Methods applied to neonatal dried blood spot samples for secondary research purposes

a scoping review

Taylor & Francis
Taylor & Francis Group

Critical Reviews in Clinical Laboratory Sciences

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/ilab20

Methods applied to neonatal dried blood spot samples for secondary research purposes: a scoping review

Jordan Canning, Rona J. Strawbridge, Zosia Miedzybrodzka, Riccardo E. Marioni, Mads Melbye, David J. Porteous, Matthew E. Hurles, Naveed Sattar, Cathie L. M. Sudlow, Rory Collins, Sandosh Padmanabhan & Jill P. Pell

To cite this article: Jordan Canning, Rona J. Strawbridge, Zosia Miedzybrodzka, Riccardo E. Marioni, Mads Melbye, David J. Porteous, Matthew E. Hurles, Naveed Sattar, Cathie L. M. Sudlow, Rory Collins, Sandosh Padmanabhan & Jill P. Pell (10 Jun 2024): Methods applied to neonatal dried blood spot samples for secondary research purposes: a scoping review, Critical Reviews in Clinical Laboratory Sciences, DOI: 10.1080/10408363.2024.2360996

To link to this article: https://doi.org/10.1080/10408363.2024.2360996

CRITICAL REVIEWS IN CLINICAL LABORATORY SCIENCES
https://doi.org/10.1080/10408363.2024.2360996

Taylor & Francis
Taylor & Francis Group

INVITED REVIEW

OPEN ACCESS Check for updates

Methods applied to neonatal dried blood spot samples for secondary research purposes: a scoping review

Jordan Canning[a], Rona J. Strawbridge[a,b], Zosia Miedzybrodzka[c,d], Riccardo E. Marioni[e], Mads Melbye[f,g,h,i], David J. Porteous[e], Matthew E. Hurles[j], Naveed Sattar[k], Cathie L. M. Sudlow[l,m], Rory Collins[n], Sandosh Padmanabhan[k] and Jill P. Pell[a]

[a]School of Health & Wellbeing, University of Glasgow, Glasgow, UK; [b]Division of Cardiovascular Medicine, Karolinska Institute, Stockholm, Sweden; [c]Department of Medical Genetics, Ashgrove House, NHS Grampian, Aberdeen, UK; [d]Medical Genetics Group, School of Medicine, Medical Sciences, Nutrition and Dentistry, University of Aberdeen, Aberdeen, UK; [e]Centre for Genomic & Experimental Medicine, Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, UK; [f]Danish Cancer Institute, Copenhagen, Denmark; [g]Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark; [h]Department of Genetics, Stanford University School of Medicine, Stanford, CA, USA; [i]K.G. Jebsen Center for Genetic Epidemiology, Norwegian University of Science and Technology, Trondheim, Norway; [j]Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridge, UK; [k]School of Cardiovascular & Metabolic Health, University of Glasgow, Glasgow, UK; [l]Usher Institute, University of Edinburgh, Edinburgh, UK; [m]Health Data Research UK, London, UK; [n]Clinical Trial Service Unit, Nuffield Department of Population Health, University of Oxford, Oxford, UK

ABSTRACT

This scoping review aimed to synthesize the analytical techniques used and methodological limitations encountered when undertaking secondary research using residual neonatal dried blood spot (DBS) samples. Studies that used residual neonatal DBS samples for secondary research (i.e. research not related to newborn screening for inherited genetic and metabolic disorders) were identified from six electronic databases: Cochrane Library, Cumulative Index to Nursing and Allied Health Literature (CINAHL), Embase, Medline, PubMed and Scopus. Inclusion was restricted to studies published from 1973 and written in or translated into English that reported the storage, extraction and testing of neonatal DBS samples. Sixty-seven studies were eligible for inclusion. Included studies were predominantly methodological in nature and measured various analytes, including nucleic acids, proteins, metabolites, environmental pollutants, markers of prenatal substance use and medications. Neonatal DBS samples were stored over a range of temperatures (ambient temperature, cold storage or frozen) and durations (two weeks to 40.5 years), both of which impacted the recovery of some analytes, particularly amino acids, antibodies and environmental pollutants. The size of DBS sample used and potential contamination were also cited as methodological limitations. Residual neonatal DBS samples retained by newborn screening programs are a promising resource for secondary research purposes, with many studies reporting the successful measurement of analytes even from neonatal DBS samples stored for long periods of time in suboptimal temperatures and conditions.

Abbreviations: DBS: Dried blood spot; DNA: Deoxyribonucleic acid; gDNA: Genomic deoxyribonucleic acid; HSA: Human serum albumin; mRNA: Messenger ribonucleic acid; miRNA: Micro ribonucleic acid; PEth: Phosphatidylethanol; PFC: Polyfluoroalkyl chemical; PKU: Phenylketonuria; RNA: Ribonucleic acid; USA: United States of America; wgaDNA: Whole genome amplified deoxyribonucleic acid

ARTICLE HISTORY
Received 29 January 2024
Revised 22 April 2024
Accepted 24 May 2024

KEYWORDS
Dried blood spots; neonatal; archived; residual; scoping review

Introduction

Neonatal dried blood spot (DBS) screening is routinely carried out within the first few days following birth to detect inherited disorders, including sickle cell disease, cystic fibrosis and congenital hypothyroidism, along with inborn errors of metabolism, such as phenylketonuria (PKU) [1]. Early detection of these serious, but potentially treatable disorders, enables treatment to be commenced before symptom-onset [2]. The process was first described in the early 1960s, when Robert Guthrie demonstrated that phenylalanine concentrations could be measured in neonates using a heel prick blood sample dried on standardized filter paper, providing a simple and convenient diagnostic tool for PKU [3]. Developed countries across the world have

CONTACT Jordan Canning Jordan.Canning@glasgow.ac.uk School of Health & Wellbeing, University of Glasgow, Clarice Pears Building, 90 Byres Road, Glasgow G12 8TB, UK

Supplemental data for this article can be accessed online at https://doi.org/10.1080/10408363.2024.2360996.

since adopted this methodology, establishing comprehensive neonatal screening programs capable of screening for over 50 conditions [1,4].

Following their initial use for screening, neonatal DBS samples are typically retained in storage for possible future clinical or quality control purposes [5]. However, they are also a potential resource for research. Repositories of neonatal DBS samples represent demographically and geographically diverse populations that are useful resources for epidemiology and population-based studies [6]. In contrast, adult cohorts are not typically representative of the general population due to selection and survival bias [7]. Analysis of residual neonatal DBS samples therefore offers a unique opportunity to capture and assess the effects of early life exposures in all individuals, including those who die early and "hard to reach" groups.

The retention and use of residual neonatal DBS samples is subject to ethical and legal considerations, particularly surrounding parental and individual consent [8]. In Denmark, residual neonatal DBS samples can be legally stored without explicit informed consent. However, later use for secondary research purposes is subject to parental consent, although this requirement may be waived by the Research Ethics Committee if the samples are anonymized [9]. Meanwhile, many states in the United States of America (USA) have implemented consent processes for the retention and use of neonatal DBS samples for research purposes, following a lawsuit in Texas that saw five million archived neonatal DBS samples destroyed on the grounds of violating constitutional privacy rights [6]. Nevertheless, public support for the use of DBS samples for secondary research purposes appears high, with the proportion of individuals opting out of DBS sample storage reported to be less than 0.1% in Denmark [9].

A scoping review, published in 2019, reported that residual neonatal DBS samples have been a well-utilized resource for secondary research, with the number of published studies increasing annually [10]. The review further reported that the secondary research uses extended beyond the diseases included in current neonatal screening programs, with analytes, deoxyribonucleic acid (DNA) and enzymes being the main target categories of investigation. However, there is currently no synthesis of the methods that have been used to analyze residual neonatal DBS samples for secondary research purposes (unrelated to newborn screening programs) and their level of success. This information is essential to inform the future storage and use of samples. Therefore, the objectives of this scoping review of the published literature were to ascertain the storage, extraction and testing methodologies used, the analytes measured and any limitations encountered when using residual neonatal DBS samples for secondary research purposes.

Methods

A scoping review of published studies was conducted. The scoping review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-analyses extension for Scoping Reviews (PRISMA-ScR) guidelines [11]. Six electronic databases were searched: Cochrane Library (Wiley; 1996-present), Cumulative Index to Nursing and Allied Health (CINAHL; EBSCOhost; 1981-present), Embase (Ovid; 1947-present), Medline (Ovid; 1966-present), PubMed (United States National Library of Medicine; 1996-present) and Scopus (Elsevier; 1996-present) on 30 August 2023. Databases were searched individually using an adapted search strategy to account for differing subject index terms and keywords used across databases. Variations of search terms used covered the following key concepts: (1) dried bloodspots, Guthrie cards or heel prick tests and (2) archived, residual, stored or biobank samples and (3) newborn, neonates or infants. Advanced search features, including multi-field searches, operators, truncations/wildcards, limits and Boolean terms were used as appropriate. Different spellings, keyword variations, synonyms and related concepts were included to ensure that the search was comprehensive. The full search strategy, including the search terms used for each electronic database, can be found in Supplemental File S1.

Original studies of any design were eligible for inclusion if they described the storage, extraction or testing methods for conducting secondary research using neonatal DBS samples; were written in or translated into English; involved human participants only and were published as full manuscripts from 1973 onwards. Studies were excluded if they were conference abstracts; focused solely on newborn screening, quality improvement in newborn screening or extensions of newborn screening programs (e.g. improving existing screening tests or developing new screening tests) or focused on the immediate diagnosis or initiation of treatment for neonatal conditions. Studies were also excluded if they focused solely on legal, social or ethical issues relating to the use of DBS samples, such as data protection, consent/permission, policy or public opinions; used only samples obtained from umbilical cords or *via* venipuncture or DBS samples obtained solely from adults or animals.

Following the database searches, records were downloaded to EndNote 21 (Bld 17096, Clarivate) and duplicate records removed. The eligibility of all remaining studies was assessed using the literature review software, DistillerSR (DistillerSR Inc., Ontario, Canada). All studies were screened in duplicate against the predefined eligibility criteria by two independent reviewers (JC and RJS) during both the title/abstract and full text

screening stages. Reviewers (JC and RJS) adopted a "Yes-No-Unclear" approach when determining if studies met each eligibility criterion, as recommended by the Joanna Briggs Institute (JBI) framework for scoping reviews [12]. Thus, studies in which the inclusion/exclusion criteria could not be determined from the title and abstract alone (deemed "Unclear") were included in the full text screening stage for consideration. Disagreements/discrepancies between reviewers were resolved through discussion, with a third reviewer available for further consultation, if required. The level of agreement between reviewers (JC and RJS) was >90% for both title/abstract and full text screening levels. Consultation with a third member of the review team was not required.

The information extracted from eligible studies included the study location and design; storage of DBS samples (duration, temperature and humidity), number and size of DBS samples used in the analyses; the analytes measured and the purpose of measuring them; the laboratory methods used; any reported DBS-specific limitations relating to methods and key study conclusions.

Results

A total of 2,877 studies were identified from the electronic databases. Following the removal of 1,679 duplicates, 1,198 studies underwent title and abstract screening, with 1,061 of these studies excluded for failing to meet the eligibility criteria. Full-text articles were therefore screened for the remaining 137 studies and 67 of these studies were deemed eligible for inclusion in the scoping review (Figure 1) [13–79].

Included studies were published between 1993 and 2023 (Table 1). Thirty-one were conducted in the USA [13–16,22,24,28,29,36,37,40,42,44,45,48–52,54–58,63,66,68,69,71,72,77], 16 in Denmark [18,20,25,27,31–34,39,46,61,62,67,74–76], five in Sweden [19,23,30,41,59], five in the UK [17,26,35,43,65], three in Canada [38,64,

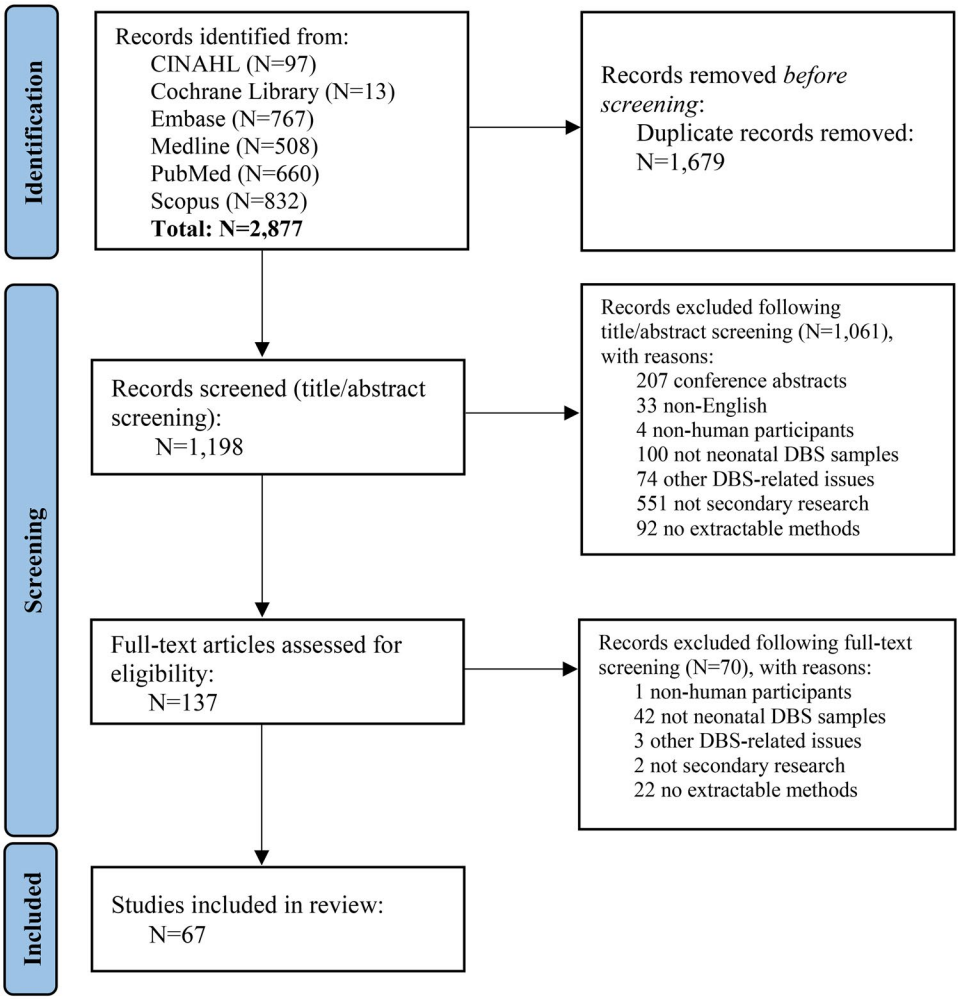

Figure 1. PRISMA 2020 flow diagram.
From: Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71. doi: 10.1136/bmj.n71.

Table 1. Key characteristics of included studies.

References	Study title	Location	Number of individuals	Study design
Makowski et al. [13]	The effect of storage on Guthrie cards: implications for deoxyribonucleic acid amplification	USA	Not stated	Methodological
Haak et al. [14]	Archived unfrozen neonatal blood spots are amenable to quantitative gene expression analysis	USA	12	Methodological
Resau et al. [15]	Evaluation of sex-specific gene expression in archived dried blood spots (DBS)	USA	106	Methodological
Slaughter et al. [16]	High correlations in gene expression between paired umbilical cord blood and neonatal blood of healthy newborns on Guthrie cards	USA	7	Methodological
Ponnusamy et al. [17]	A study of microRNAs from dried blood spots in newborns after perinatal asphyxia: a simple and feasible biosampling method	UK	30	Methodological
Bybjerg-Grauholm et al. [18]	RNA sequencing of archived neonatal dried blood spots	Denmark	10	Methodological
Gauffin et al. [19]	Quantitation of RNA decay in dried blood spots during 20 years of storage	Sweden	25	Methodological
Grauholm et al. [20]	Gene expression profiling of archived dried blood spot samples from the Danish Neonatal Screening Biobank	Denmark	20	Methodological
Chaisomchit et al. [21]	Stability of genomic DNA in dried blood spots stored on filter paper	Thailand	100	Methodological
Hardin et al. [22]	Whole genome microarray analysis, from neonatal blood cards	USA	24	Methodological
Klassen et al. [23]	Visual automated fluorescence electrophoresis provides simultaneous quality, quantity, and molecular weight spectra for genomic DNA from archived neonatal blood spots	Sweden	16	Methodological
Lane et al. [24]	Maximizing deoxyribonucleic acid yield from dried blood spots	USA	4,000	Methodological
Pedersen et al. [25]	The iPSYCH2012 case-cohort sample: new directions for unravelling genetic and environmental architectures of severe mental disorders	Denmark	77,639	Case-control
Rajatileka et al. [26]	Isolation of human genomic DNA for genetic analysis from premature neonates: a comparison between newborn dried blood spots, whole blood and umbilical cord tissue	UK	115	Methodological
Sjöholm et al. [27]	Assessing quality and functionality of DNA from fresh and archival dried blood spots and recommendations for quality control guidelines	Denmark	30	Methodological
Sok et al. [28]	Utilization of archived neonatal dried blood spots for genome-wide genotyping	USA	399	Methodological
St. Julien et al. [29]	High quality genome-wide genotyping from archived dried blood spots without DNA amplification	USA	1,773	Methodological
Hannelius et al. [30]	Phenylketonuria screening registry as a resource for population genetic studies	Sweden	76	Methodological
Hollegaard et al. [31]	Genome-wide scans using archived neonatal dried blood spot samples	Denmark	24	Methodological
Hollegaard et al. [32]	High-throughput genotyping on archived dried blood spot samples	Denmark	29	Methodological
Hollegaard et al. [33]	Genotyping whole-genome-amplified DNA from 3- to 25-year-old neonatal dried blood spot samples with reference to fresh genomic DNA	Denmark	29	Methodological
Hollegaard et al. [34]	Robustness of genome-wide scanning using archived dried blood spot samples as a DNA source	Denmark	4,641	Methodological
Auma et al. [35]	Using dried blood spots for a sero-surveillance study of maternally derived antibody against Group B Streptococcus	UK	450	Case-control
Mei et al. [36]	Effect of specimen storage conditions on newborn dried blood spots used to assess Toxoplasma gondii immunoglobulin M (IgM)	USA	858	Case-control
He et al. [37]	Metabolite stability in archived neonatal dried blood spots used for epidemiological research	USA	899	Methodological
Murphy et al. [38]	Metabolic profiles derived from residual blood spot samples: a longitudinal analysis	Canada	307	Methodological
Ottosson et al. [39]	Effects of long-term storage on the biobanked neonatal dried blood spot metabolome	Denmark	200	Methodological
Petrick et al. [40]	An untargeted metabolomics method for archived newborn dried blood spots in epidemiologic studies	USA	106	Methodological
Yu et al. [41]	Untargeted metabolomics profiling and hemoglobin normalization for archived newborn dried blood spots from a refrigerated biorepository	Sweden	15	Methodological
Asrani et al. [42]	DNA methylome profiling on the Infinium HumanMethylation450 Array from limiting quantities of genomic DNA from a single, small archived bloodspot	USA	1	Methodological
Cunningham-Burley et al. [43]	Feasibility and ethics of using data from the Scottish newborn blood spot archive for research	UK	56	Methodological
Ghantous et al. [44]	Optimized DNA extraction from neonatal dried blood spots: application in methylome profiling	USA	Not stated	Methodological
Ghantous et al. [45]	DNA methylation analysis from blood spots: increasing yield and quality for genome-wide and locus-specific methylation analysis	USA	Not stated	Methodological

(*Continued*)

Table 1. Continued.

References	Study title	Location	Number of individuals	Study design
Hollegaard et al. [46]	DNA methylome profiling using neonatal dried blood spot samples: a proof-of-principle study	Denmark	2	Methodological
Zar Kyaw et al. [47]	The utility of post-test newborn blood spot screening cards for epigenetic association analyses: association between HIF3A methylation and birth weight-for-gestational age	Japan	300	Methodological
Bakhireva et al. [48]	The validity of phosphatidylethanol in dried blood spots of newborns for the identification of prenatal alcohol exposure	USA	60	Case-control
Bakhireva et al. [49]	Stability of phosphatidylethanol in dry blood spot cards	USA	5	Prospective cohort
Henderson et al. [50]	Radioimmunoassay screening of dried blood spot materials for benzoylecgonine	USA	>500	Prospective cohort
Spector et al. [51]	Detection of cotinine in newborn dried blood spots	USA	20	Case-control
Yang et al. [52]	Levels of cotinine in dried blood specimens from newborns as a biomarker of maternal smoking close to the time of delivery	USA	428	Methodological
Di Martino et al. [53]	EDTA is essential to recover lead from dried blood spots on filter paper	Italy	20	Methodological
Funk et al. [54]	Hemoglobin adducts of benzene oxide in neonatal and adult dried blood spots	USA	9	Methodological
Funk et al. [55]	Quantification of arsenic, lead, mercury and cadmium in newborn dried blood spots	USA	49	Methodological
Kato et al. [56]	Analysis of blood spots for polyfluoroalkyl chemicals	USA	98	Methodological
Kim et al. [57]	Method for the determination of iodide in dried blood spots from newborns by high performance liquid chromatography tandem mass spectrometry	USA	20	Methodological
Ma et al. [58]	Analysis of polyfluoroalkyl substances and bisphenol A in dried blood spots by liquid chromatography tandem mass spectrometry	USA	192	Methodological
Björkesten et al. [59]	Stability of proteins in dried blood spot biobanks	Sweden	5	Methodological
Dijkstra et al. [60]	Important lessons on long-term stability of amino acids in stored dried blood spots	The Netherlands	2,170	Retrospective cohort
Klamer et al. [61]	Adiponectin levels measured in dried blood spot samples from neonates born small and appropriate for gestational age	Denmark	122	Case-control
McGuire et al. [62]	Screening newborns for candidate biomarkers of type 1 diabetes	Denmark	162	Case-control
Mihalopoulos et al. [63]	Validity and reliability of perinatal biomarkers of adiposity after storage as dried blood spots on paper	USA	45	Methodological
Mineyko et al. [64]	Association of neonatal inflammatory markers and perinatal stroke subtypes	Canada	197	Ambi-directional cohort
Raha-Chowdhury et al. [65]	Blood ferritin concentrations in newborn infants and the sudden infant death syndrome	UK	82	Case-control
Samenuk et al. [66]	Rapid method towards proteomic analysis of dried blood spots by MALDI mass spectrometry	USA	10	Methodological
Skogstrand et al. [67]	Simultaneous measurement of 25 inflammatory markers and neurotrophins in neonatal dried blood spots by immunoassay with xMAP technology	Denmark	66	Methodological
Yano et al. [68]	Untargeted adductomics of Cys34 modifications to human serum albumin in newborn dried blood spots	USA	49	Retrospective cohort
Yeung et al. [69]	Newborn adipokines and birth outcomes	UA	3,625	Cross-sectional
Durie et al. [70]	Quantification of DNA in neonatal dried blood spots by adenine tandem mass spectrometry	Canada	501	Methodological
Bassaganyas et al. [71]	Whole exome and whole genome sequencing with dried blood spot DNA without whole genome amplification	USA	24	Methodological
Ding et al. [72]	Scalable, high quality, whole genome sequencing from archived, newborn, dried blood spots	USA	29	Methodological
Nagy et al. [73]	Minimally invasive genetic screen for GJB2 related deafness using dried blood spots	Hungary	576	Methodological
Hollegaard et al. [74]	Archived neonatal dried blood spot samples can be used for accurate whole genome and exome-targeted next-generation sequencing	Denmark	2	Methodological
Poulsen et al. [75]	High-quality exome sequencing of whole-genome amplified neonatal dried blood spot DNA	Denmark	22	Methodological
Winkel et al. [76]	Whole-genome amplified DNA from stored dried blood spots is reliable in high resolution melting curve and sequencing analysis	Denmark	10	Methodological
Needham et al. [77]	Maternal social disadvantage and newborn telomere length in archived dried blood spots from the Michigan Neonatal Biobank	USA	192	Retrospective cohort
Barco et al. [78]	A validated LC-MS/MS method for the quantification of piperacillin/tazobactam on dried blood spot	Italy	7	Methodological
Rovito et al. [79]	Impact of congenital cytomegalovirus infection on transcriptomes from archived dried blood spots in relation to long-term clinical outcome	The Netherlands	18	Methodological

70], two in Italy [53,78], two in The Netherlands [60,79] and one each in Hungary [73], Japan [47] and Thailand [21]. All neonatal DBS samples used in studies were sourced from the country of study origin, apart from two USA-based studies that used neonatal DBS samples from both the USA and Australia [44,45] and a study based in Sweden that used samples sourced from both Sweden and Denmark [59]. The number of individuals from whom DBS samples were collected ranged from one to 77,639, with the number of individuals not clearly specified in three studies [13,44,45]. Studies were predominately methodological ($N = 52$) in nature [13–24,26–34,37–47,52–59,63,66,67,70–76,78,79], reporting either the development, validation or comparison of methods. Of the remainder, eight were case-control studies [25,35,36,48,51,61,62,65], six cohort studies [49,50,60,64,68,77] and one cross-sectional [69].

Storage conditions

The storage of neonatal DBS samples was highly variable across included studies, with the duration of storage ranging from two weeks to over 40 years (Table 2). Neonatal DBS samples were stored solely at room temperature in 28 studies [13–17,21–24,26,35,38,43–45,48,50,53,55,56,63–66,70,72,73,79] and in cold storage (4 °C) in eight [19,30,41,51,57,58,69,78]. Twenty-four studies used frozen DBS samples [18,25,29,31–34,38–40,42,46,47,49,52,54,61,62,67,68,71,74–76], with temperatures ranging from −20 °C to −80 °C. Two studies did not state the exact frozen temperature [42,54]. Storage conditions in three studies changed due to updated storage policies in the respective biobank facilities [28,59,60]. This largely involved moving samples to colder conditions and controlling humidity. Four studies compared the effects of storage temperature and duration by comparing frozen and room temperature-stored samples (Table 4) [20,27,36,77]. Humidity control was inconsistently reported across studies, with the majority of studies not reporting this aspect. Two studies simply stated that conditions were "humidity-controlled" [19,77], four reported the use of a desiccant to control humidity [36,71,72,78] and three studies provided humidity levels which ranged from <30% to 70% [21,28,59].

Extraction

The number and size of DBS samples used for testing varied. Six studies reported using one or more full DBS samples in their analyses [36,54,56,61,65,79] but the majority of studies used only part of the DBS sample. Most punched between one and 12 disks with diameters ranging from 1 mm to 16 mm (Table 2) [13–35,37–47,49,50,52,53,57,58,60,62–64,67–78]. One study reported using half the DBS sample [55] and two studies reported using one-quarter [51,66]. Two studies did not specify the number or size of DBS samples used [48,59].

Analytes

Of the 67 studies, 37 measured nucleic acids and nucleic acid types (Table 2). Fourteen measured DNA [13,22,24,25,27,28,44,45,47,70–73,77], eight whole genome amplified DNA (wgaDNA) [30–34,74–76], six RNA [14,15,18–20,79], four genomic DNA (gDNA) [21,23,26,29], three methylated DNA [42,43,46], one messenger RNA (mRNA) [16] and one microRNA (miRNA) [17]. These were measured for a variety of purposes, including DNA amplification, gene expression, genotyping, DNA methylation, DNA quantification, DNA sequencing, telomere length measurement and transcriptome analysis. Two studies measured maternally-derived antibodies; one against Group B Streptococcus [35] and the other against Toxoplasma Gondii [36]. Metabolomics were used in five studies to measure a range of 39–1,107 metabolites [37–41]. Six studies measured markers of prenatal exposure to substance use, including alcohol (phosphatidylethanol; PEth) [48,49], cocaine (benzoylecgonine) [50] and tobacco (cotinine [37,51,52] and Cys34 human serum albumin (HSA) adducts [68]). A further six studies measured analytes indicative of prenatal exposure to environmental pollutants, including lead [53,55], hemoglobin adducts of benzene oxide [54], arsenic [55], mercury [55], cadmium [55], polyfluoroalkyl chemicals (PFCs) [56,58] and iodide [57]. A variety of proteins were measured across studies, including oncology-related proteins [59], amino acids [37,41,60], cytokines/hormones [61,63,64,67,69], ferritin [65] and unspecified proteins [62,66]. The antibiotics, piperacillin and tazobactam, were also measured in one study for therapeutic drug monitoring purposes [78]. Studies were largely able to measure the analytes of interest using the methods described (Table 3), although a number of studies noted lower quantity and quality of some analytes as a result of the storage methods used (Table 2).

DNA and RNA appeared to be stable in neonatal DBS samples stored at differing temperatures for long periods of time, with storage at −20 °C seemingly improving the preservation of samples [20,27,77]. Proteins were shown to remain detectable after prolonged periods of storage, but storage temperature significantly impacted detectability. Proteins were better preserved when DBS samples were stored frozen, while storage at 4 °C resulted in the degradation of many proteins [59]. Amino acids stored at room temperature were subject to substantial degradation

Table 2. Summary of methods used in included studies.

Purpose	Analyte(s)	Storage		DBS puncture size	Method(s)	Limitation(s)	Conclusion(s)	References
		Duration	Temperature / Humidity					
Amplification	DNA	1–30 months	Room	1 × 3 mm	Water-based extraction method to elute whole blood PCR inhibitors (protein, hemoglobin, iron) and retain nucleic acid, followed by PCR by direct amplification.	Nucleic acid may become "fixed" to filter paper matrix. Eluting natural PCR inhibitors (protein, hemoglobin and iron) can be used to overcome this.	Possible to amplify two regions encoding cystic fibrosis mutation from DBS samples stored up to 30 months at room temperature.	Makowski et al. [13]
Gene expression	RNA	≤9 years	Room	8 × 3 mm	Parallel microarray-based gene expression and quantitative PCR analyses of RNA.	DNA contamination may occur when using small amounts of RNA. Treatment with RNase-free DNase recommended for future experiments.	RNA isolated and amplified from DBS samples archived at room temperature for up to 9 years can be analyzed by microarray and quantitative PCR.	Haak et al. [14]
	RNA	2.5–15.6 years	Room	3 × 3 mm	Samples were homogenized before total RNA extraction using the illustra RNAspin Mini RNA Isolation Kit.	None stated	Sex-specific RNA gene expression is preserved in DBS stored at room temperature up to 15.6 years.	Resau et al. [15]
	mRNA	5 months	Room	3 × 3 mm	RNA isolation, copy DNA synthesis, and microarray procedures were conducted according to published protocols.	None stated	Abundant gene expression observed in newborn blood on Guthrie cards.	Slaughter et al. [16]
	miRNA	6 months	Room	1 × 6 mm	Total RNA isolation was carried out using the MirVana PARIS isolation kit.	Circulating RNases may have degraded large RNAs into small RNAs.	Candidate miRNAs were extractable from DBS samples stored at room temperature for up to 6 months.	Ponnusamy et al. [17]
	RNA	Not stated	−20 °C	2 × 3.2 mm	Next generation sequencing for RNA expression.	RNA input for sequencing library preparation was restricted to two 3.2 mm DBS punches for each sample, lessening library output.	Feasible to use DBS for RNA extraction and expression studies.	Bybjerg-Grauholm et al. [18]
	RNA	1–20 years	4 °C (since 1981); controlled humidity (since 1996)	5 × 3 mm	RNA was isolated from each sample, quantitated by spectrophotometry and reverse transcribed following DNase I treatment. Amplifiable copy DNA was subsequently detected by real-time PCR using primers specific for transcripts encoding b-actin.	None stated	No evidence of significant RNA decay in DBS samples stored up to 20 years.	Gauffin et al. [19]
	RNA	12–30 years	−20 °C and Room	2 × 3.2 mm	RNA amplification, whole transcriptome amplification and gene expression profiling analysis.	Use of multiple whole transcriptome amplification kits may introduce significant differences in expression. The use of a single kit is recommended.	Length of storage is secondary to the storage temperature; no significant differences between samples when stored at −20 °C. Storing DBS samples at −20 °C improves the preservation of RNA.	Grauholm et al. [20]
Genotyping	gDNA	4–14 years	28 °C; 70% humidity; stored in sealed plastic bags	1 × 4.8 mm	DNA extraction method involved two steps: (1) DBS treated with methanol and (2) Genomic DNA extracted with Tris buffer in a heat incubation step.	Ambient conditions may not be appropriate for DBS storage for more than 10 years if studying long DNA sequences.	DNA in DBS samples are stable under tropical conditions for at least 10 years.	Chaisomchit et al. [21]
	DNA	≥10 years	Room	2 × 3 mm	GenSolve technology was used in combination with standard DNA purification, followed by analysis on the Illumina 610 bead array microarray platform.	None stated	The larger surface area of 2 × 3 mm dia. punches or 1 × 6 mm dia. punch may be more dependable to produce enough DNA for microarray analysis. High quality DNA can be obtained from standard neonatal DBS samples stored dry for at least 10 years.	Hardin et al. [22]

(*Continued*)

Table 2. Continued.

Purpose	Analyte(s)	Storage		DBS puncture size	Method(s)	Limitation(s)	Conclusion(s)	References
		Duration	Temperature / Humidity					
	gDNA	>30 years	Room	2×3 mm	Visual automated fluorescence electrophoresis (VAFE).	Lack of systematic quality control for sample preparation and assessment.	Addition of VAFE measures in downstream quality control increases confidence in the validity of genetic data and allows cost-effective downstream analysis of gDNA. DBS is a good resource for DNA for genotyping studies.	Klassen et al. [23]
	DNA	>10 years	Room	2×5 mm	Standard methods used for DNA extraction and purification with a modified elution step. Linear array method was used for HLA (human leukocyte antigen) genotyping.	Residual DBS samples are nonrenewable. Thus, DNA yield should be maximized.	Inclusion of modified elution step increased DNA yield. Commercial extraction protocols can be modified to maximize DNA yield from stored DBS samples.	Lane et al. [24]
	DNA	13–37 years	−20 °C	2×3.2 mm	DNA was extracted from neonatal DBS samples using the Extract-N-Amp Blood PCR kit and genotyped using the Illumina PsychChip.	No DBS-specific limitations stated	Genotyping was successful for 90% of DBS samples using described method.	Pedersen et al. [25]
	gDNA	3–22 years	Room; stored in boxes	1–3×3.2 mm	DNA isolated using the QIAmp DNA Micro Kit following manufacturer's guidelines.	None stated	Storage duration did not affect total amount of DNA recovered but significantly reduced purity over time.	Rajatileka et al. [26]
	DNA	3 months; 22 years; 26 years	−20 °C and Room	1×3 mm	EZNA method for DNA extraction, followed by multiple displacement amplification (MDA).	The degree of fragmentation of DNA extracted from archival DBS increased with increasing age of the samples.	Room temperature-stored DBS samples performed poorly (likely degradation of DNA during storage). DBS storage at −20 °C recommended to prevent DNA degradation.	Sjöholm et al. [27]
	DNA	9–25 years	21 °C, 35% humidity (for 12 years); room, 35% humidity (for 4 years)	6×3 mm	DNA extraction performed using GenTegra GenSolve Whole Blood DNA recovery kit according to manufacturer protocol.	Unable to evaluate quality of low frequency variants as in other genome-wide association studies due to limited sample size.	DBS samples stored in a temperature- and humidity-controlled environment yielded higher quality DNA. Storage duration negatively affected DNA quality regardless of environment. However, samples stored in an uncontrolled environment were acceptable for most genotyping applications.	Sok et al. [28]
	gDNA	Not specified	−20 °C	2–5×2 mm	Genome-wide genotyping of DNA isolated from DBS samples was done by Illumina using their Infinium single nucleotide extension SNP genotyping assay and a cytoSNP or Omni bead microarray.	None stated	The described method provides sufficient unamplified DNA for genome-wide genotyping.	St. Julien et al. [29]
	wgaDNA	10 years; 25 years	4 °C	1×3 mm	Saponin and chelex-100 based method and preamplified by improved primer preamplification.	None stated	Whole genome amplification makes large genetic epidemiological studies feasible, even using 25-year-old samples.	Hannelius et al. [30]

(*Continued*)

Table 2. Continued.

Purpose	Analyte(s)	Storage		DBS puncture size	Method(s)	Limitation(s)	Conclusion(s)	References
		Duration	Temperature / Humidity					
	wgaDNA	15–25 years	−24 °C	1–3×3.2 mm	DNA extraction and whole genome amplification methods using standard test kits.	Call-rate cutoff that would ensure reliable genotyping could not be clearly defined due to few samples being available to calculate correlation coefficient. Limited number of samples in each comparison group is a noted limitation.	WgaDNA from one 3.2 mm punch of DBS sample stored at −24 °C for more than 20 years is suited for reliable genotyping.	Hollegaard et al. [31]
	wgaDNA	19–27 years	−24 °C	1×3.2 mm	Genomic DNA was extracted and amplified using GPlex WGA kit (selected over AmpliQ Genomic Amplifier kit following pilot study).	The early storage policy, where DBS samples were stored at 4 °C for up to 1 year, may have caused some degradation of the gDNA.	WgaDNA from DBS samples for genotyping studies is feasible despite years of storage.	Hollegaard et al. [32]
	wgaDNA	3–25 years	−24 °C	1×3.2 mm	Extract-N-Amp kit and WGA reactions using GPlex kit.	WgaDNA made from DBSs may result in scattered genotype plots. This is probably due to poor quality and/or low amounts of input DNA resulting in an unequal amplification of the alleles.	Genotyping quality dependent upon extraction and amplification method. Storage duration did not appear to influence results. One 3.2 mm punch sufficient for multiple amplifications.	Hollegaard et al. [33]
	wgaDNA	5–29 years	−20 °C	2×3.2 mm	DNA extraction, whole genome amplification and SNP genotyping.	Sample set limited by difficulty obtaining neonatal-adult matched samples.	Next generation sequencing (NGS) of neonatal DBS samples does not compromise NGS accuracy. Archived DBS samples represent a reliable source of DNA for whole genome amplification. Two 3.2 mm DBS punches sufficient for whole genome amplification and subsequent sequencing.	Hollegaard et al. [34]
Maternally-derived antibodies	Invasive Group B Streptococcus	5 years	Room	2×3 mm	Multiplex immunoassay.	Poor sample storage conditions resulted in few viable DBS sample cards.	DBS storage at room temperature was stable for 3 months compared with storage from collection at −20 °C and rapidly degraded thereafter. Poor sample storage conditions resulted in few viable DBS samples. DBS samples must be stored at −20 °C for long term preservation of antibody.	Auma et al. [35]
	Toxoplasma gondii	3–13 years	−20 °C with desiccant; room temperature with no desiccant; −1 °C to 40 °C (no humidity control)	1 x full DBS (approx. 13 mm)	Time-resolved immunofluorometric assay.	Relatively small number of DBS samples tested. Due to limited number of punches available, not all analytes could be tested from the subset.	Ability to recover IgM and other biomarkers related to storage conditions, independent of sample age or storage time. When stored at −20 °C with desiccant, Toxo-IgM was found to be stable for at least 2 years in DBS.	Mei et al. [36]

(*Continued*)

Table 2. Continued.

Purpose	Analyte(s)	Storage		DBS puncture size	Method(s)	Limitation(s)	Conclusion(s)	References
		Duration	Temperature / Humidity					
Metabolomics	39 metabolites related to nutrition and health	~30 years	−20 °C	1×5 mm	High-resolution metabolomics with untargeted LC-MS/MS.	Metabolite detection may be affected by sample-handling issues during collection, transport, extraction, storage and analysis, duration of storage, lipid content and hematocrit impacting stability of metabolites during spot drying and delivery, resolubilization/loss of metabolites due to free-radical polymerization and protein trapping.	Storage at −20 °C for almost 30 years did not affect the deterioration of most of the metabolites. Stability over the period was seen for 17 of the 39 selected metabolites. Degradation appears minimal for amino acids, likely due to storage temperature. Caffeine was detected in 82% of samples indicating breastfeeding-related exposures. Cotinine and hydroxycotinine remain detectable in DBS after storage for up to 29 years. Lipid metabolites appeared less stable.	He et al. [37]
	48 metabolites	2–12 months	Room	8×3.2 mm	High performance liquid chromatography; Immunoassays; electrospray ionization tandem mass spectrometry; quantitative polymerase chain reaction.	Effects of extreme environmental storage conditions were not explored. Samples providing negative screening used. Unclear if analytes exhibit similar rates of degradation in infants with congenital conditions.	Largest changes in analyte levels were observed after 12 months of storage. Duration of sample storage, independent of temperature and humidity affects newborn screening profiles and should be considered when using DBS samples for research purposes.	Murphy et al. [38]
	731 metabolites	1–10 years	−20 °C	1×3.2 mm	Untargeted LC-MS/MS.	Short term effects of storage (< 1 year) not investigated. Transportation and storage time may influence metabolome.	Majority (>71%) of DBS metabolome is stable during storage at −20 °C for up to 10 years. Untargeted metabolomics of DBS samples, with long-term storage, is suitable for retrospective epidemiological studies.	Ottosson et al. [39]
	1,107 metabolites	9–32 years	−20 °C	1×4.7 mm	Untargeted LC-HRMS.	Nuisance technical variation may occur. Measuring potassium in each DBS punch as a proxy for hematocrit, used to adjust for this.	Method allowed >1000 prevalent and robust features representing all of the major subclasses of metabolites to be measured.	Petrick et al. [40]
	137 metabolites	≤21 years	4 °C	1×5 mm	LC-MS.	Small sample size (N=15). Unknown volumes of blood and hematocrit are noted issues. Further evaluations should utilize larger numbers of archived DBS and samples with extreme values of hematocrit.	39% of metabolite features were associated with storage year. Amino acids and peptides were particularly sensitive to storage duration.	Yu et al. [41]
Methylation	Methylated DNA	14–31 years	Frozen (temperature not stated)	1×2 mm	DNA methylation analysis on the Illumina 450k platform	None stated	DNA isolated from stored DBS sample yields methylation profiles adequate for methylation studies.	Asrani et al. [42]
	Methylated DNA	32–38 years	Room	6–8×3 mm	DNA methylation was profiled using the Infinium HumanMethylationEPIC v1.0 BeadChip.	None stated	Pilot studies demonstrated sufficient quantity and quality of DNA could be extracted for whole genome/exome sequencing from 94% of DBS cards. Genome-wide methylation data, along with baseline smoking behavior information, reflected maternal smoking.	Cunningham-Burley et al. [43]

(*Continued*)

Table 2. Continued.

Purpose	Analyte(s)	Storage		DBS puncture size	Method(s)	Limitation(s)	Conclusion(s)	References
		Duration	Temperature / Humidity					
	DNA	>20 years	Room	2×1 mm	10 different DNA extraction protocols were tested. GQ was found to be the most robust protocol. For GQ, Phase I method: GenSolve and Phase II method: QIAamp (DNA Micro kit). DNA quality was assessed by PCR (gel electrophoresis) and DNA integrity and size range (bioanalyser analyses).	None stated	GQ method was able to detect small methylation differences in a consistent manner and with low inter-replicate variation. This optimized method for DNA extraction has applications in methylation analyses for diagnostic and research purposes.	Ghantous et al. [44]
	DNA	>20 years	Room	2×1 mm	Lysis-based DNA extraction methods from DBS using combinations of in-house derived and modified commercial extraction kits were compared.	None stated	Both protocols were more efficient relative to other published methods, one protocol yields less DNA compared to the other, but shows improved quality.	Ghantous et al. [45]
	Methylated DNA	26–28 years	−20 °C	2×3.2 mm	DNA extraction and methylation, using standard kits and protocols.	None stated	Choice of DNA extraction method is important. Neonatal DBS samples stored for 26 to 28 years can be used for DNA methylome profiling, using only two 3.2 mm punches.	Hollegaard et al. 2013a [46]
	DNA	>3 years	−20 °C	8×3 mm	DNA bisulfite treatment, amplification, reverse transcription and cleavage, applied to a 384 SpectroCHIP, for mass-based fragment separation using a Matrix Assisted Laser Desorption-Ionization Time of Flight platform.	None stated	DBS samples retained sufficient quantity and quality of DNA to conduct both genome-wide and epigenome-wide studies.	Zar Kyaw et al. [47]
Prenatal substance use	Phosphatidyl -ethanol (PEth; ethanol metabolite)	2 weeks	Room	3 x size not specified	LC-MS/MS.	Unable to detect PEth beyond 2–3 weeks prior to delivery. No agreement regarding cutoff concentration for PEth when assessing prenatal alcohol exposure in newborns.	Specificity of PEth-DBS in this study was 100% and sensitivity was 32.1%, believed to reflect moderate alcohol consumption. DBS samples can be used to detect PEth for prenatal alcohol exposure analyses. The stability of PEth long-term remains unknown.	Bakhireva et al. [48]
	Phosphatidyl-ethanol (PEth; ethanol metabolite)	4 months	−80 °C	3×3.175 mm	LC-MS/MS.	Higher humidity can potentially accelerate PEth degradation, although not taken into account in study as DBS samples stored at −80 °C less likely to be affected by humidity.	Insignificant degradation of PEth after storage for 4 months at −80 °C. Can be used for prenatal alcohol exposure analyses but long-term stability of analyte is unknown.	Bakhireva et al. [49]
	Benzoylecgonine (BE; cocaine metabolite)	≥30 days	Room	1×6.35 mm	Modified commercial radioimmunoassay (RIA) kit.	None stated	DBS samples stored at ambient temperature and room humidity show no diminution in BE levels measured over 30 days.	Henderson et al. [50]
	Cotinine (tobacco-specific biomarker)	7 years	4 °C	¼ x DBS	GC-MS.	Factors that may affect cotinine levels in DBS include storage, handling and blood volume. It is also unclear whether cotinine may transfer between DBS stored adjacently without being individually sealed or whether handling of DBS by smokers could contaminate samples. Although study assumed a constant blood volume to calculate cotinine, in reality, the amount of blood may vary with spot size, punch location and filter paper batch.	Mean cotinine in newborn DBS of infants of smoking mothers was significantly higher than in those of infants with nonsmoking mothers (five-fold difference). The extent to which cotinine in DBS may degrade under improper storage conditions is not known.	Spector et al. [51]

(*Continued*)

Table 2. Continued.

Purpose	Analyte(s)	Storage		DBS puncture size	Method(s)	Limitation(s)	Conclusion(s)	References
		Duration	Temperature / Humidity					
	Cotinine (tobacco-specific biomarker)	7 months	−20 °C	1×6.35 mm	LC-MS/MS.	The accuracy of DBS cotinine in measurement of maternal smoking is expected to be lower with increasing time of collection after delivery because of cotinine metabolism in the newborn and may vary among racial/ethnic groups. Contamination is a theoretical concern though not documented previously as almost impossible for cotinine to migrate between samples.	Measurements of cotinine in DBS samples had high sensitivity (92.3%) and specificity (99.7%) in the prediction of maternal active smoking. Cotinine levels in DBS samples are an accurate biomarker of maternal smoking close to the time of delivery. Study could not prove the cotinine stability in DBS over long-term storage at room or refrigerator temperatures.	Yang et al. [52]
Prenatal environmental pollutant exposure	Lead	6 months	Room	1×6.3 mm	Furnace atomic absorption spectrophotometry.	None stated	Residual DBS samples could be useful tools for epidemiological survey of lead pollution. Lead must be extracted from DBS in the presence of optimal amounts of EDTA.	Di Martino et al. [53]
	Hemoglobin adducts of benzene oxide	30 days	Frozen (temperature not specified)	1 x DBS	GC-MS.	None stated	Levels in neonatal DBS comparable to adult DBS or fresh blood. Can be used as a measure of the air pollutant, benzene, in neonatal DBS samples.	Funk et al. [54]
	Arsenic, lead, mercury and cadmium	4–10 years	Room	½ x DBS	ICP-MS (inductively coupled plasma mass spectrometry).	Results suggest contamination may be an issue during blood collection and storage.	Arsenic concentrations were low in samples, with 82% of samples below the limit of detection. Lead was detectable in all samples. Mercury levels were all below the detection limit. Cadmium was detectable in 67% of samples after pair-wise subtraction. However, recovery does not appear to decrease over time and heavy metal concentrations are expected to be higher and above the detection threshold in exposed populations.	Funk et al. [55]
	PFCs; PFHS; PFOA; PFOS; perfluorononanoate	≤2 years	Room	1 x DBS	SPE-HPLC-MS/MS (solid phase extraction high performance liquid chromatography tandem mass spectrometry).	Different combinations of extraction procedures and amplification may impact performance.	PFOS and PFOA were detected in all DBS samples at concentration in the low $ngml^{-1}$ range.	Kato et al. [56]
	Iodide	≤8 years	4 °C	1×16 mm	Ultrasonic extraction and HPLC-ESI-MS/MS (high performance liquid chromatography electron spray ionization tandem mass spectrometry).	Actual volume of whole blood spotted on DBS is not known. Efforts to measure the actual volume of blood present are needed. Development of appropriate internal standards for iodide analysis required to eliminate the need for using a matrix-matched calibration method of quantification.	Iodide stability in DBS specimens was tested and determined to be sufficient at storage conditions of 4 °C.	Kim et al. [57]

(*Continued*)

Table 2. Continued.

Purpose	Analyte(s)	Storage		DBS puncture size	Method(s)	Limitation(s)	Conclusion(s)	References
		Duration	Temperature / Humidity					
	BPA (bisphenol A), PFOA, PFOS	2–5 years	4 °C	1 × 16 mm	Liquid-liquid extraction and HPLC-MS/MS.	Quantification of exact volume of blood in each DBS sample remains challenging, along with potential issues arising from background levels of contamination.	Method is reliable but dependent on volume of blood used which is difficult to quantify. Background levels of contamination may also cause issues.	Ma et al. [58]
Protein biomarkers	92 oncology-related proteins	0.5–40.5 years	Room temperature (for 5 years), then 4 °C at < 70% humidity (for 13 years), then 4 °C at < 30% humidity to present	Not specified	Multiplex PEA (proximity extension assay).	None stated	61 of 92 proteins investigated degraded when stored at 4 °C. Storage at −24 °C better preserves protein levels after 10 years in storage. Many proteins remained detectable even after 30 years of storage, but temperature is a major factor in detectability.	Björkesten et al. [59]
	23 amino acids	5 years	4 °C for 1 year, then room temperature for 4 years	1 × 3.2 mm	LC-MS/MS.	Short-term instability could not be investigated.	Amino acids in residual DBS stored at room temperature are subject to substantial degradation. Except for aspartate (stable for 4 years, all other amino acid concentrations significantly decreased within one year of storage.	Dijkstra et al. [60]
	Adiponectin	Not specified	−24 °C	2 x full DBS samples	Luminex Sandwich immunoassay.	None stated	Reliable quantification of adiponectin in stored DBS samples is feasible.	Klamer et al. [61]
	Untargeted proteomics	6 years	−25 °C	1 × 3.2 mm	SELDI-TOF-MS (surface enhanced laser desorption/ionization time of flight mass spectrometry)	Little sample volume means it was not possible to identify any other proteins.	Hemoglobin is the dominant protein in samples. Method developed allows for the depletion of hemoglobin to measure other proteins.	McGuire et al. [62]
	Adiponectin, leptin and insulin	≤6 months	Room	1 × 3.2 mm	Chip-based immunoaffinity capillary electrophoresis (CB-ICE) with on-line laser-induced fluorescence detection.	None stated	Strong correlations between serum and DBS measures of adiponectin, leptin and insulin for up to 6 months storage.	Mihalopoulos et al. [63]
	64 cytokines	7 years	Room	1 × 10 mm	Luminex multiplex assay.	Retrospective analysis of DBS samples may result in decay of cytokines.	Quantified measures of cytokines were consistently and reliably obtained from neonatal DBS samples. 91% of cytokines were measurable in samples stored up to 10 years.	Mineyko et al. [64]
	Ferritin	≥11 months	Room	1 x DBS punch	Ferritin eluted and assayed as per previously published method using a mouse monoclonal antibody directed against human spleen ferritin.	Small sample size.	Recovery of ferritin was very low from DBS samples stored for more than 6 months at room temperature. A recovery of about 25% expected from DBS samples stored for prolonged periods of time at room temperature.	Raha-Chowdhury et al. [65]
	25 proteins	2 years	Room	¼ x DBS punch	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).	Storage in less-than-ideal conditions may have caused certain protein to degrade.	Proteins were identified based on their respective peptide fragments following enzymatic digestion. Study suggests method can be used to analyze and identify certain proteins and other molecular species even after 2 years storage at room temperature.	Samenuk et al. [66]

(*Continued*)

Table 2. Continued.

Purpose	Analyte(s)	Storage		DBS puncture size	Method(s)	Limitation(s)	Conclusion(s)	References
		Duration	Temperature / Humidity					
	Inflammatory markers, neurotrophins, CRP and TREM-1	1 month – 23 years	−24 °C	2×3.2 mm	Luminex multiplex assay.	Interactions between analytes and blood proteins/cells may have affected recovery of some analytes	The measurable amounts of most cytokines were nearly constant in DBS samples stored up to 23 years. A marked decrease over time was observed in measurable concentrations of interleukin (IL)-1β, IL-8, soluble (s)IL-6rα, matrix metallopeptidase (MMP)-9, triggering receptor expressed on myeloid cells (TREM)-1, CRP, Brain Derived Neurotrophic Factor (BDNF) and neurotrophin (NT)-4 in stored DBS samples.	Skogstrand et al. [67]
	Cys34 adducts in human serum albumin (HAS)	14–32 years	−20 °C	1×4.7 mm	LC-MS.	Possible that some HSA modifications may be artifacts of sample processing or storage.	Significantly higher levels of the Cys34 cyanide adduct were detected among newborns whose mother's smoked during pregnancy. DBS adductomics is suitable for investigating in utero exposures to reactive chemicals and metabolites, such as smoking, in the month prior to birth.	Yano et al. [68]
	Adipokines (adiponectin, adipsin, resistin, leptin, CRP, serpin E1)	8 months	4 °C	1×3.2 mm	Luminex Human obesity panel.	Difficulties in measuring leptin may be due to the use of multiplex, which has less sensitivity than ELISA. Initial cold storage prior to freezing may have contributed to low measures of leptin.	Leptin and CRP were too low to detect in majority of samples. Mean levels of DBS adipokines differ from previously reported levels using cord or venous blood in this study.	Yeung et al. [69]
Quantification (DNA)	DNA	3 months	Room	1×3.2 mm	Adenine tandem MS.	Method measures total DNA present in sample regardless of source, including by contamination with microbial or viral content. Likewise, degraded and/or fragmented DNA will yield similar measurements as higher molecular weight DNA.	Method provides reliable quantification of DNA from neonatal DBS samples in high throughput settings using instruments currently used in NBS laboratories.	Durie et al. [70]
Sequencing	DNA	>20 years	−20 °C (stored in containers with desiccant)	2×3.2 mm	Automated DNA extraction protocol without amplification.	None stated	Properly stored residual newborn DBS samples are a satisfactory source of DNA for genetic studies.	Bassaganyas et al. [71]
	DNA	≤20 years	Room (in a desiccator)	6–12×3 mm	Genomic DNA extraction and whole genome sequencing using a number of methods.	None stated	High quality DNA can be obtained from DBS samples stored up to 20 years without need to amplify.	Ding et al. [72]
	DNA	5–10 years	Room	1×4 mm	AS-PCR (allele-specific polymerase chain reaction).	Further investigation on DBS and corresponding whole-blood samples required.	Blood stored on Guthrie cards can be used to store DNA samples for at least 10 years. Even under suboptimal storage conditions the samples' DNA remains intact for detecting 35delG mutation in GJB2 (Cx26) gene.	Nagy et al. [73]

(*Continued*)

Table 2. Continued.

Purpose	Analyte(s)	Storage		DBS puncture size	Method(s)	Limitation(s)	Conclusion(s)	References
		Duration	Temperature / Humidity					
	wgaDNA	27–29 years	−20 °C	2×3.2 mm	DNA extraction, whole genome amplification, then whole genome sequencing and exome sequencing	Small sample size noted.	Reliable DNA obtained for whole genome and exome sequencing using a small fraction of archived neonatal DBS sample.	Hollegaard et al. [74]
	wgaDNA	Not specified	−20 °C	2×1.6 mm; 2×3.2 mm	Samples were set up for library preparation using different kits in 3 x pilot studies, before sequencing and data analysis.	Overall exome coverage may be affected by reduced quality of input DNA used for WGA or amplification bias (due to WGA kit used of reduced quality input DNA).	WgaDNA of neonatal DBS samples performs with great accuracy and efficiency in exome sequencing and performed similarly to matched high-quality reference (whole blood DNA). No differences were observed substituting 2×3.2 mm with 2×1.6 mm punches, allowing for reduction of sample material.	Poulsen et al. [75]
	wgaDNA	≤28 years	−24 °C	2×3.2 mm	DNA was extracted using an "in-house" technique based on Extract-N-amp Blood PCR Kit. WGA was performed in triplicates by the multi-displacement amplification method using the REPLI-g kit. 20 ng/µL used for subsequent exon sequencing.	None stated	WgaDNA works well for exome sequencing, similar to gDNA.	Winkel et al. [76]
Telomere length	DNA	5–9 years	−20 °C and Room; humidity-controlled	6×3.175 mm	QIAamp DNA Investigator Kit, PCR and telomere length to standard ratio assessed.	Small sample size, cross-sectional design and lack of detailed information on time in storage.	Room temperature associated with degraded samples. −20 °C is a better temperature for storage. Year of birth associated with telomere length.	Needham et al. [77]
Therapeutic drug monitoring	Antibiotics (Piperacillin and Tazobactam)	16 days	4 °C (stored in sealed bag with desiccant)	1×3.2 mm	LC-MS/MS.	Hematocrit correction not considered.	Developed method allows simultaneous identification of piperacillin and tazobactam from a low volume of blood. Piperacillin and tazobactam resulted to be stable in DBS for prolonged storage at −20 °C, while a rapid degradation occurred at 25 °C. Shipment and storage of DBS must be performed at −20 °C.	Barco et al. [78]
Transcriptome analysis	RNA	8 years	Room	1 x full DBS	RNA extracted using the Nucleospin miRNA kit according to manufacturer's instruction with minor modification (pre-incubating DBS with lysis buffer).	RNA degradation may result from ribonucleases, pH, humidity or UV light, although RNA contamination considered negligible in this study.	RNA assumed to be less prone to degradation and relatively stable for long periods of time at room temperature in this study.	Rovito et al. [79]

CRP = C-reactive protein; DBS = dried blood spot; DNA = deoxyribonucleic acid; DNase = deoxyribonuclease; GC-MS = gas chromatography-mass spectrometry; gDNA = genomic DNA; LC-MS/MS = liquid chromatography-mass spectrometry; LC-HRMS = liquid chromatography-high resolution mass spectrometry; mRNA = messenger RNA; miRNA = microRNA; PCR = polymerase chain reaction; PFCs = polyfluoroalkyl chemicals; PFHS = perfluorohexane sulfonate; PFOA = perfluorooctanoate; PFOS = perfluorooctane sulfonate; RNA = ribonucleic acid; RNase = ribonucleases; wgaDNA = whole genome amplified DNA; SNP = single nucleotide polymorphism.

Table 3. Summary of analytes successfully measured using neonatal dried blood spot (DBS) samples stored at varying temperatures and their maximum duration of storage as reported in the included studies.

			Maximum storage duration studied					
Category	Analytes measured	Storage conditions	< 1 year	1 to 5 years	> 5 to 10 years	> 10 to 20 years	> 20 to 30 years	> 30 years
Genomic	DNA	Room	✓	✓	✓	✓	✓	
		Frozen		✓	✓		✓	✓
	gDNA	Room				✓		✓
	Methylated DNA	Room				✓		✓
		Frozen					✓	✓
	wgaDNA	Cold					✓	
	RNA	Room			✓	✓		
		Cold				✓		
		Frozen					✓	
	mRNA	Room	✓					
	miRNA	Room	✓					
Proteins	Ferritin	Room		✓				
	Cytokines	Room			✓			
	Untargeted proteomics	Room		✓				
		Frozen			✓			
	Aspartate	Room		✓				
	Adiponectin	Room	✓					
		Frozen	✓					
	Leptin	Room	✓					
	Insulin	Room	✓					
	Adipokines	Cold	✓					
	Oncology-related	Cold						✓
	Neurotrophins	Frozen					✓	
	Inflammatory markers	Frozen					✓	
	Toxoplasma gondii IgM	Frozen		✓				
Metabolites	Untargeted metabolomics	Room		✓				
		Cold					✓	
		Frozen						✓
	Nutrition and health status-related	Frozen					✓	
Environmental pollutants	Lead	Room	✓		✓			
	Arsenic	Room			✓			
	Cadmium	Room			✓			
	Polyfluoroalkyl chemicals	Room		✓				
		Cold		✓				
		Frozen		✓				
	Iodide	Cold			✓			
	Cys34 adducts of HSA	Frozen						✓
	Hb adducts of benzene oxide	Frozen	✓					
Substance use in pregnancy	Phosphatidylethanol (PEth)	Room	✓					
		Frozen	✓ ✓					
	Benzoylecgonine	Room	✓					
	Cotinine	Cold			✓			
		Frozen	✓					
Medications	Piperacillin/tazobactam	Cold	✓					

DNA = deoxyribonucleic acid; gDNA = genomic DNA; Hb = hemoglobin; HSA = human serum albumin; IgM = immunoglobulin M; mRNA = messenger RNA; miRNA = microRNA; RNA = ribonucleic acid; wgaDNA = whole genome amplified DNA.

Table 4. Summary of studies directly comparing methods used on neonatal dried blood spot (DBS) samples.

References	Methods compared	Results	Conclusion(s)/Recommendation(s)
Grauholm et al. [20]	Study examined the effect on gene expression profiles (GEPs) when two different whole transcriptome amplification (WTA) kits were used on samples stored for ~5 years at −20 °C.	Use of two different WTA kits (WT-Ovation Pico RNA amplification system and Signa-Aldrich's complete WTA kit) resulted in a 13% variation in the principal component analysis.	Consistent use of a single WTA kit throughout a study is recommended to avoid introduction of significant differences in expression.
Klassen et al. [23]	The novel quality control (QC) technology, visual automated fluorescence electrophoresis (VAFE), was performed before and after whole genome amplification in parallel with traditional QC methods to compare quantity, quality, and integrity of nucleic acid from downstream genomic technologies.	VAFE QC data were correlated with subsequent sample performance in PCR, sequencing, and high-density comparative genome hybridization array.	Addition of VAFE measures in QC increases confidence in the validity of genetic data and allows cost-effective downstream analysis of gDNA for investigational and diagnostic applications.
Lane et al. [24]	Commercial methods of DNA extraction from DBS samples were compared with and without protocol modifications to assess if DNA yield is improved.	The addition of a purpose developed GenSolve reagent and a pre-warmed elution buffer and 70 °C soaking step increased DNA yield versus following the commercial protocol alone.	DNA yield can be improved with modifications to commercially available DNA extraction kits.
Hollegaard et al. [31]	Study assessed the quality of DNA obtained from different amplification protocols, comparing: two commercial DNA extraction procedures (Extract-N-Amp Blood PCR Kit [Sigma-Aldrich] or QIAamp DNA Blood Micro Kit [Qiagen]) and three WGA procedures (REPLI-g kit [Qiagen], GenomePlex® Complete WGA Kit [GPlex2, Sigma-Aldrich] or GenomePlex® Single Cell Whole Genome Amplification Kit [GPlex4, Sigma-Aldrich]), the effect of number of 3.2 mm DBS samples punches used and the effect of protein extraction prior to gDNA extraction.	The Extract-N-Amp Blood PCR Kit combined with the REPLI-g kit WGA featured the highest call-rates and the lowest conflict rates. Use of one or three DBS sample punches did not affect extraction. Protein extraction did not impair genotyping of the produced wgaDNA.	The Extract-N-Amp Blood PCR Kit is recommended for DNA extraction combined with GPlex2 or GPlex4 for WGA. Tests should be performed to determine the suitability of WGA procedures based on the given task, with one to three DBS sample punches being sufficient for analysis.
Hollegaard et al. [33]	Three DNA extraction methods were compared: QIAamp DNA Blood Mini Kit (Qiagen), Extract-N-Amp Blood PCR Kit (Sigma-Aldrich) and an "in-house" method based on Chelex 100 beads (Sigma-Aldrich). QIAamp and Extract-N-Amp DNA extractions were performed according to the kit instructions. The "in-house" gDNA extraction was carried out by adding 200 ml washing buffer (PBS, 0.5% TWEEN20) to each well, containing one 3.2 mm DBS punch. Four WGA kits and one re-amplification kit were used: REPLI-g (RepliG, Qiagen), AmpliQ Genomic Amplifier Kit (AmpliQ, Ampliqon), GenomePlexs Complete WGA Kit (GPlex2, Sigma-Aldrich), GenomePlexs Single Cell Whole Genome Amplification Kit (GPlex4, Sigma-Aldrich), and GenomePlexs WGA Reamplification Kit (GPlex3, Sigma-Aldrich). WGA reactions were performed according to the kit instructions.	Using the Extract-N-Amp kit for extraction of gDNA and performing the maximal number of WGA reactions on the extract using the GPlex kit will give a total yield of approximately 50 mg wgaDNA. The wgaDNA can be re-amplified, producing virtually infinite amounts of wgaDNA. The two OmniPlex kits, GPlex2 and GPlex4, performed well with all three extraction methods, indicating that they tolerated lower quality and smaller amounts of input gDNA better than the two multi-displacement amplification kits.	Genotyping performance is dependent on the combination of extraction procedure and amplification method.
Hollegaard et al. [34]	Genome-wide association studies using three different types of array were examined. The three studies, "610k", "Omni1"z, and "Axiom" used an Infinium Human610-Quad chip array (Illumina), an Infinium HD HumanOmni1-Quad chip array (Illumina) and an Axiom Genome-Wide CEU Array chip (Affymetrix), respectively.	Type of filter paper used for DBS samples was significantly associated with genotyping call rate (GCR) when using the "Omni1" array. Samples stored at −20 °C genotyped by the "Axiom" array had significantly higher GCRs than samples stored at 4 °C. Storage time and conditions did not affect the GCR when measured using the "610k" array.	Overall, of the three arrays tested, the Illumina "Omni1" array performed best. Compared with the other arrays, "Omni1" had the highest mean GCR, and the highest sample success rate. Although none of the arrays performed poorly and the effects of the different variables on the GCR were minimal, even when statistically significant. All three arrays should be considered usable for GWS of DBS samples.

(*Continued*)

Table 4. Continued.

References	Methods compared	Results	Conclusion(s)/Recommendation(s)
Ghantous et al. [44]	A range of DNA extraction methods from neonatal DBS samples, individually or in combinations, were developed and tested. The DNA extraction protocol was split into two phases: phase I included blood extraction from DBS samples, cell lysis and protease digestion and phase II included DNA precipitation, purification and elution. Ten method combinations were tested to optimize DNA extraction.	Protocol GQ used the commercially available kits, GenSolve for phase I and QIAamp (DNA Micro Kit) for phase II. DNA yield was consistently better with less DNA degradation/ fragmentation when using this protocol compared to others.	Protocol GQ appears to be the most robust among the tested methods across all tested DNA quantity and quality parameters.
Ghantous et al. [45]	Two robust and efficient protocols that increase DNA yield from DBS while minimizing DNA degradation were presented. One protocol, termed GenSolve-Qiagen, represents a combination of (a) GenSolve material in Phase I, (b) Qiagen material in Phase II and (c) in-house modifications. The other protocol, termed Qiagen-Ethanol, involves (a) Qiagen consumables in phases I and II, (b) ethanol as a precipitation buffer and (c) in-house modifications.	The Qiagen-Ethanol protocol extracted approximately twice the amount of DNA relative to the GenSolve-Qiagen protocol, although the latter protocol still represents a highly efficient protocol yielding quality DNA.	Either protocol could be used according to specific needs of the study, with both protocols being more efficient relative to other published methods.
Di Martino et al. [53]	Lead from DBS samples was measured using furnace atomic absorption spectrophotometry with or without the addition of 5 mmol/L EDTA to eluting solution.	The median optical density at 283.3 nm was significantly higher in DBS samples than controls in the presence of EDTA. When EDTA was absent from eluting solution, controls had a higher optical density than DBS samples.	Adding EDTA to the eluting solution is essential for the optimal recovery of lead during the extraction step from DBS samples.

within one year of storage (although aspartate appeared to be stable for four years) [60], while degradation was minimal for amino acids stored at −20 °C [37]. Cytokines were measurable in samples stored at room temperature for ten years [64] and frozen samples for 20 years and appeared to be well preserved [67]. Inflammatory markers and neurotrophins were well preserved and detectable in equal concentrations over time in frozen samples stored for longer than 20 years [67]. Ferritin recovery was low from samples stored at room temperature for more than six months, with expected recovery of ferritin from samples stored for longer periods at room temperature estimated to be around 25% [65]. Adipokines (adiponectin, resistin and adipsin, but not leptin) were measurable in cold storage samples but levels differed from previously reported levels using cord or venous blood [69]. Adiponectin, leptin and insulin were measurable in samples stored at room temperature for up to six months, with similar levels to those observed in serum samples [63]. Antibodies stored at room temperature were subject to degradation within months but may be better preserved for longer when samples are stored at −20 °C [35,36].

Storage of samples at −20 °C was shown to protect against the deterioration of most metabolites studied for almost 30 years, although lipid metabolites appeared less stable [37]. For samples stored at room temperature, the largest changes in metabolite levels were observed after 12 months [38]. Untargeted metabolomics of DBS samples stored for long periods appeared feasible, with the majority of DBS metabolomes stable for up to ten [39] and 30 [40] years if stored at −20 °C. In studies assessing prenatal environmental pollutant exposure, lead was measurable in samples stored at room temperature for up to six months but needed to be extracted from DBS samples in the presence of optimal amounts of EDTA (ethylenediaminetetraacetic acid) [53]. In another study, lead and cadmium (but not mercury) were detectable in samples stored at room temperature for over ten years but arsenic concentrations were low, with potential contamination during blood sample collection and storage noted as an issue [55]. Hemoglobin adducts of benzene oxide, a marker of exposure to the air pollutant benzene, were measurable in neonatal DBS samples after one month of frozen storage [54]. The levels measured were comparable to adult DBS samples and recovery did not appear to decrease over time, although contamination may again be an issue and no longer-term studies of stability were found. Iodide was found to be stable in samples stored at 4 °C for up to eight years [57], although no studies assessing iodide in samples stored frozen or at room temperature were found. PFC extraction was possible using samples stored at room temperature for up to two years but was dependent on the extraction and amplification procedures used [56]. The extraction method used for samples stored at 4 °C for up to five years was deemed reliable but dependent on the number and size of DBS samples used. Background levels of contamination may also cause issues with accurate quantification [58].

A number of studies used neonatal DBS samples to detect prenatal exposures to substances consumed or used by the mother, such as alcohol and tobacco. PEth (a direct ethanol metabolite that can be used to detect prenatal alcohol exposure) was detectable in samples stored at room temperature for two weeks [48] and in samples stored at −80 °C for four months [49], with insignificant degradation noted. However, the stability of PEth in samples stored at these temperatures for longer durations remains unknown. Cotinine and hydroxycotinine (markers of maternal smoking) remained detectable in frozen samples stored for up to 30 years [37]. Measurement of these markers of tobacco was found to be reliable but may be affected by the amount of DBS sample used and background levels of contamination [51,52]. Cotinine stability in DBS samples stored long-term at room temperature or 4 °C is not known, although another study demonstrated that methylated DNA extracted from room temperature samples stored for over 30 years, could be used to indicate maternal smoking [43]. Cys34 HSA adducts (used to investigate in-utero exposure to smoking in the month prior to birth) were measurable in samples stored at −20 °C for over 30 years [68], although it was reported that quantification of Cys34 HSA adducts was harder when hemoglobin was oxidized at room temperature. Samples stored at room temperature showed no decrease in the direct cocaine metabolite, benzoylecgonine, when measured over 30 days [50]. However, no studies assessing long-term stability were found. Finally, the antibiotics, piperacillin and tazobactam, were stable in samples stored at −20 °C but rapid degradation occurred at room temperature [78].

Comparisons

A total of nine studies compared methods used on neonatal DBS samples (Table 4) [20,23,24,31,33,34,44,45,53]. These studies predominately compared the use of commercially available kits with or without modifications to the available protocol. Such modifications typically improved the efficiency of the method and were recommended for use in future studies. Full descriptions of the methods that were compared along with the results and conclusions/recommendations are contained in Table 4.

Limitations

Methodological limitations relating to the use of DBS samples were noted for each study (Table 2). Contamination of samples may occur during sample collection, transportation, storage or handling and was a noted limitation in a number of studies. Depending on the analytes being measured, contaminants included DNA [14,70], RNAases [17,79], lipids [37], proteins [13,67], volume of red blood cells (hematocrit) [13,37,67,78] and microbial or viral content [70], although studies largely did not speculate as to the potential sources of contamination. The number and size of DBS samples used further restricted investigations or outputs in a number of studies [18,28,31,33,34,36,41,62,65,74,77]. This was due to finite availability of DBS samples or restrictions imposed by biobank facilities concerning the number of samples/punches that could be used for secondary research purposes. The volume of blood spotted onto DBS samples is largely unknown and may have varied between samples due to DBS size, punch location and filter paper type [41,51,57,58]. Storage duration and conditions were further noted as limitations in included studies. Suboptimal conditions, along with long storage times, may impact the viability of DBS samples and/or recovery of analytes due to degradation [27,32,35,37–39,64,66,68,69]. Results may also be impacted by direct limitations of the methods applied (for example, some methods may be less sensitive when measuring certain analytes), along with a lack of appropriate standards and systematic quality control for sample preparation and assessment [23,69,75].

Discussion

Summary of evidence

This scoping review is the first evidence synthesis, to our knowledge, covering the methodological designs that have been successfully used on residual neonatal DBS samples for secondary research purposes. We have presented the approaches used in the storage, extraction and testing of residual neonatal DBS samples, along with the analytes measured and the limitations encountered, as reported in the published literature. As expected, the methodological designs were highly variable across the included studies. The duration and temperature of neonatal DBS sample storage were notable factors in the detectability of many analytes, with studies emphasizing the need to consider this when choosing optimal methods for planned analyses, particularly when using samples stored for extended periods of time in suboptimal conditions. Contamination, particularly from endogenous components, was a further concern when performing analyses using neonatal DBS samples and appropriate corrections should be made where possible, along with use of quality assurance measures. Further investigation is required to assess the long-term stability of certain analytes, such as those

reflecting substance use and environmental pollutant exposure during pregnancy. Additional validation studies using neonatal DBS samples may also be required. For example, the long-term preservation of RNA in neonatal DBS samples stored at room temperature was a particularly intriguing finding given the known instability of RNA. Nevertheless, this review presents encouraging evidence supporting the use of residual neonatal DBS samples for a range of secondary research purposes, with potential for high throughput omics analyses (including DNA, methylation, RNA and proteins) and methods that are scalable to population-level datasets for molecular epidemiology.

Whilst recognizing that issues of consent and access approval for research purposes are yet to be resolved in many jurisdictions [80], using residual neonatal DBS samples for research has a number of advantages. Compared with venous and cord blood samples, DBS samples are more easily transported and stored [81]. Secondary use of neonatal DBS samples collected for screening obviates the need for recruitment and provides large-scale, unselected sampling frames which are continually being expanded. As analytical techniques continue to improve, along with updated biobank policies regarding the optimal storage of samples, analytes may be measurable with high throughput and increased specificity and sensitivity [82], further expanding the scope of research that can be performed. Finally, the linkage of stored DBS samples and healthcare registries would provide resources for studies, including case series, case-control and retrospective cohort studies, to address research questions relating to epidemiology, genotype/phenotype interactions and treatment outcomes, with the potential to inform future policy and practice [83].

Limitations

Due to the broad nature of this scoping review and the breadth of related literature, some articles may have been missed during the search process. However, efforts were made to minimize the risk of unfound articles, such as searching multiple databases using a comprehensive search strategy to identify additional relevant studies, as well as members of the review team performing article screening in duplicate. Likewise, the depth of analysis was limited due to the broad focus of this scoping review that resulted in a large number of included studies. Similarly, this review did not assess risk of bias or quality in the included studies, which is a limitation of scoping reviews in general. Studies were also limited to those published in the English language, although a systematic literature review published in 2012, reported no evidence of systematic bias as a result of English-language restrictions [84].

This scoping review also searched for studies published from 1973 onwards, meaning that relevant studies prior to this year may have been missed. However, newborn screening for inherited inborn errors of metabolism and other metabolic disorders was in its infancy in the 1950s and 1960s, with particular focus on testing for PKU [85,86]. Subsequent neonatal DBS-related research predominately focused on adding conditions to newborn screening panels [87], with the World Health Organization (WHO) commissioning a report in 1968 to develop screening criteria to guide and evaluate the selection of conditions for screening [88]. This focus on the expansion of newborn screening programs may explain the lack of eligible studies prior to 1993 in this scoping review, which was interested in secondary research (unrelated to newborn screening) using neonatal DBS samples. Another reason for the inclusion of studies that were newer in origin may be that advances in methodologies, e.g. the introduction of tandem mass spectrometry in the early 1990s, increased the use of neonatal DBS samples for secondary research purposes by broadening what was technologically possible at this time [85].

The non-reporting of failed experiments and publication bias may also be an issue, in that failed attempts at measuring specific analytes of interest may not have been reported. Delays in sending samples for analysis or in performing clinical testing itself (which remains the priority of neonatal DBS sample collection), may have also impacted the reported storage durations, with such delays unlikely to be reported in studies. Nevertheless, we have attempted to collate the results of studies to demonstrate the feasibility of measuring analytes of interest which may act as an important first step in the standardization of neonatal DBS methodologies for secondary research purposes.

Conclusions

Neonatal DBS samples have been used successfully to measure a variety of analytes for different purposes. Storage duration, temperature and potential contamination limit some potential uses of DBS samples and should be considered when planning research studies and selecting appropriate methods.

Disclosure statement

JC, RJS, ZM, DJP, CLMS, RC, SP and JPP declare no relevant competing interests. REM is a scientific advisor to Optima Partners and the Epigenetic Clock Development Foundation. MM is a co-founder of Mirvie Inc. (San Fransisco) that creates

precise, actionable, noninvasive tests for maternal-fetal health. MEH is a founder of, director of, consultant to and holds shares in Congenica Ltd. and is a consultant to the AstraZeneca Centre for Genomics Research. NS has consulted for and/or received speaker honoraria from Abbott Laboratories, Abbvie, Amgen, AstraZeneca, Boehringer Ingelheim, Eli Lilly, Hanmi Pharmaceuticals, Janssen, Menarini-Ricerche, Novartis, Novo Nordisk, Pfizer, Roche Diagnostics and Sanofi, and received grant support paid to his University from AstraZeneca, Boehringer Ingelheim, Novartis and Roche Diagnostics outside the submitted work.

Funding

No external funding. RJS was supported by UKRI Innovation-HDR-UK (MR/S003061/1) and University of Glasgow Lord Kelvin Adam Smith Fellowships.

ORCID

Jordan Canning http://orcid.org/0000-0002-1432-1302
Rona J. Strawbridge http://orcid.org/0000-0001-8506-3585

References

[1] Therrell BL, Padilla CD, Loeber JG, et al. Current status of newborn screening worldwide: 2015. Semin Perinatol. 2015;39(3):171–187. doi: 10.1053/j.semperi.2015.03.002.
[2] Wilcken B, Wiley V. Newborn screening. Pathology. 2008;40(2):104–115. doi: 10.1080/00313020701813743.
[3] Guthrie R, Susi A. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. Pediatrics. 1963;102(1):236–237. doi: 10.1542/peds.32.3.338.
[4] Benkendorf J, Goodspeed T, Watson MS. Newborn screening residual dried blood spot use for newborn screening quality improvement. Genet Med. 2010;12(12 Suppl):S269–S272. doi: 10.1097/GIM.0b013e3181fea489.
[5] Caggana M, Jones EA, Shahied SI, et al. Newborn screening: from guthrie to whole genome sequencing. Public Health Rep. 2013;128(Suppl 2):14–19. doi: 10.1177/00333549131280S204.
[6] Botkin JR, Goldenberg AJ, Rothwell E, et al. Retention and research use of residual newborn screening bloodspots. Pediatrics. 2013;131(1):120–127. doi: 10.1542/peds.2012-0852.
[7] Kingston A, Jagger C. Review of methodologies of cohort studies of older people. Age Ageing. 2018;47(2):215–219. doi: 10.1093/ageing/afx183.
[8] Tarini BA. Storage and use of residual newborn screening blood spots: a public policy emergency. Genet Med. 2011;13(7):619–620. doi: 10.1097/GIM.0b013e31822176df.
[9] Hougaard DM, Bybjerg-Grauholm J, Christiansen M, et al. Response to "newborn dried blood spot samples in Denmark: the hidden figures of secondary use and research participation. Eur J Hum Genet. 2019;27(11):1625–1627. doi: 10.1038/s41431-019-0437-y.
[10] Rothwell E, Johnson E, Riches N, et al. Secondary research uses of residual newborn screening dried bloodspots: a scoping review. Genet Med. 2019;21(7):1469–1475. doi: 10.1038/s41436-018-0387-8.
[11] Tricco AC, Lillie E, Zarin W, et al. PRISMA extension for scoping reviews (PRISMA-ScR): checklist and explanation. Ann Intern Med. 2018;169(7):467–473. doi: 10.7326/M18-0850.
[12] Peters MDJ, Casey M, Tricco AC, et al. Updated methodological guidance for the conduct of scoping reviews. JBI Evid Implement. 2021;19(1):3–10. doi: 10.1097/XEB.0000000000000277.
[13] Makowski GS, Davis EL, Hopfer SM. The effect of storage on Guthrie cards: implications for deoxyribonucleic acid amplification. Ann Clin Lab Sci. 1996;26(5):458–469.
[14] Haak PT, Busik JV, Kort EJ, et al. Archived unfrozen neonatal blood spots are amenable to quantitative gene expression analysis. Neonatology. 2009;95(3):210–216. doi: 10.1159/000155652.
[15] Resau JH, Ho NT, Dykema K, et al. Evaluation of sex-specific gene expression in archived dried blood spots (DBS). Int J Mol Sci. 2012;13(8):9599–9608. doi: 10.3390/ijms13089599.
[16] Slaughter J, Wei C, Korzeniewski SJ, et al. High correlations in gene expression between paired umbilical cord blood and neonatal blood of healthy newborns on Guthrie cards. J Matern Fetal Neonatal Med. 2013;26(18):1765–1767. doi: 10.3109/14767058.2013.804050.
[17] Ponnusamy V, Kapellou O, Yip E, et al. A study of microRNAs from dried blood spots in newborns after perinatal asphyxia: a simple and feasible biosampling method. Pediatr Res. 2016;79(5):799–805. doi: 10.1038/pr.2015.276.
[18] Bybjerg-Grauholm J, Hagen CM, Khoo SK, et al. RNA sequencing of archived neonatal dried blood spots. Mol Genet Metab Rep. 2016;10:33–37. doi: 10.1016/j.ymgmr.2016.12.004.
[19] Gauffin F, Nordgren A, Barbany G, et al. Quantitation of RNA decay in dried blood spots during 20 years of storage. Clin Chem Lab Med. 2009;47(12):1467–1469. doi: 10.1515/CCLM.2009.351.
[20] Grauholm J, Khoo SK, Nickolov RZ, et al. Gene expression profiling of archived dried blood spot samples from the Danish neonatal screening biobank. Mol Genet Metab. 2015;116(3):119–124. doi: 10.1016/j.ymgme.2015.06.011.
[21] Chaisomchit S, Wichajarn R, Janejai N, et al. Stability of genomic DNA in dried blood spots stored on filter paper. Southeast Asian J Trop Med Public Health. 2005;36(1):270–273.
[22] Hardin J, Finnell RH, Wong D, et al. Whole genome microarray analysis, from neonatal blood cards. BMC Genet. 2009;10(1):38. doi: 10.1186/1471-2156-10-38.
[23] Klassen TL, Drabek J, Tomson T, et al. Visual automated fluorescence electrophoresis provides simultaneous quality, quantity, and molecular weight spectra for genomic DNA from archived neonatal blood spots. J Mol Diagn. 2013;15(3):283–290. doi: 10.1016/j.jmoldx.2013.01.003.
[24] Lane JA, Noble JA. Maximizing deoxyribonucleic acid yield from dried blood spots. J Diabetes Sci Technol. 2010;4(2):250–254. doi: 10.1177/193229681000400204.
[25] Pedersen CB, Bybjerg-Grauholm J, Pedersen MG, et al. The iPSYCH2012 case-cohort sample: new directions for unravelling genetic and environmental architectures of severe mental disorders. Mol Psychiatry. 2018;23(1):6–14. doi: 10.1038/mp.2017.196.
[26] Rajatileka S, Luyt K, El-Bokle M, et al. Isolation of human genomic DNA for genetic analysis from premature neo-

nates: a comparison between newborn dried blood spots, whole blood and umbilical cord tissue. BMC Genet. 2013;14(1):105. doi: 10.1186/1471-2156-14-105.
[27] Sjöholm MI, 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):1401–1407. doi: 10.1373/clinchem.2007.087510.
[28] Sok P, Lupo PJ, Richard MA, et al. Utilization of archived neonatal dried blood spots for genome-wide genotyping. PLoS One. 2020;15(2):e0229352. doi: 10.1371/journal.pone.0229352.
[29] St Julien KR, Jelliffe-Pawlowski LL, Shaw GM, et al. High quality genome-wide genotyping from archived dried blood spots without DNA amplification. PLoS One. 2013;8(5):e64710. doi: 10.1371/journal.pone.0064710.
[30] Hannelius U, Lindgren CM, Melén E, et al. Phenylketonuria screening registry as a resource for population genetic studies. J Med Genet. 2005;42(10):e60–e60. doi: 10.1136/jmg.2005.032987.
[31] Hollegaard MV, Grauholm J, Børglum A, et al. Genome-wide scans using archived neonatal dried blood spot samples. BMC Genomics. 2009a;10(1):297. doi: 10.1186/1471-2164-10-297.
[32] Hollegaard MV, Grove J, Thorsen P, et al. High-throughput genotyping on archived dried blood spot samples. Genet Test Mol Biomarkers. 2009b;13(2):173–179. doi: 10.1089/gtmb.2008.0073.
[33] Hollegaard MV, Thorsen P, Norgaard-Pedersen B, et al. Genotyping whole-genome-amplified DNA from 3- to 25-year-old neonatal dried blood spot samples with reference to fresh genomic DNA. Electrophoresis. 2009c;30(14):2532–2535. doi: 10.1002/elps.200800655.
[34] Hollegaard MV, Grove J, Grauholm J, et al. Robustness of genome-wide scanning using archived dried blood spot samples as a DNA source. BMC Genet. 2011;12(1):58. doi: 10.1186/1471-2156-12-58.
[35] Auma E, Hall T, Chopra S, et al. Using dried blood spots for a Sero-Surveillance study of maternally derived antibody against group B Streptococcus. Vaccines (Basel). 2023;11(2):357. doi: 10.3390/vaccines11020357.
[36] Mei JV, Li L, Rasmussen SA, et al. Effect of specimen storage conditions on newborn dried blood spots used to assess Toxoplasma gondii immunoglobulin M (IgM). Clin Chim Acta. 2011;412(5-6):455–459. doi: 10.1016/j.cca.2010.11.028.
[37] He D, Yan Q, Uppal K, et al. Metabolite stability in archived neonatal dried blood spots used for epidemiological research. Am J Epidemiol. 2023;192(10):1720–1730. doi: 10.1093/aje/kwad122.
[38] Murphy MSQ, Hawken S, Cheng W, et al. Metabolic profiles derived from residual blood spot samples: a longitudinal analysis. Gates Open Res. 2018;2:28. doi: 10.12688/gatesopenres.
[39] Ottosson F, Russo F, Abrahamsson A, et al. Effects of long-term storage on the biobanked neonatal dried blood spot metabolome. J Am Soc Mass Spectrom. 2023;34(4):685–694. doi: 10.1021/jasms.2c00358.
[40] Petrick L, Edmands W, Schiffman C, et al. An untargeted metabolomics method for archived newborn dried blood spots in epidemiologic studies. Metabolomics. 2017;13(3):27. doi: 10.1007/s11306-016-1153-z.
[41] Yu M, Dolios G, Yong-Gonzalez V, et al. Untargeted metabolomics profiling and hemoglobin normalization for archived newborn dried blood spots from a refrigerated biorepository. J Pharm Biomed Anal. 2020;191:113574. doi: 10.1016/j.jpba.2020.113574.
[42] Asrani K, Shaw GM, Rine J, et al. DNA methylome profiling on the infinium HumanMethylation450 array from limiting quantities of genomic DNA from a single, small archived bloodspot. Genet Test Mol Biomarkers. 2017;21(8):516–519. doi: 10.1089/gtmb.2017.0019.
[43] Cunningham-Burley S, McCartney DL, Campbell A, et al. Feasibility and ethics of using data from the scottish newborn blood spot archive for research. Commun Med (Lond). 2022;2(1):126. doi: 10.1038/s43856-022-00189-2.
[44] Ghantous A, Saffery R, Cros MP, et al. Optimized DNA extraction from neonatal dried blood spots: application in methylome profiling. BMC Biotechnol. 2014;14(1):60. doi: 10.1186/1472-6750-14-60.
[45] Ghantous A, Hernandez-Vargas H, Herceg Z. DNA methylation analysis from blood spots: increasing yield and quality for genome-wide and locus-specific methylation analysis. Methods Mol Biol. 2018;1708:605–619. doi: 10.1007/978-1-4939-7481-8_31.
[46] Hollegaard MV, Grauholm J, Nørgaard-Pedersen B, et al. DNA methylome profiling using neonatal dried blood spot samples: a proof-of-principle study. Mol Genet Metab. 2013a;108(4):225–231. doi: 10.1016/j.ymgme.2013.01.016.
[47] Zar Kyaw T, Yamaguchi S, Imai C, et al. The utility of post-test newborn blood spot screening cards for epigenetic association analyses: association between HIF3A methylation and birth weight-for-gestational age. J Hum Genet. 2019;64(8):795–801. doi: 10.1038/s10038-019-0621-5.
[48] Bakhireva LN, Leeman L, Savich RD, et al. The validity of phosphatidylethanol in dried blood spots of newborns for the identification of prenatal alcohol exposure. Alcohol Clin Exp Res. 2014;38(4):1078–1085. doi: 10.1111/acer.12349.
[49] Bakhireva LN, Shrestha S, Gutierrez HL, et al. Stability of phosphatidylethanol in dry blood spot cards. Alcohol Alcohol. 2016;51(3):275–280. doi: 10.1093/alcalc/agv120.
[50] Henderson LO, Powell MK, Hannon WH, et al. Radioimmunoassay screening of dried blood spot materials for benzoylecgonine. J Anal Toxicol. 1993;17(1):42–47. doi: 10.1093/jat/17.1.42.
[51] Spector LG, Hecht SS, Ognjanovic S, et al. Detection of cotinine in newborn dried blood spots. Cancer Epidemiol Biomarkers Prev. 2007;16(9):1902–1905. doi: 10.1158/1055-9965.
[52] Yang J, Pearl M, Jacob P, 3rd, et al. Levels of cotinine in dried blood specimens from newborns as a biomarker of maternal smoking close to the time of delivery. Am J Epidemiol. 2013;178(11):1648–1654. doi: 10.1093/aje/kwt182.
[53] Di Martino MT, Michniewicz A, Martucci M, et al. EDTA is essential to recover lead from dried blood spots on filter paper. Clin Chim Acta. 2004;350(1-2):143–150. doi: 10.1016/j.cccn.2004.07.019.
[54] Funk WE, Waidyanatha S, Chaing SH, et al. Hemoglobin adducts of benzene oxide in neonatal and adult dried blood spots. Cancer Epidemiol Biomarkers Prev. 2008;17(8):1896–1901. doi: 10.1158/1055-9965.EPI-08-0356.

[55] Funk WE, McGee JK, Olshan AF, et al. Quantification of arsenic, lead, mercury and cadmium in newborn dried blood spots. Biomarkers. 2013;18(2):174–177. doi: 10.3109/1354750X.2012.750379.
[56] Kato K, Wanigatunga AA, Needham LL, et al. Analysis of blood spots for polyfluoroalkyl chemicals. Anal Chim Acta. 2009;656(1–2):51–55. doi: 10.1016/j.aca.2009.10.007.
[57] Kim UJ, Kannan K. Method for the determination of iodide in dried blood spots from newborns by high performance liquid chromatography tandem mass spectrometry. Anal Chem. 2018;90(5):3291–3298. doi: 10.1021/acs.analchem.7b04827.
[58] Ma W, Kannan K, Wu Q, et al. Analysis of polyfluoroalkyl substances and bisphenol a in dried blood spots by liquid chromatography tandem mass spectrometry. Anal Bioanal Chem. 2013;405(12):4127–4138. doi: 10.1007/s00216-013-6787-3.
[59] Björkesten J, Enroth S, Shen Q, et al. Stability of proteins in dried blood spot biobanks. Mol Cell Proteomics. 2017;16(7):1286–1296. doi: 10.1074/mcp.RA117.000015.
[60] Dijkstra AM, de Blaauw P, van Rijt WJ, et al. Important lessons on long-term stability of amino acids in stored dried blood spots. Int J Neonatal Screen. 2023;9(3):34. doi: 10.3390/ijns9030034.
[61] Klamer A, Skogstrand K, Hougaard DM, et al. Adiponectin levels measured in dried blood spot samples from neonates born small and appropriate for gestational age. Eur J Endocrinol. 2007;157(2):189–194. doi: 10.1530/EJE-06-0710.
[62] McGuire JN, Eising S, Wägner AM, et al. Screening newborns for candidate biomarkers of type 1 diabetes. Arch Physiol Biochem. 2010;116(4-5):227–232. doi: 10.3109/13813455.2010.501801.
[63] Mihalopoulos NL, Phillips TM, Slater H, et al. Validity and reliability of perinatal biomarkers of adiposity after storage as dried blood spots on paper. Am J Hum Biol. 2011;23(5):717–719. doi: 10.1002/ajhb.21199.
[64] Mineyko A, Nettel-Aguirre A, de Jesus P, et al. Association of neonatal inflammatory markers and perinatal stroke subtypes. Neurology. 2020;95(9):e1163–e1173. doi: 10.1212/WNL.0000000000010309.
[65] Raha-Chowdhury R, Moore CA, Bradley D, et al. Blood ferritin concentrations in newborn infants and the sudden infant death syndrome. J Clin Pathol. 1996;49(2):168–170. doi: 10.1136/jcp.49.2.168.
[66] Samenuk GM, Kelley AR, Perry G, et al. Rapid method towards proteomic analysis of dried blood spots by MALDI mass spectrometry. Clin Mass Spectrom. 2019;12:30–36. doi: 10.1016/j.clinms.2019.03.002.
[67] Skogstrand K, Thorsen P, Nørgaard-Pedersen B, et al. Simultaneous measurement of 25 inflammatory markers and neurotrophins in neonatal dried blood spots by immunoassay with xMAP technology. Clin Chem. 2005;51(10):1854–1866. doi: 10.1373/clinchem.2005.052241.
[68] Yano Y, Grigoryan H, Schiffman C, et al. Untargeted adductomics of Cys34 modifications to human serum albumin in newborn dried blood spots. Anal Bioanal Chem. 2019;411(11):2351–2362. doi: 10.1007/s00216-019-01675-8.
[69] Yeung EH, McLain AC, Anderson N, et al. Newborn adipokines and birth outcomes. Paediatr Perinat Epidemiol. 2015;29(4):317–325. doi: 10.1111/ppe.12203.
[70] Durie D, Yeh E, McIntosh N, et al. Quantification of DNA in neonatal dried blood spots by adenine tandem mass spectrometry. Anal Chem. 2018;90(1):801–806. doi: 10.1021/acs.analchem.7b03265.
[71] Bassaganyas L, Freedman G, Vaka D, et al. Whole exome and whole genome sequencing with dried blood spot DNA without whole genome amplification. Hum Mutat. 2018;39(1):167–171. doi: 10.1002/humu.23356.
[72] Ding Y, Owen M, Le J, et al. Scalable, high quality, whole genome sequencing from archived, newborn, dried blood spots. NPJ Genom Med. 2023;8(1):5. doi: 10.1038/s41525-023-00349-w.
[73] Nagy AL, Csáki R, Klem J, et al. Minimally invasive genetic screen for GJB2 related deafness using dried blood spots. Int J Pediatr Otorhinolaryngol. 2010;74(1):75–81. doi: 10.1016/j.ijporl.2009.10.021.
[74] Hollegaard MV, Grauholm J, Nielsen R, et al. Archived neonatal dried blood spot samples can be used for accurate whole genome and exome-targeted next-generation sequencing. Mol Genet Metab. 2013b;110(1-2):65–72. doi: 10.1016/j.ymgme.2013.06.004.
[75] Poulsen JB, Lescai F, Grove J, et al. High-quality exome sequencing of whole-genome amplified neonatal dried blood spot DNA. PLoS One. 2016;11(4):e0153253. doi: 10.1371/journal.pone.0153253.
[76] Winkel BG, Hollegaard MV, Olesen MS, et al. Whole-genome amplified DNA from stored dried blood spots is reliable in high resolution melting curve and sequencing analysis. BMC Med Genet. 2011;12(1):22. doi: 10.1186/1471-2350-12-22.
[77] Needham BL, Hicken MT, Govia IO, et al. Maternal social disadvantage and newborn telomere length in archived dried blood spots from the Michigan neonatal biobank. Biodemography Soc Biol. 2017;63(3):221–235. doi: 10.1080/19485565.2017.1300520.
[78] Barco S, Risso FM, Bruschettini M, et al. A validated LC-MS/MS method for the quantification of piperacillin/tazobactam on dried blood spot. Bioanalysis. 2014;6(21):2795–2802. doi: 10.4155/bio.14.205.
[79] Rovito R, Warnatz HJ, Kiełbasa SM, et al. Impact of congenital cytomegalovirus infection on transcriptomes from archived dried blood spots in relation to long-term clinical outcome. PLoS One. 2018;13(7):e0200652. doi: 10.1371/journal.pone.0200652.
[80] Cunningham S, O'Doherty KC, Sénécal K, et al. Public concerns regarding the storage and secondary uses of residual newborn bloodspots: an analysis of print media, legal cases, and public engagement activities. J Community Genet. 2015;6(2):117–128. doi: 10.1007/s12687-014-0206-0.
[81] Zakaria R, Allen KJ, Koplin JJ, et al. Advantages and challenges of dried blood spot analysis by mass spectrometry across the total testing process. EJIFCC. 2016;27(4):288–317.
[82] Lehmann S, Delaby C, Vialaret J, et al. Current and future use of "dried blood spot" analyses in clinical chemistry. Clin Chem Lab Med. 2013;51(10):1897–1909.) doi: 10.1515/cclm-2013-0228.
[83] Lombardo S, Seedat F, Elliman D, et al. Policy-making and implementation for newborn bloodspot screening in Europe: a comparison between EURORDIS principles and UK practice. Lan Reg Health Eur. 2023;33:100714. doi: 10.1016/j.lanepe.2023.100714.

[84] Morrison A, Polisena J, Husereau D, et al. The effect of english-language restriction on systematic review-based meta-analyses: a systematic review of empirical studies. Int J Technol Assess Health Care. 2012;28(2):138–144. doi: 10.1017/S0266462312000086.

[85] Bhattacharya K, Wotton T, Wiley V. The evolution of blood-spot newborn screening. Transl Pediatr. 2014;3(2):63–70. doi: 10.3978/j.issn.2224-4336.2014.03.08.

[86] Downing M, Pollitt R. Newborn bloodspot screening in the UK – past, present and future. Ann Clin Biochem. 2008;45(Pt 1):11–17. doi: 10.1258/acb.2007.007127.

[87] Grüner N, Stambouli O, Ross RS. Dried blood spots–preparing and processing for use in immunoassays and in molecular techniques. J Vis Exp. 2015;(97):e52619. doi: 10.3791/52619.

[88] Wilson JMG, Jungner G. Principles and practice of screening for disease. Geneva: WHO; 1968. Available from: http://www.who.int/bulletin/volumes/86/4/07-050112BP.pdf