lysosomal storage diseases, allowing the determination of the genotype of the individuals. DNA was extracted from 3 3-mm diameter DBS punches. Quality, purity, and concentration were determined, and method performance was assessed by standard polymerase chain reaction, restriction length polymorphism, Sanger sequencing, and targeted NGS.

Results: Results were compared with the ones obtained from DNA samples extracted following the internally validated in-house extraction protocol that used 6 3-mm punches of DBS and samples extracted from whole blood.

Conclusion: This organic method proved to be effective in obtaining high-quality DNA from DBS, being compatible with several downstream molecular applications, in addition to having a lower cost per sample.

Keywords: *DNA extraction; dried blood spots; molecular genetic testing; newborn screening; targeted next-generation sequencing*

INTRODUCTION

The collection of dried blood spots (DBS) on filter paper is a useful approach for large scale analysis, such as screening programs. DBS was first introduced in 1963 as an alternative to blood sampling and screening of phenylketonuria in a large population of newborn infants[1]. DBS sampling has since emerged as an appropriate method for the collection, transportation, analysis, and storage of biological fluids due to its simplicity, reduced invasiveness, and low cost[2-4]. It is also the best option when venipuncture, transportation, and storage conditions are not favorable[5], which is why DBS are still used as samples for newborn screening (NBS)[6].

The use of DBS for diagnosis has increased throughout the years[7] and, despite some limitations (small amount of available material and risk of DNA degradation, among others), its performance in downstream molecular processes is similar or only slightly inferior to gold-standard sample types[2]. To date, DNA obtained from DBS has been used for diagnostic purposes using different molecular biology techniques, such as standard polymerase chain reaction (PCR)[5], real-time PCR[8], multiplex ligation-dependent probe

ISSN 2357-9730

amplification[9], high-resolution melting analysis[10], and different next-generation sequencing (NGS) approaches such as targeted NGS (TNGS), whole-exome sequencing, and whole-genome sequencing[11-15].

The performance and reliability of different molecular downstream processes are highly influenced by both the quantity and quality of DNA[16]. Therefore, an efficient methodology for DNA extraction that maximizes DNA yield and quality with minimal co-extraction of inhibitors should be established[17]. To achieve these parameters, there is a wide range of commercial and noncommercial methods for purifying DNA from DBS[5,18-20]. Organic extraction, a noncommercial method, is well-known for having high extraction efficiency and for removing the majority of PCR inhibitors in comparison with other methods[21].

The Medical Genetics Service of the Hospital de Clínicas de Porto Alegre (HCPA) is a reference center in the diagnosis of lysosomal storage diseases (LSD) in Brazil and Latin America[22] that receives a large number of samples from different services in this region. Thus, an effective collection method that allows for convenient worldwide transportation and requires only a minimal amount of blood, such as DBS, should be established. We have recently started using genomic DNA (gDNA) extracted from DBS by an organic extraction method[23] that was internally validated using 6 3-mm punches. For the potential inclusion of LSD, a group of rare diseases[24,25], in Brazil's neonatal screening program, the minimum amount of sampling needed to obtain a good gDNA yield to perform different molecular assays should be determined, since sample availability is limited and it is not always possible to obtain new DBS samples from babies.

The aim of the present study was to standardize the organic extraction method previously used for isolating gDNA from DBS, using 3 3-mm punches instead of 6, to make it suitable to be used in NBS. Method performance was tested in different molecular biology processes, including NGS, to verify sampling quality and efficiency.

METHODS

Sample collection

Twenty control samples provided by the manufacturer of the NBS kit (Perkin Elmer®, USA) that also served as biochemical controls were used for method standardization. Additionally, to validate the method's clinical applicability, 3 newborns with a previous biochemical diagnosis of Fabry disease, Pompe disease, and Mucopolysaccharidosis type I were evaluated. The newborns were part of a project evaluating a potential NBS program for LSD, and their DBS samples were stored at HCPA's Medical Genetics Service. Control samples were collected in a sterile environment using venipuncture. Blood was spotted on PerkinElmer® 226 Paper Grade and dried for 3 hours at room temperature before storage. The samples were stored at 4°C until analysis.

DNA Extraction

The organic extraction (GE Healthcare, Reliable extraction of DNA from Whatman™ FTA™ cards) was performed using three punches (3-mm). Three discs were punched out from a full dried blood spot into a 1.5 mL microcentrifuge tube using a metal hole puncher and six blank discs were punched between each sample to avoid cross-contamination. DBS punches were incubated at 56°C overnight in constant agitation of 650 RPM with 500 µL of Extraction Buffer (10 mM Tris-HCl, pH 8.0; 10 mM EDTA, disodium salt, pH 8.0; 100 mM NaCl; and 2% w/v SDS) and 6 µL of proteinase K (10 mg/mL) (Promega, USA). The lysate was treated with the same volume of buffered phenol (pH 8.0) (Sigma®, Germany) followed by vortexing and centrifugation at 14,000 rpm for 10 minutes at room temperature. The aqueous phase was transferred to a fresh 1.5 mL microcentrifuge tube with 500 µL of chloroform (Sigma®) for subsequent vortexing and centrifugation at 14,000 rpm for 10 minutes at room temperature. The upper aqueous phase was transferred to a new 1.5 mL microcentrifuge tube and precipitated with 45 µL of 3 M sodium acetate (pH 5.2) and 100% absolute ethanol (Merck, Germany) and incubated at −20°C overnight. On the next day, the solution was centrifuged at 14,000 rpm for 30 minutes at 4°C to recover the DNA. The supernatant was removed, and the pellet was washed with 70% ethanol and centrifuged again at 14,000 rpm for 30 minutes at 4°C. The supernatant was removed again, and the pellet was dried out at 60°C for 30 minutes. DNA was resuspended in 13 µL of water.

DNA yield and quality

DNA integrity (5 µL) was assessed by 0.8% agarose gel electrophoresis. Concentration was measured using Qubit® double-stranded DNA (dsDNA) High Sensitivity (HS) Assay kit (Invitrogen – Thermo Fisher Scientific, USA), which calculates concentration based on the fluorescence of a dye that binds to double-stranded DNA. Concentration values provided by Qubit® dsDNA HS were used as reference for TNGS analysis. Additionally, the concentration was measured using NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific, USA), which also provides DNA purity (A260/A280 nm ratio).

As calculated by Saavedra-Matiz et al.[20], a 100% efficient DNA extraction is expected to yield

3.47 ng/μL of DNA per 3-mm DBS. Accordingly, a yield of approximately 10.41 ng/μL is expected for 3 3-mm punches; this will be our reference to calculate extraction efficiency.

Molecular biology techniques

To evaluate the quality of the DNA extracted by this standardized method and to determine whether any inhibitory material was interfering with the reaction, PCR was performed using Veriti 96-Well Thermal Cycler (Applied Biosystems®, USA) of different-sized fragments using primers flanking exonic and intronic regions of the following genes: 297 bp (*TPP1*, exon 8), 367 bp (*GAPDH*, exon 4), and 1315 bp (*IDS*, intron 7). The products were assessed by 1.5% agarose gel to demonstrate amplification. PCR of the *TPP1* gene was followed by BigDye sequencing using the Applied Biosystems® 3500xL 96-capillary DNA analyzer (Thermo Fisher Scientific), and the sequence was analyzed on the BioEdit Sequence Alignment Editor. Restriction fragment length polymorphism (RFLP) was performed using *Hinf*1 following the amplification of intron 7 of the *IDS* gene. The reaction was incubated at 37°C overnight, and the product was assessed by 3.0% agarose gel to demonstrate digestion.

Targeted next-generation sequencing (TNGS)

Briefly, 10 ng of DNA samples were used to prepare target enrichment with the specifically customized panels, consisting of 2 different PCR primer pools (Thermo Fisher Scientific). Amplification was achieved by multiplex PCR using the Ion AmpliSeq™ Library Kit (Thermo Fisher Scientific), followed by the connection to Ion Xpress™ barcodes (Thermo Fisher Scientific). These unamplified libraries were purified with magnetic beads (Agencourt™ AMPure™ XP Reagent, Beckman Coulter, USA) and quantified (Qubit® dsDNA HS kit, Thermo Fisher Scientific). The quantified libraries were prepared and pooled in equimolar concentrations of 100 pM each. Barcoded libraries underwent template preparation in the Ion Chef™ Instrument (Thermo Fisher Scientific), where the Ion 510™ chip (Thermo Fisher Scientific) is loaded. The chip was transferred to the Ion S5™ Sequencer (Thermo Fisher Scientific), where the libraries are sequenced. Raw sequencing data were processed and analyzed using the Torrent Suite Software (Thermo Fisher Scientific). A list of detected sequence variants, including single nucleotide polymorphisms and small insertions/deletions, were subsequently imported into the Ion Reporter™ Software (Thermo Fisher Scientific) for interpretation. Alignments were visually verified with the Integrative Genomics Viewer (IGV, USA) v2.3. Run metrics and coverage analyses were performed to identify technical deficiencies.

We used two different customized gene panels[26]: Panel I (26.75 Kb) – with 8 targets and 138 amplicons to analyze sample 1 (Mucopolysaccharidosis type 1); and Panel II (13.1 Kb) – with 4 targets and 72 amplicons to analyze samples 2 (Pompe disease) and 3 (Fabry disease). Because of internal planning and budgeting, sample 1 was analyzed in 2 separate runs. It was first sequenced with DNA extracted from 6 3-mm punches and then with DNA extracted from 3 3-mm punches. Samples 2 and 3 were sequenced in the same run using both 6 and 3 punches.

Ethical approval

This study was approved in 2019 by HCPA's Ethics Committee (Project: 19-0754).

RESULTS: EXTRACTED DNA EVALUATION

DNA yield and quality

An extraction efficiency of 63.6% was achieved – mean 6.62 ng/μL with the DNA extracted from 3 3-mm punches, provided by Qubit® dsDNA HS (Invitrogen, Thermo Fisher Scientific). A mean concentration of 16.6 ng/μL was provided by NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific). Purity was approximately 1.8 (A260/A280 nm ratio), indicating good quality of the extracted DNA (Figure 1). The quality of the DNA was also verified by its ability to be amplified by different molecular assays, as reported below.

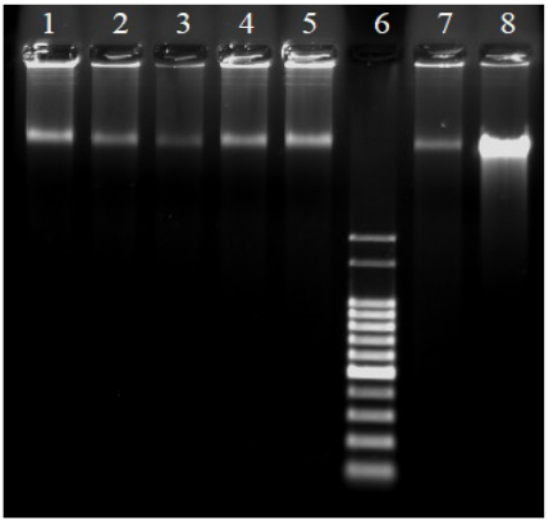

Figure 1: Quality of genomic DNA assessed by 0.8% agarose gel.

Lane 1-5: DNA extraction using the standardized organic method (3 3-mm punches); Lane 7: DNA extraction using the previous internally validated organic method (6 3-mm punches); Lane 8: DNA extraction from whole blood. Lane 1: 34.4 ng; Lane 2: 22.6 ng; Lane 3: 18.05 ng; Lane 4: 34.4 ng; Lane 5: 34.2 ng; Lane 6: 100 bp mass ladder (Invitrogen™, USA); Lane 7: 24.5 ng; Lane 8: 35.0 ng.

Molecular biology techniques performed

All samples had amplified PCR products, with recognizable bands of the different targeted genomic regions. Figure 2 shows the amplification of different PCR products from 1315 bp to 297 bp in the *IDS* and *TPP1* genes, respectively, comparing the amplification of a PCR product obtained by amplification of DNA extracted from DBS using 6 punches and DNA extracted from whole blood (WB), which is the gold standard. Figure 3 shows the *Hinf1* digestion of the intron 7 of the *IDS* gene by RFLP. Figure 4 shows BigDye sequence analysis for the beginning of exon 8 of the *TPP1* gene, which is a homopolymeric region of difficult amplification.

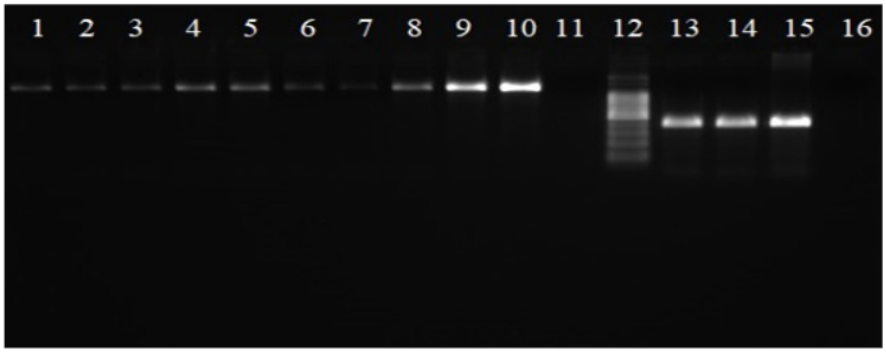

Figure 2: Quality of DNA extracted from 3 punches of dried blood spots (DBS) compared with 6 punches and whole blood (WB), assessed by 1.5% agarose gel.

Lane 1-7: *IDS*, intron 7 (1315 bp), with DNA from 3 punches; Lane 8: *IDS*, intron 7 (1315 bp), with DNA from 6 DBS punches; Lane 9-10: *IDS*, intron 7 (1315 bp), with DNA from WB; Lane 12: 100 bp ladder (Invitrogen™); Lane 13: *TPP1*, exon 8 (297 bp), with DNA from 3 punches; Lane 14: *TPP1*, exon 8 (297 bp), with DNA from 6 punches; Lane 15: *TPP1*, exon 8 (297 bp), with DNA from WB; Lanes 11 and 16: no DNA template control.

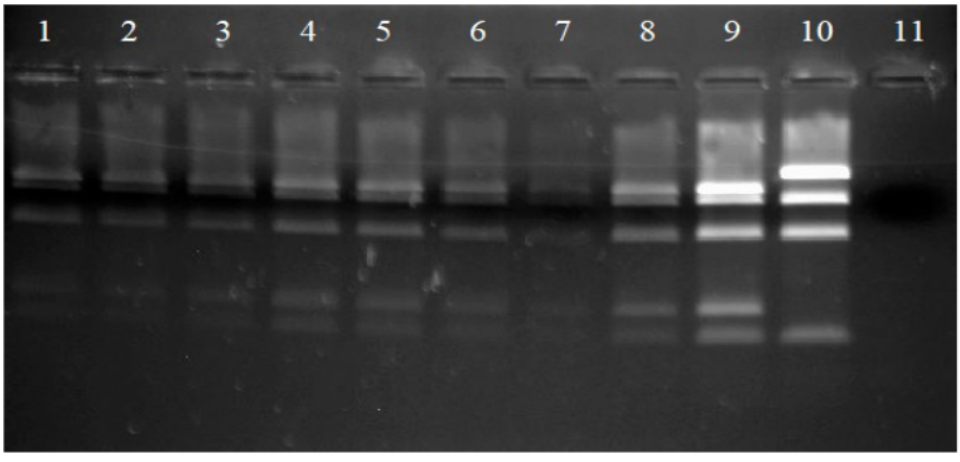

Figure 3: Restriction fragment length polymorphism products of the *IDS* gene assessed by 3.0% agarose gel.

Lane 1-7: DNA from dried blood spots (DBS) using 3 3-mm punches; Lane 8: DNA from DBS using 6 3-mm punches; Lane 9: DNA from whole blood (WB); Lane 10: DNA from WB showing a control; Lane 11: no DNA template control.

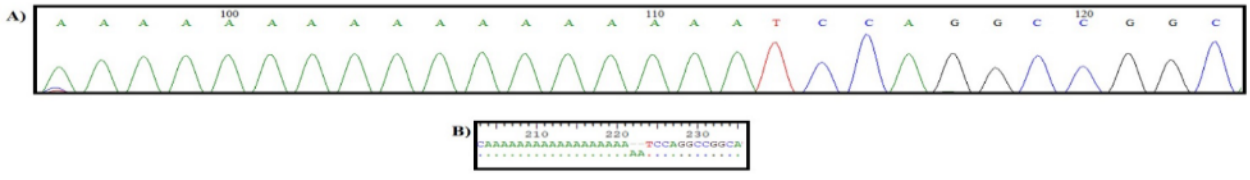

Figure 4: Sanger sequencing of exon 8 of *TPP1* gene using genomic DNA extracted from 3 dried blood spot punches.

A: BigDye sequence analysis showing the chromatogram peaks of the homopolymeric region in exon 8 of the *TPP1* gene (amplicon size = 297 bp); B: BigDye sequence analysis showing alignment with the reference sequence. Polymerase chain reaction products were purified with ExoSAP-IT™ (Thermo Fisher Scientific) and cycle sequenced according to standard manufacturer recommendations.

Targeted next generation sequencing (TNGS)

Results from TNGS have shown good sequencing data, adequate depth coverage for variant calling, and acceptable concordance between the extractions from the same DBS sample using 6 and 3 3-mm punches (Table 1). Furthermore, TNGS using DNA obtained from DBS has shown high similarity with results obtained from TNGS using DNA extracted from WB. The mean coverage of DBS samples was 195.88; likewise, the mean coverage of WB samples was 200.71. These values were obtained using a 100× coverage of the amplicons.

Table 1: Targeted next-generation sequencing results from the Ion Torrent Reporter™.

	Sample 1		Sample 2		Sample 3	
	6 punches	3 punches	6 punches	3 punches	6 punches	3 punches
Number of mapped reads	56,312	118,058	29,904	21,481	66,679	35,520
On-target reads, %	36.41%	27.73%	98.57%	98.96%	97.00%	95.51%
Average base cover depth	150.7	233.3	401.3	290.2	895.6	451.1
Uniformity of base coverage, %	94.24%	95.42%	91.41%	91.92%	98.99%	98.88%

DISCUSSION

Extraction of pure, intact, and double-stranded DNA is necessary for successful and reliable downstream molecular applications[27]. Peripheral blood is the preferred material for DNA extraction regarding the type of collection matrix; however, when collection is not feasible, blood can be collected as dried spots on filter paper[28], as in NBS, in which DBS sampling by heel prick is the gold standard.

Several methods for DNA isolation from DBS have been described[5,19-20,28-30], overcoming the limitations presented by this type of sampling. Some of the limitations include the low amount of DNA available in the sample, which may hamper quantification of extracted DNA, making it difficult to standardize the amount of DNA per reaction[5]. Additionally, the chemicals included in some protocols, such as phenol and salt, can be a source of contamination and cause problems if not removed properly, since they are considered PCR assay inhibitors[19,28]. When considering blood amount or viscosity, cold or dehydrated patients may lead to unequal saturation of the filter paper, which in turn may lead to an imprecise estimation of starting volume from a DBS punch[31]. Open-air drying before storage may also be a source of contamination, and drying may be impacted by surrounding temperature and humidity conditions. Open-air drying also precludes the retention of volatile organic compounds, which are often lost during drying; besides, even though most of the pathogens are inactivated by drying, some pathogens remain active for a few days, such as dengue, hepatitis B, and group A streptococci[31]. Additionally, the general recommendation is to store samples at −20°C to prevent DNA degradation[32]. However, when the budget is considered, which is the case of our laboratory, storing DBS at room temperature provides economic benefits like reduced cost and space requirements[30]. Despite these limitations, DBS sampling allows for convenient transportation, without the need for refrigeration, which facilitates transportation compared with WB, which requires refrigeration and biological material safety conditions.

To this extent, the implementation of an efficient and cost-effective standardized DNA extraction method is crucial for the molecular diagnosis of genetic diseases in routine laboratories like ours. Considering the dynamics of our center, in which enzymatic assays are performed first and samples are received from several places, including very distant places (making it more difficult to collect more DBS samples), a DNA extraction method that could be adapted to the minimum volume of sample available and provide suitable DNA to perform molecular assays should be developed. Several commercial kits are available[28], and some of them have been tested in comparison with the organic extraction method, indicating that the Chelex-100 kit would be the optimal choice for DNA extraction[33]. However, considering the reality of our laboratory, buying commercial kits is expensive, given that our demand does not support a large-scale purchase of these kits.

Some studies have shown that different modified phenol-chloroform methods yielded suitable amounts of DNA from several sample types[19,34-37]. In this study, standardization of a phenol-chloroform extraction method was evaluated for effectiveness and efficiency in extracting and purifying DNA from DBS using a minimum sample amount, equivalent to 3 3-mm punches.

The DNA concentration obtained from most DBS samples was 6.62 ng/µL. We observed a good quality of DNA, which was not degraded after undergoing vortexing, repeated pipetting, and other forms of mechanical stress (Figure 1). The housekeeping gene *GAPDH* was used as an internal control for normalization of the gDNA obtained by the standardized organic extraction method, which obtained amplified PCR products, indicating that the DNA is amplifiable for specific short regions (data not shown). Neduvat et al.[38] used the coagulation factor XIII (*F13*) gene as an internal control for normalization of gDNA and cited some of the housekeeping genes that are frequently used as internal control in mammalian cells: *GAPDH*, β-actin, *β2-microglobulin*, *cyclooxygenase 1*, *hypoxanthine phosphoribosyltransferase 1*, *glucose-6-phosphate dehydrogenase*, *cyclophilin A*, *tubulin*, *transferrin receptor*, and *18S ribosomal RNA*.

Different studies have demonstrated the possibility of obtaining DNA with sufficient quality and quantity from DBS for use in different molecular techniques[11,12,14,20,39]. The molecular processes assessed in this study (conventional PCR followed by RFLP, conventional PCR followed by Sanger sequencing, and TNGS) produced similar results to those obtained from DNA isolated from WB (Figures 2-4).

Regarding TNGS, which was used to prove the clinical applicability of the standardized extraction method, although the data obtained from the sequencing performed using 3 punches of DBS appeared to be inferior to the data obtained from the sequencing performed using 6 punches of DBS (Table 1), the same variants were identified in all samples. This indicates that the DNA extracted by the standardized method can be used for TNGS and provide molecular diagnosis. Clearly, sample 1 demonstrated low run metrics values; this is because it is a fragmented, poor-quality sample. However, it was still possible to amplify the sample, conduct TNGS, and identify the molecular variants related to the disease according to the biochemical pattern previously obtained.

Our laboratory has employed this method for more than 3 years to extract DNA using 6 3-mm punches as internal validation, mainly for TNGS for LSD, allowing molecular diagnosis. The opportunity to standardize this method using a minimum amount of sample and still reach a molecular diagnosis is an important achievement, given that these molecular assays are currently used as second-tier tests.

NBS programs based on biochemical methods are progressively adding molecular tests as second-tier tests, generating the need for a reliable, inexpensive, and practical method for DNA extraction from DBS[20]. In addition, the possibility of including lysosomal diseases in Brazil's NBS program emphasizes the demand for an optimal DNA extraction method that requires small sample amounts. As previously stated, the application of a DBS-based TNGS assay for the precise and rapid diagnosis of inborn errors of metabolism, which include LSD, is viable, efficient, and beneficial[12,40,41].

In summary, this study has shown that the organic method of DNA extraction from DBS using only 3 3-mm punches provides an acceptable yield of DNA concentration and purity, allowing its use for TNGS analysis. This method combines simplicity and cost effectiveness and is a suitable alternative for reference laboratories in low- and middle-income countries.

Funding

This work was funded by the FIPE/HCPA (Project number 19-0754). DRM received a scholarship from Innovate Peru (N°073-FINCYT-BDE-2014). FK received a scholarship from CAPES, Brazil. We also acknowledge the support from INAGEMP (grants CNPq 465549/2014-4, CAPES 88887.136366/2017-00, FAPERGS 17/2551-0000521-0).

REFERENCES

1. Guthrie R, Susi A. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. *Pediatrics*. 1963;32:338-43.
2. Smit PW, Elliott I, Peeling RW, Mabey D, Newton PN. An overview of the clinical use of filter paper in the diagnosis of tropical diseases. *Am J Trop Med Hyg*. 2014;90(2):195-210.
3. Knudsen RC, Slazyk WE, Richmond JY, Hannon WH. Guidelines for the shipment of dried blood spot specimens. *Infant Screen*. 1993;16(1):1-3.
4. Lakshmy R. Analysis of the use of dried blood spot measurements in disease screening. *J Diabetes Sci Technol*. 2008;2(2):242-3.
5. Karthipan SN, George E, Jameela S, Lim WF, Teh LK, Lee TY, et al. An assessment of three noncommercial DNA extraction methods from dried blood spots for beta-thalassaemia mutation identification. *Int J Lab Hematol*. 2011;33(5):540-4.
6. Clinical and Laboratory Standards Institute. *Blood collection on filter*

paper for newborn screening programs; approved standard. 6th ed. Wayne: CLSI; 2013.

7. Pollard L, Huang R, Wood T. Increased utility of dried blood spots in clinical diagnostic testing. *Mol Genet Metab*. 2018;123(2):S120.

8. Boppana SB, Ross SA, Novak Z, Shimamura M, Tolan RW Jr, Palmer AL, et al. Dried blood spot real-time polymerase chain reaction assays to screen newborns for congenital cytomegalovirus infection. *JAMA*. 2010;303(14):1375-82.

9. Sørensen KM, Agergaard P, Olesen C, Andersen PS, Larsen LA, Østergaard JR, et al. Detecting 22q11.2 deletions by use of multiplex ligation-dependent probe amplification on DNA from neonatal dried blood spot samples. *J Mol Diagn*. 2010;12(2):147-51.

10. Er TK, Kan TM, Su YF, Liu TC, Chang JG, Hung SY, et al. High-resolution melting (HRM) analysis as a feasible method for detecting spinal muscular atrophy via dried blood spots. *Clin Chim Acta*. 2012;413(21-22):1781-5.

11. Lefterova MI, Shen P, Odegaard JI, Fung E, Chiang T, Peng G, et al. Next-generation molecular testing of newborn dried blood spots for cystic fibrosis. *J Mol Diagnostics*. 2016;18(2):267-82.

12. Qian J, Wang X, Liu J, Zhong J, Le Y, Tellier LCAM, et al. Applying targeted next generation sequencing to dried blood spot specimens from suspicious cases identified by tandem mass spectrometry-based newborn screening. *J Pediatr Endocrinol Metab*. 2017;30(9):979-88.

13. Hollegaard MV, Grauholm J, Børglum A, Nyegaard M, Nørgaard-Pedersen B, Ørntoft T, et al. Genome-wide scans using archived neonatal dried blood spot samples. *BMC Genomics*. 2009;10:297.

14. Boemer F, Fasquelle C, d'Otreppe S, Josse C, Dideberg V, Segers K, et al. A next-generation newborn screening pilot study: NGS on dried blood spots detects causal mutations in patients with inherited metabolic diseases. *Sci Rep*. 2017;7(1):17641.

15. Bassaganyas L, Freedman G, Vaka D, Wan E, Lao R, Chen F, et al. Whole exome and whole genome sequencing with dried blood spot DNA without whole genome amplification. *Hum Mutat*. 2018;39(1):167-71.

16. Wang TY, Wang L, Zhang JH, Dong WH. A simplified universal genomic DNA extraction protocol suitable for PCR. *Genet Mol Res*. 2011;10(1):519-25.

17. Shams SS, Vahed SZ, Soltanzad F, Kafil V, Barzegari A, Atashpaz S, et al. Highly effective DNA extraction method from fresh, frozen, dried and clotted blood samples. *BioImpacts*. 2011;1(3):183-7.

18. Chaisomchit S, Wichajarn R, Chowpreecha S, Chareonsiriwatana W. A simple method for extraction and purification of genomic DNA from dried blood spots on filter paper. *Southeast Asian J Trop Med Public Health*. 2003;34(3):641-5.

19. Hue NT, Chan NDH, Phong PT, Linh NTT, Giang ND. Extraction of human genomic DNA from dried blood spots and hair roots. *Int J Biosci Biochem Bioinform*. 2012;2(1):21-6.

20. Saavedra-Matiz CA, Isabelle JT, Biski CK, Duva SJ, Sweeney ML, Parker AL, et al. Cost-effective and scalable DNA extraction method from dried blood spots. *Clin Chem*. 2013;59(7):1045-51.

21. Thompson J, Kibler J, Kupferschmid T. *Comparing five forensic DNA extraction methods* [Internet]. Salt Lake City: Sorenson Forensics; 2009 [cited 2022 Dec 13]. Available from: http://www.uniscience.co.kr/ZyGEM/Comparing%20Five%20Forensic%20DNA%20Extraction%20Methods.pdf

22. Giugliani R, Federhen A, Michelin-Tirelli K, Riegel M, Burin M. Relative frequency and estimated minimal frequency of lysosomal storage diseases in Brazil: report from a reference laboratory. *Genet Mol Biol*. 2017;40(1):31-9.

23. GE Healthcare. *Reliable extraction of DNA from Whatman™ FTA™ cards (28-9822-22 AA)* [Internet]. Chicago: GE Healthcare; 2010 [cited 2022 Dec 13]. Available from: https://beta-static.fishersci.com/content/dam/fishersci/en_EU/promotions/GEHealthcareLifeSciences/Whatman_Extraction_FTA.pdf

24. Futerman AH, van Meer G. The cell biology of lysosomal storage disorders. *Nat Rev Mol Cell Biol*. 2004;5(7):554-65.

25. Ballabio A. The awesome lysosome. *EMBO Mol Med*. 2016;8:73-76.

26. Málaga DR, Brusius-Facchin AC, Siebert M, Pasqualim G, Saraiva-Pereira ML, Souza CFM, et al. Sensitivity, advantages, limitations, and clinical utility of targeted next-generation sequencing panels for the diagnosis of selected lysosomal storage disorders. *Genet Mol Biol*. 2019;42(1 Suppl 1):197-206.

27. Psifidi A, Dovas CI, Bramis G, Lazou T, Russel CL, Arsenos G, et al. Comparison of eleven methods for genomic DNA extraction suitable for large-scale whole-genome genotyping and long-term DNA banking using blood samples. *PLoS One*. 2015;10(1):e0115960.

28. Hue NT, Phong PT, Chan NDH, Hoan NKH, Thuy HTT. An efficiency human genomic DNA extraction from dried blood spots. *Procedia Environ Sci*. 2011;8:179-85.

29. Fischer A, Lejczak C, Lambert C, Servais J, Makombe N, Rusine J, et al. Simple DNA extraction method for dried blood spots and comparison of two PCR assays for diagnosis of vertical human immunodeficiency virus type 1 transmission in Rwanda. *J Clin Microbiol*. 2004;42(1):16-20.

30. Choi EH, Lee SK, Ihm C, Sohn YH. Rapid DNA extraction from dried blood spots on filter paper: potential applications in biobanking. *Osong Public Heal Res Perspect*. 2014;5(6):351-7.

31. Freeman JD, Rosman LM, Ratcliff JD, Stricklan PT, Graham DR, Silbergeld EK. State of the science in dried blood spots. *Clin Chem*. 2018;64(4):659-79.

32. Sjöholm MIL, Dillner J, Carlson J. Assessing quality and functionality of DNA from fresh and archival dried blood spots and recommendations for quality control guidelines. *Clin Chem*. 2007;53(8):1401-7.

33. Panda BB, Meher AS, Hazra RK. Comparison between different methods of DNA isolation from dried blood spots for determination of malaria to determine specificity and cost effectiveness. *J Parasit Dis*. 2019;43(3):337-42.

34. Zhang BW, Li M, Ma LC, Wei FW. A widely applicable protocol for DNA isolation from fecal samples. *Biochem Genet*. 2006;44(11-12):503-12.

35. Ghatak S, Muthukumaran RB, Nachimuthu SK. A simple method of genomic DNA extraction from human samples for PCR-RFLP analysis. *J Biomol Tech*. 2013;24(4):224-31.

36. Barnett R, Larson G. A phenol-chloroform protocol for extracting DNA from ancient samples. *Methods Mol Biol*. 2012;840:13-9.

37. Siembieda S, Pocernich C, Chittur S, Knudtson K. Comparison of genomic DNA extraction methods. *J Biomol Tech*. 2020;31(Suppl):S11.

38. Neduvat AC, Murthy PM, Sundarrajan S, Padmanabhan S. Use of coagulation factor XIII (F13) gene as an internal control for normalization of genomic DNA's for HLA typing. *MethodsX*. 2018;5:881-9.

39. Bhattacharjee A, Sokolsky T, Wyman SK, Reese MG, Puffenberger E, Strauss K, et al. Development of DNA confirmatory and high-risk diagnostic testing for newborns using targeted next-generation DNA sequencing. *Genet Med*. 2015;17(5):337-47.

40. Park KJ, Park S, Lee E, Park JH, Park JH, Park HD, et al. A population-based genomic study of inherited metabolic diseases detected through newborn screening. *Ann Lab Med*. 2016;36(6):561-72.

41. van Campen JC, Sollars ESA, Thomas RC, Bartlett CM, Milano A, Parker MD, et al. Next generation sequencing in newborn screening in the United Kingdom National Health Service. *Int J Neonatal Screen*. 2019;5(4):40.

Received: Jun 24th, 2021
Accepted: May 16th, 2022