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Review

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Multi-omics analysis from archival neonatal dried blood spots: limitations and opportunities

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Abstract: Newborn screening (NBS) programs operate in many countries, processing millions of dried bloodspot (DBS) samples annually. In addition to early identification of various adverse health outcomes, these samples have considerable potential as a resource for population-based research that could address key questions related to child health. The feasibility of archival DBS samples for emerging targeted and untargeted multi-omics analysis has not been previously explored in the literature. This review aims to critically evaluate the latest advances to identify opportunities and challenges of applying omics analyses to NBS cards in a research setting. Medline, Embase and PubMed databases were searched to identify studies utilizing DBS for genomic, proteomic and metabolomic assays. A total of 800 records were identified after removing duplicates, of which 23 records were included in this review. These papers consisted of one combined genomic/metabolomic, four genomic, three epigenomic, four proteomic and 11 metabolomic studies. Together they demonstrate that the increasing sensitivity of multi-omic analytical techniques makes the broad use of NBS samples achievable for large cohort studies. Maintaining the pre-analytical integrity of the DBS sample through storage at temperatures below −20 °C will enable this important resource to be fully realized in a research capacity.

Keywords: biobank; dried blood spot(s); emerging technologies; epigenomics; genomics; GenV; metabolomics; newborn screening; omics; proteomics.

Introduction

Understanding interrelated biological impacts on early life-course health and other outcomes requires universally available samples, ideally in the newborn period, that are valid for omics analyses. Until now, only the first of these two requisites have existed, in the form of newborn bloodspot screening (NBS).

NBS are clinical screening programs offered to millions of babies globally whose primary purpose is to identify a set of treatable medical conditions with potentially grave implications [1]. Performed on a dried blood spot(s) (DBS) collected onto a Guthrie card soon after birth, it is analysed for specified biological markers of inborn errors in metabolism (IEMs) that, if detected early, can vastly improve the rate of morbidity and mortality [2]. Whilst DBS are conventionally used in the setting of neonatal screening, they serve as a fitting medium for future research in view of their advantages: (a) ease of collection and transport; (b) smaller volume of blood required compared to traditional venepuncture; (c) high analyte conservation in long term storage; and (d) perhaps most important of all – whole population collection and storage, often for many years.

Whilst the intent of NBS programs is not to support research, a number of jurisdictions have considered research access (always with informed consent) and the development of biobanks for their archival DBS cards [3–6]. Biobanks represent a collection of appropriately stored biological samples for potential future research use, that is governed by institutional and/or legislative regulations. A scoping study conducted in 2017, estimated that approximately 40% of the 2.1 million DBS samples stored in the Danish NBS Biobank had contributed to published research [7]. With appropriate biobank storage and sample volume, NBS biobanking has the

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potential to answer important early life research questions at a population level.

Generation Victoria (GenV) represents a state-wide program led by the Murdoch Children's Research Institute (MCRI) that aims to facilitate research on children's health and development problems [8]. With informed written consent from parents, GenV plans to collect and store punches from DBS cards from approximately 100,000 Victorian infants over a two-year period. Future use of these limited samples in biological assays must be carefully considered to fully realise the potential of such valuable resources.

Recent advances in the sensitivity and cost of a range of omics technologies has greatly improved the analytical sensitivity for a range of biological measures, enabling application of such approaches, via microsampling, to sample such as DBS. In conjunction with the rapidly evolving practices in bioinformatics, omics technologies have the capacity to generate and analyse vast pools of data for specific biological components. These include, but are not limited to, information carried by genes (genomics), RNAs (transcriptomics), proteins (proteomics) and metabolites (metabolomics) (Table 1) [9]. Whether it be targeted or untargeted, these analytical techniques possess extensive clinical utility and it is anticipated that novel breakthroughs in disease pathology and diagnosis can be expedited with these emerging tools. Despite this, there is currently no publication synthesising existing literature regarding application of multi-omics technology on DBS in the context of large cohort population studies.

The aim of this review is to critically evaluate relevant literature to identify potential opportunities and limitations of using NBS cards for omics analyses, and thus determine the suitability of this procedure for our GenV biobank. In addition, we will discuss the timing of performing this analysis and establish whether it is beneficial to commence analysis of the banked samples immediately, using current technologies, or to wait for further translation of emerging technologies.

Table 1: Classification of omics.

Omics	Molecule	Description	Example NBS targeted omics applications	Example literature applications
Genomics	DNA	Structural and functional analysis of genome. Genetic profiling and mapping.	Cystic fibrosis transmembrane conductance regulator mutations–second tier panel	[10–13]
Epigenomics	DNA	Covalent methylation of DNA.	Currently not applicable	[14–16]
Transcriptomics	mRNA	RNA sequencing, gene expression, and transcriptional regulation.	Currently not applicable	[17, 18]
Proteomics	Proteins and peptides	Protein identification, quantification, and post-translational modification.	Haemoglobinopathies e.g. sickle cell disease	[19–22]
Metabolomics[a]	Metabolites	Profiling of metabolites, hormones and signalling molecules.	Amino acids and acylcarnitine disorders e.g. medium chain acyl carnitine deficiency detected by an increase in C8-acylcarnitine	[23–28]
Lipidomics[b]	Lipids	Quantitative measurement of lipids and identifying alterations in lipid metabolism.	Not routine e.g. peroxisomal disorders	[26, 29]
Glycomics[b]	Carbohydrates	Systematic study of glycans (sugars).	Currently not applicable	[30, 31]
Steroidomics[bc]	Steroids	Study of steroids in an organism.	Disorders of sex development e.g. congenital adrenal hyperplasia due to 21 hydroxylase deficiency- second tier panel with an increase in 21 deoxycortisol and other steroids.	[32, 33]
Exposomics	Exposures	Study of the interaction between all internal/external exposures and health.	Currently not applicable	[34]

The overarching classes of omics categorised according to their approximate chronological synthesis and function. For newborn bloodspot screening, targeted metabolomic and steroidomic applications are routine for many jurisdictions. To a lesser extent, targeted proteomics is applied for hemoglobinopathy screening. A specific genomic panel for cystic fibrosis screening is applied as a second-tier panel in some jurisdictions based on prevalence. We predict that expanded targeted genomics panels will be incorporated into NBS in the coming decade. The composition of these panels is currently not harmonised between NBS jurisdictions. [a]Targeted panel of amino acids and acylcarnitine's routinely available for NBS applications since early 2000s. [b]Subfield of metabolomics. [c]Targeted steroid panel for NBS applications to improve specificity for congenital adrenal hyperplasia (CAH) screening has more recently become available due to improvements in mass spectrometry instrument sensitivity. This has led to improved positive predictive value for CAH screening.

Literature review

Search strategy

We performed a systematic search (23/07/2021) on the following electronic databases: Medline, Embase and PubMed. Search terms covered combinations of expressions used to describe the sample (e.g., dried blood spot, neonatal blood spot, newborn blood spot, archival blood spot) and the analytical technologies (e.g., omic, multiomic, genomic, proteomic, metabolomic, lipidomic). The full search string for each database is available in Table 2. Title and abstract were first screened, followed by full text review of relevant studies. Reference lists were also hand searched for potential inclusion.

Eligibility criteria

Included studies, all from primary references, featured application of omics analyses on human dried blood spots in English, dating from 2011 to current due to the rapidly

Table 2: Search strategy for the three databases interrogated.

Steps	OVID medline	Embase	Pubmed
1	*Dried blood spot testing/	*Dried blood spot testing/	"Dried-blood-spot*" OR "dry-blood-spot*" OR "neonatal-blood-spot*" OR "newborn-blood-spot*" OR "newborn-screen*" OR "neonatal-screen*"
2	(Dried-blood-spot* or dry-blood-spot* or neonatal-blood-spot* or newborn-blood-spot* or archival-blood-spot* or newborn-screen* or neonatal-screen*).tw,kf.	(Dried-blood-spot* or dry-blood-spot* or neonatal-blood-spot* or newborn-blood-spot* or archival-blood-spot* or newborn-screen* or neonatal-screen*).tw,kw,dq.	"Omic*" OR "genomic*" OR "proteomic*" OR "metabolomic*" OR "transcriptomic*" OR "phenomic*" OR "lipidomic*" OR "steroidomic*" OR "glycomic*" OR "exomic*"
3	Exp *genomics/or exp *metabolomics/or exp *phenomics/	*Omics/or exp genomics/or lipidomics/or exp metabolomics/or *multiomics/or peptidomics/or exp proteomics/or exp transcriptomics/	NOTNLM OR publisher [sb] OR inprocess [sb] OR pubmednotmedline [sb] OR indatareview [sb] OR pubstatusaheadofprint
4	(Genomic*or proteomic* or metabolomic* or transcriptomic* or phenomic* or lipidomic* or steroidomic* or glycomic* or exomic* or omic*).tw,kf.	(Genomic* or proteomic* or metabolomic* or transcriptomic* or phenomic* or lipidomic* or steroidomic* or glycomic* or exomic* or omic*).tw,kw,dq.	"Animal" OR "animals" OR "rat" OR "rats" OR "mouse" OR "mice" OR "rodent*" OR "swine" OR "porcine" OR "murine" OR "sheep" OR "lamb" OR "lambs" OR "pig" OR "pigs" OR "piglet" OR "piglets" OR "rabbit" OR "rabbits" OR "cat" OR "cats" OR "dog" OR "dogs" OR "cattle" OR "bovine" OR "monkey" OR "monkeys" OR "trout" OR "marmoset" OR "marmosets"
5	(1 or 2) and (3 or 4)	(1 or 2) and (3 or 4)	(#1 AND #2 AND #3) NOT #4
6	(Exp animals/or (rat or rats or mouse or mice or rodent* or swine or porcine or murine or sheep or lamb or lambs or pig or pigs or piglet or piglets or rabbit or rabbits or cat or cats or dog or dogs or cattle or bovine or monkey or monkeys or trout or marmoset or marmosets).ti.) not human*.sh.	Limit 5 to (conference abstract or conference paper or "conference review" or editorial or letter)	Limited to English; in the last 10 years Excluded: Books & documents OR case reports OR editorial OR guideline OR letter OR practice guideline
7	Limit 5 to (case reports or comment or editorial or guideline or letter or practice guideline)	(Rat or rats or mouse or mice or rodent* or swine or porcine or murine or sheep or lamb or lambs or pig or pigs or piglet or piglets or rabbit or rabbits or cat or cats or dog or dogs or cattle or bovine or monkey or monkeys or trout or marmoset or marmosets).ti. and animal experiment/	
8	5 not (6 or 7)	Animal experiment/not (human experiment/or human/)	
9	Limit 8 to (English language and yr="2011–current")	5 not (6 or 7 or 8)	
10		Limit 9 to (English language and yr="2011–current")	

progressing field of omics research. Case reports, comments, conference papers, editorials, letters, and practice guidelines were excluded as they either contribute little in terms of robust research or are out of date. Publications were further filtered by excluding studies focused on purely serum, plasma or other dried fluid matrices (e.g., urine) as opposed to whole blood, and studies relating to public health policies or populations that do not include newborns.

Study selection

Two independent reviewers (YZ and YM) autonomously screened all the titles and abstracts of the identified studies. Following the initial screening phase, each reviewer independently evaluated the full texts of accessible reports to assess their eligibility for inclusion in the final review. Cases of dissensus were resolved in discussion with a third reviewer (RG).

Data extraction

Analysis (by YZ) involved extraction of fundamental properties of the experimental study and sample preparation based on the information as follows: sample collection, filter paper, storage temperature, sample size, omic type, analytical technique, research objectives, and key findings. Study limitations were also noted if present. A summary of the core outcomes resulting from this synthesis is available in Table 3.

Results

In total, 1,363 publications were identified from the preliminary literature search, including 414 from Medline, 471 from Embase, 464 from PubMed, and 14 from grey literature. After removal of 563 duplicates, 800 papers remained. This list was refined through successive screening of title and abstract (reducing the list to 187) and then the full text (leaving 23 for inclusion). Detailed specifications of the search strategy are illustrated in a PRISMA diagram [41] (Figure 1).

All 23 papers underwent full data extraction [10–16, 19–28, 35–40]. Whatman 903 Protein Saver cards were used in the majority (n=13) for depositing DBS samples. The types of omics analyses gathered are: one combined genomic/metabolomic, four genomic, three epigenomic, four proteomic and 11 metabolomic (Table 3). Of the proteomic studies, three were untargeted and one targeted, whereas there were seven and four in the metabolomic studies respectively.

Sample size

The included studies implemented various DBS sample sizes of 3–3,000 excluding unknowns. Punch diameter ranged from 2–10 mm with a mode of 3.2 mm, typically performed as 1–5 punches from each DBS up to a maximum of 30 (Tables 3).

Genomic outcomes

Analytical techniques used for genomic studies include next generation sequencing, spectrophotometer, Qubit fluorometer and Illumina arrays. Whole genome amplified DNA from archived neonatal DBS samples was found to provide results of equivalent quality to genomic DNA, however, the concentration and purity of extracted genomic DNA may be subject to variation from the cellulose component of filter paper, small blood volume and storage duration, etc. [11, 13].

Epigenomic outcomes

Infinium HumanMethylation450 and Infinium HumanMethylation27 were employed in epigenomic studies. No statistically significant difference ($p<0.001$) was detected between the methylation profiles of the whole blood sample and DBS, in addition to minimal change of the global DNA methylation profiles despite storage of the neonatal DBS samples for 26–28 years [14]. Identification of specific loci associated with participant characteristics could be achieved through epigenomic analyses [15, 16].

Proteomic outcomes

Liquid chromatography with tandem mass spectrometry (LC-MS/MS), multiple reaction monitoring MS (MRM-MS) and antibody array platforms (sandwich, quantitative and biotin-label-based) formed the basis of examining proteomes. The accessibility of identifiable proteins in plasma, serum and whole blood with the dried fluid spot equivalents were found to be highly correspondent (83%, 88% and 70% respectively according to Chamber's study) [19, 21]. A total of 120–253 unique proteins were identified

Table 3: Summary of omics analyses on dried blood spots, identified by systematic literature search.

Author (year); journal	Sample collection	DBS sample size; punch size	Filter paper; storage temperature	Type of Omic; analytical technique	Aim of study	Key findings	Limitations
Kerkhofs et al. (2020)	Not stated	168 DBS of 97 patients with known IEMs.	Not stated	Genomic & metabolomic, untargeted	To develop a cross-omics method to improve the diagnostic yield for IEM.	For accurate prioritisation of disease-causing genes in IEMs, it is essential to consider not only the proteins involved in the primary reaction, but a larger network of potentially affected metabolites.	Genes without a gene-specific metabolic set cannot be accounted for, even if they might cause IEMs.
Metabolites [10]		30 DBS from control patients without IEMs. Size and number of punches per sample not stated.	Not stated	NGS: WES and DI-HRMS		Diagnostic value of the cross-omics method was highest for metabolites that participate in up to 15 reactions.	
Hollegaard et al. (2013)	1. Reference DBS: Venous blood sample from two adult siblings, male and female. 50 µL was transferred to filter paper, stored over three years at −20 °C. 2. Neonatal DBS: Samples from the same individuals were isolated from the DNSB.	8 DBS	Whatman 903 protein saver card, Danish DBS cards	Genomic	To investigate whether wgaDNA extracted from 3.2 mm discs of archived DBS can be used for reliable WES and WGS.	Error rates of the three sample types strongly indicate that the wgaDNA samples provide SNV results that are as reliable as the unamplified high quality gDNA samples.	Small sample set due to difficulty in obtaining neonatal-adult matched samples.
Molecular Genetics and Metabolism [11]		Two 3.2 mm punches from each of the reference and neonatal DBS samples.	−20 °C	NGS: HumanOmni2.5-quad BeadChip array (Illumina Inc.)		Reliable WGS and WES can be conducted on archived neonatal DBS samples using only a fraction of the accessible material. Potential for neonatal DBS samples in future genetics research, diagnostics and screening projects.	
Kumar et al. (2018)	DBS samples collected during health check-up at Bangalore centre between 2005 and 2007 and stored at 4 °C.	3000 DBS	Whatman 903 protein saver card	Genomic	To optimise the methods of gDNA extraction from stored DBS and explore its utility in large scale epidemiological studies.	Extracted gDNA amount 2.15–24 ng/µl is insufficient for whole genome sequencing or genotyping.	Degree of degradation expected from 5–8 years' storage at 4 °C and temporary RT storage.

Table 3: (continued)

Author (year); journal	Sample collection	DBS sample size; punch size	Filter paper; storage temperature	Type of Omic; analytical technique	Aim of study	Key findings	Limitations
Gates Open Research [13]	This was followed by transportation at RT to Mumbai at RT in 2013 and stored at −80 °C until laboratory experiments conducted in 2016.	6 mm diameter punches obtained from 1-4 DBS.	−80 °C	Spectrophotometer, Qubit fluorometer		However, these can be achieved by whole genome amplification of extracted gDNA to increase template copy number. DBS potentially a robust sample source for bio banking in field epidemiological studies.	Large variation in concentration and purity of extracted gDNA due to interference from cellulosic component of filter paper, small blood volume, long term storage, loss during storage, loss during assays, cell debris etc.
St. Julien et al. (2013)	DBS extracted from n=1773 BPD cases and controls (921 cases, 852 controls), retrieved from the californian NBS program.	Exact number of DBS not stated.	Guthrie card	Genomic	To determine if unamplified genomic DNA extracted from DBS could be used for genome-wide SNP genotyping in a case-control study investigating bronchopulmonary dysplasia.	Sufficient gDNA was extracted from a 96 well protocol providing robust, high accuracy, genome-wide SNP genotyping of DBS.	Wide range of gDNA yield which may reflect differences in leukocyte levels, gDNA stability or differences in Guthrie card construction.
PLoS ONE [35]		~30 × 2 mm diameter punches obtained from each 1.2 cm diameter DBS.	Not stated	Qubit fluorometer, Illumina		Unamplified gDNA can be used for analyses that may be problematic for wgaDNA due to potential DNA amplification bias. wgaDNA is preferred when only a small fraction of DBS is available, or DNA isolation time is critical. Costs are comparable between the two.	
Winkel et al. (2011)	Whole blood from n=10 patients with lone atrial fibrillation (mean age 22.3 years); matched newborn DBS samples from DNSB.	Exact number of DBS not stated.	Danish NBS cards	Genomic	To compare melting curves and sequencing results from wgaDNA derived from DBS samples with gDNA derived from whole blood.	Altered melting curves present in 85 and 81 of the wgaDNA and gDNA samples respectively.	Possibility of loss of heterozygosity in wild type alleles in low concentrated samples.
BMC Medical Genetics [12]		2 × 3.2 mm disc punches from each DBS.	−24 °C	HRMCA and sequencing analysis		Exact same 31 variants found in wgaDNA and gDNA groups.	

Table 3: (continued)

Author (year); journal	Sample collection	DBS sample size; punch size	Filter paper; storage temperature	Type of Omic; analytical technique	Aim of study	Key findings	Limitations
						Reliable use of tri-amplified DNA in HRMCA and direct sequence analysis as an alternative to whole blood.	
Hollegaard et al. (2013) Molecular Genetics and Metabolism [14]	1. Reference DBS: Venous blood sample from two adult siblings. 50 µL was transferred to filter paper, stored over three years at −20 °C. 2. Neonatal DBS: Samples from the same individuals were isolated from the DNSB.	8 DBS 2 × 3.2 mm punches from each of the reference and neonatal DBS samples.	Whatman 903 protein saver card, Danish DBS cards −20 °C	Epigenomic Infinium HumanMethylation27 (Illumina Inc.)	To address whether two 3.2 mm disks punched from a neonatal dried blood spot sample contain sufficient reliable DNA for genome-wide methylome profiling.	The methylation profile of the whole blood sample and the dried blood spots demonstrated no statistically significant differences ($p<0.001$). Therefore, there is sufficient material in two 3.2 mm disks for reliable methylome profiling. Storing the whole blood sample on neonatal dried blood spot filter paper for 3 years does not interfere with the outcome of the analysis. Despite 26–28 years of storage and suboptimal DNA quality, the global DNA methylation profile of the samples underwent minimal change; thus, archived neonatal dried blood spot samples remain suitable for methylome profiling.	–

Table 3: (continued)

Author (year); journal	Sample collection	DBS sample size; punch size	Filter paper; storage temperature	Type of Omic; analytical technique	Aim of study	Key findings	Limitations
Van Dijk et al. (2018)	NBS samples of n=438 children were collected as a component of the growth and insulin resistance follow-up study.	438 DBS	Guthrie card	Epigenomic	To assess whether epigenetic marks in blood of newborn children are associated with body mass index (BMI) and insulin sensitivity later in childhood.	No individual methylation site was found to be associated with obesity or insulin sensitivity measures at 5 years.	The methylome obtained from blood may not necessarily reflect the situation in tissues that play a role in obesity and T2DM development.
International Journal of Obesity [16]		3 × 3 mm punches from each card.	Not stated	Infinium HumanMethylation450 BeadChip (Illumina Inc.)		DNA methylation in 69 genomic regions at birth were associated with BMI z-scores at 5 years. In particular, a region near the non-coding RNA nc886 (VTRNA2-1) revealed a clear link with childhood BMI (P=0.001). Otherwise, methylation changes were generally small (<5%). DNA methylation was found to be associated with maternal smoking and birth weight.	Differences between individual cell populations (affected by gestational age) may affect blood methylome.
Walker et al. (2019)	Whole blood and DBS samples from n=62 participants in the Generation	100 µL (~2 DBS) per participant. Exact number of DBS not stated (estimated at ~124).	Whatman FTA card	Epigenomic	To determine whether DNA stored in DBS samples at RT can be used to generate DNA methylation profiles comparable to that of frozen EDTA tube blood samples.	DNA methylation profiles obtained from DBS samples were of good quality and highly correlated with matched EDTA tube samples.	Inability to separate sample into separate blood cell types to address cellular heterogeneity due to using Whatman FTA cards–this can be managed with algorithms.
Wellcome Open Research [15]	Scotland: Scottish Family health study collected from 2006 to 2011. DBS cards were stored at RT for 5–	Size of punches per sample not stated.	RT	Infinium HumanMethylation450 BeadChip (Illumina Inc.)		The overall rate of site detection was slightly better in DBS samples. No significant effect of storage time up to eight years on the	

Table 3: (continued)

Author (year); journal	**Sample collection**	**DBS sample size; punch size**	**Filter paper; storage temperature**	**Type of Omic; analytical technique**	**Aim of study**	**Key findings**	**Limitations**
	10 years before extraction.					success of methylation profiling. The most significant locus identified in smokers was cg05575921 in AHRR, which was hypomethylated in smokers, and has been suggested as a biomarker of smoking status. The locus cg16867657 in ELOVL2 showed positive correlation between methylation and age.	
Chambers et al. (2013)	Matching plasma, serum and whole blood samples from n=10 healthy adults (five male, five female).	15 µL spots. Exact number of DBS not stated (estimated at 10 unique DBS).	Whatman 903 protein saver card	Proteomic, untargeted	To compare protein identified in plasma, serum and whole blood with their and dried fluid spot counterparts by tandem MS.	Many proteins currently analysed via plasma/serum/whole blood by MS methods are also readily accessible in DPS, DSS and DBS samples.	Addition of E2-EDTA to prevent coagulation during storage and transport slightly alters protein expression in whole blood.
Journal of the American society for Mass spectrometry [19]		Size and number of punches per sample not stated.	Not stated	LC-MS/MS		The average number of proteins identified in whole blood and DBS samples was 223 and 253 respectively. High degree of overlap in protein accessibility between the proteins identified in plasma, serum and whole blood with their dried fluid spot equivalents (83%, 88% and 70% respectively). Proteins were unbiased with regards to	Whole blood was refrigerated instead of frozen to prevent red blood cell lysis, which may have yielded higher activity of endogenous proteases.

Table 3: (continued)

Author (year); journal	Sample collection	DBS sample size; punch size	Filter paper; storage temperature	Type of Omic; analytical technique	Aim of study	Key findings	Limitations
						various chemical and physical properties: Molecular weight, isoelectric point, aliphatic index and grand average hydrophobicity.	
Jiang et al. (2014)	Serum samples from healthy subjects (number not stated).	~6 DBS per subject.	Whatman 903 protein saver card	Proteomic, untargeted	To investigate the potential application of dried blood spot samples in protein expression profiling using 3 types of antibody array technology.	Detection of circulating proteins in DBSS correlated well for both low- and high-abundance proteins in comparison to results obtained from paired serum samples.	Some discrepancy between DBS and serum samples in a few markers.
Journal of Immunological Methods [21]	Paired DBSS were prepared by pricking the two middle fingers of the same subject, and blotting 3 drops per finger.	10 mm punches.	−80 °C	Antibody array platforms		Three antibody array platforms produced equivalent results (sandwich-based antibody arrays, quantitative antibody arrays and biotin-label-based antibody array).	Reasons: Protein marker shielding; eluted samples from DBS may not contain these markers as they remained on the filter paper; underlying differences between serum and whole blood samples; smaller volume of blood in DBS.
Martin et al. (2013)	n=3 normal adult DBS specimens collected via finger prick onto Guthrie cards.	3 DBS	Ahlstrom grade 226 filter paper	Proteomic, untargeted	To analyse dried blood spots via an untargeted bottom-up proteomics approach and discuss the potential use of identified proteins as biomarkers in screening programs.	120 proteins identified via simple automated tryptic digestion, with a concentration range over four orders of magnitude. Most abundant were haemoglobin and albumin.	–
Journal of the American society		Size of punches per sample not stated.	RT (23–24 °C)	LC-MS/MS		Several proteins are potential disease biomarkers in newborn	

Table 3: (continued)

Author (year); journal	Sample collection	DBS sample size; punch size	Filter paper; storage temperature	Type of Omic; analytical technique	Aim of study	Key findings	Limitations
for Mass spectrometry [20]						screening programs. E.g. ceruloplasmin and alpha-1-antitrypsin. Optimum digestion time was 1 h, which offers potential for automated parallel analysis on a large scale. Suggestion of using proteases with broader specificity to enable higher protein identification.	
Eshghi et al. (2020)	Whole blood collected from commercial vendors.	100 DBS	Whatman 903 protein saver card	Proteomic, targeted	To develop precise, sensitive and multiplexed methods for measuring endogenous protein concentrations in DBS.	Repeatable quantification of 200 proteins in DBS via MRM assays.	Not all MRM assays could be tested for parallelism, but of those tested, majority displayed parallelism indicating accurate measurement of protein concentration.
Molecular & cellular proteomics [22]	DBS from n=20 caucasian males, 50 µL of blood per spot for five spots.	5 × 6 mm punches per DBS.	RT	MRM-MS		Measured protein concentrations remained stable in DBS stored at ambient temperatures for up to 2 months.	Drying process induced conformational change in protein structure that may have affected digestion efficiency.
Petrick et al. (2017)	n=106 control DBS samples collected from 1985 to 2006 for the California childhood leukemia study, obtained from the California birth registry.	106 DBS	Not stated	Metabolomic, untargeted	To develop a quantitative untargeted analysis method of archived newborn DBS in an epidemiological study	Detection of significant associations with ethnicity (3 metabolites) and birth weight (15 metabolites) of the >1,000 small molecules tested.	Age of spot affects extraction efficiency.
Metabolomics [25]		4.7 mm punches (equivalent to ~8 µL whole blood). Number of punches per sample not stated.	−20 °C	LC-HRMS		Adjusted for Hct variation through measurement of potassium in each punch.	

Table 3: (continued)

Author (year); journal	Sample collection	DBS sample size; punch size	Filter paper; storage temperature	Type of Omic; analytical technique	Aim of study	Key findings	Limitations
Petrick et al. (2019)	n=656 DBS collected from 1985 to 2005 for the California childhood leukemia study, obtained from the California birth registry.	656 DBS	Not stated	Metabolomic, untargeted	To discover associations between foetal metabolomic (lipidomic) NBS and ALL via untargeted analysis.	Noted putative lipid modifications associated with ALL that differed between early and late diagnosis of ALL and were negatively correlated with breastfeeding duration. This suggests that breastfeeding is protective for ALL.	Limited to a single sample of ALL cases and matched controls; requires validation in independent cohorts.
Cancer letters [26]	These include n=332 who developed acute lymphoblastic leukaemia (ALL) and n=324 healthy controls.	4.7 mm punches (equivalent to ~8 µL whole blood). Number of punches per sample not stated.	−20 °C	LC-HRMS			Annotations of lipid features limited by LC-HRMS platform and online databases.
Rus et. al (2021)	Stability study (n=27)	60 µL spots. Exact number of DBS not stated.	CentoCard	Metabolomic, untargeted	To increase the efficiency and reliability of the developed methods for biomarker development for rare diseases [1]; assessing stability of metabolites over time [2]; investigating suitable extraction solvents for metabolomics studies.	Storage at −20 °C over six years in six controls has a major impact on metabolite stability.	Limited number of samples.
Metabolites [27]	Extraction study (n=33). Cumulative study (n=95)	5 × 3.2 mm centre punches from each card.	−20 °C	LC-MS/MS		Variations in stability also occur in storage at RT compared to −20 °C for three days. Metabolite yield is affected by storage conditions, sample age and type. Methanol was the most effective extraction buffer for metabolome coverage and detects high-abundance metabolites.	
Tobin et al. (2021)	Archived paired maternal plasma and DBS from n=79 pregnant women living with HIV.	158 DBS	Whatman 903 protein saver card	Metabolomics, untargeted	To compare the ability of DBS- and plasma-based assays to characterise maternal metabolites in pregnant women with HIV.	Total of 984 biochemicals detected across plasma and DBS samples, of which 627 were detected in both.	Relatively small sample sizes make it difficult to draw meaningful conclusions from HIV study.

Table 3: (continued)

Author (year); journal	**Sample collection**	**DBS sample size; punch size**	**Filter paper; storage temperature**	**Type of Omic; analytical technique**	**Aim of study**	**Key findings**	**Limitations**
Metabolomics [23]		2 × 6 mm centre punches extracted per card.	− 20 °C,	UPLC-MS/MS		Metabolite profiles were strongly concordant after standardisation.	
			− 80 °C			Lipids were preferentially detected in plasma, and dipeptides in DBS. Non-standardised metabolic profiles were markedly different between paired plasma and DBS samples, but the observed bias was removed when standardised values were applied.	
Trifonova et al. (2019)	All samples collected from the same person (male) at the same time for each matrix.	Whatman 903 paper imprinted with four half-inch circles.	HemaSpot-HF blood collection Device;	Metabolomic, untargeted	To determine the comparability of four different types of DBS sampling for analysis of the global metabolite profile.	Analytical performance of all sampling materials showed consistent results across variety of detected metabolites with only minor differences.	Specific contaminants from the DBS matrix may affect ionisation of compounds, and thus reduce detectable limit and resulting metabolite profile– may be attenuated with appropriate data processing.
		Card Immuno-Health imprinted with five half-inch circles.	Whatman 903 protein saver snap Apart card;			Significant difference of metabolite profiles after four weeks of storage at RT. Degradation process was initiated at different time points for different sampling material.	
Metabolites [28]			Card ImmunoHealth; Glass fiber strip ImmunoHealth RT (20–22 °C)	Direct injection MS		Minor differences in analytical performance for most clinically relevant compounds.	

Table 3: (continued)

Author (year); journal	Sample collection	DBS sample size; punch size	Filter paper; storage temperature	Type of Omic; analytical technique	Aim of study	Key findings	Limitations
	20 µL aliquots pipetted for each material.	Size and number of punches per sample not stated.					
Ward et al. (2021) Journal of mass spectrometry [24]	Exact number of samples not stated. BioIVT whole blood samples in EDTA tubes spotted as DBS. 50 µL of blood pipetted per DBS.	3 DBS for each optimisation condition. 5 × 3 mm punches per DBS, with one central and four peripheral locations.	Whatman 903 protein saver card −20 °C	Metabolomic, untargeted LC-MS/MS	To analyse the potential of DBS as a biosample storage device for non-targeted LCMS compared to plasma samples and assess intra-spot variation and stability of metabolites in DBS stored at room temperature over seven days.	DBS captures a metabolomic profile comparable to traditional plasma samples. Over 80% of metabolites were chemically stable in DBS stored at room temperature for up to a week. Polar metabolites changed minimally, whereas lipophilic molecules were more prone to fluctuation. Small polar bioactive molecules had the greatest change due to non-enzymatic oxidation.	–
Yu et al. (2020) Journal of Pharmaceutical & Biomedical analysis [36]	DBS obtained from Swedish NBS program. Three anonymous samples from different subjects collected on five different years: 1998, 2005, 2008, 2015, 2018 (n=15).	>15 DBS Single 5 mm punches.	Guthrie card (schleicher & schuell 2,992, schleicher & schuell 903, Ahlströmer 226) −80 °C	Metabolomic, untargeted LC-HRMS	To propose a proof-of-concept workflow for the normalisation of metabolite measurements from DBS archived for decades at 4 °C.	Feasibility of generating robust metabolomics profiles of archived DBS from a refrigerated repository of up to 21 years. Hb measured by the Hb sodium lauryl sulfate method can effectively minimise variation associated with DBS spot age and blood volume and may be incorporated into normalisation schemes for DBS metabolomics.	Limited by small sample size.

Table 3: (continued)

Author (year); journal	Sample collection	DBS sample size; punch size	Filter paper; storage temperature	Type of Omic; analytical technique	Aim of study	Key findings	Limitations
Jacob et al. (2018) Analytica chimica Acta [37]	Whole blood from 20 healthy adult individuals; 100 μL aliquoted into Eppendorf tubes; 100 μL as DBS. 200 healthy control DBS samples from newborn screening panel in Saudi Arabia. 56 abnormal DBS samples from patients with 8 different IEM.	Estimate of 80–100 DBS excluding control and abnormal samples. 5 × 3.1 mm punches from each DBS.	Guthrie card (Perkin Elmer 226) −80 °C	Metabolomic, targeted LC-MS/MS	To develop an LC-MS/MS-based comprehensive targeted metabolomics panel of 220 analytes involved in various clinical and biochemical genetic disorders.	For DBS samples, most metabolites were stable with minimal variance when analysed after 2 and 4 months. DBS showed maximum quantifiable level of metabolic analytes compared to serum and blood samples. This targeted panel can be useful as a second-tier assay and as a screening tool for IEM.	–
Li et al. (2020) Metabolites [38]	DBS samples prepared by either pipetting 70 μL of Li-heparin whole blood or EDTA whole blood onto the card, or directly via fingerprick.	Total of 184 DBS divided between different studies. Single 6.3 mm punches.	Whatman 903 protein saver card −80 °C	Metabolomic, targeted LC-MS/MS	To develop a DBS targeted metabolomic workflow detecting 430 metabolites in a single injection LS-MS/MS analysis.	Excellent correlation between DBS metabolome and other blood sample types. Blood volume and hct variations had no significant impact on the assay for a spot volume >60 μL and hct >30%. DBS samples were most stable when stored in −80C.	Stability analysis only performed for up to one year, thus no conclusion can be drawn regarding stability beyond one year. No RT condition without desiccant or an oxygen scavenger.
Prentice et al. (2013) Future science [39]	Anonymised whole blood from heparinised blood tubes divided into three batches of 50 μL blood spots.	Exact number of DBS not stated. 3.2 mm punches.	Whatman 903 protein saver card RT (~21 °C), −20 °C, −80 °C	Metabolomic, targeted LC ESI-MS/MS	To investigate the effect of storage temperature on DBS using a prospective storage experiment over a 2-year period.	Storage at room temperature results in significant time-dependent changes in concentration for most metabolites investigated. Storage of DBS at −20 °C or −80 °C for up to 2 years was only associated with	Experiment results only apply to the metabolites detected, which was limited by instrument sensitivity. No information on the effect of moisture at various storage temperatures, nor the

Table 3: (continued)

Author (year); journal	Sample collection	DBS sample size; punch size	Filter paper; storage temperature	Type of Omic; analytical technique	Aim of study	Key findings	Limitations
		Number of punches per sample not stated.				relatively minor changes in glutamine and methionine; metabolite recovery was largely preserved. Not possible to generalise the effects of storage for all analytes. Glutamine always shows degradation with time and could be used to determine efficacy of storage conditions. Creatinine and free carnitine increase with time at ambient temperature.	benefit of using desiccant. Samples were only run in duplicate.
Zukunft et al. (2013) Chromatographia [40]	Venous blood from one male and one female volunteer collected in 9 mL EDTA tubes or lithium-heparin tubes. 80 μL aliquots of freshly taken blood were spotted onto filter paper.	Exact number of DBS not stated. 3 mm punches from the centre. One punch per DBS.	Whatman 903 protein saver card RT	Metabolomic, targeted LC-MS/MS, FIA-MS/MS	To adapt the AbsoluteIDQ (TM) p180 kit from Biocrates to the DBS matrix instead of plasma for the quantification of metabolites.	Almost the same number of metabolites were quantified in DBS compared to plasma (137 vs 155). High level of concordance between DBS and plasma metabolite concentrations. Best long-term storage of DBS at −80C to maintain metabolite stability.	–

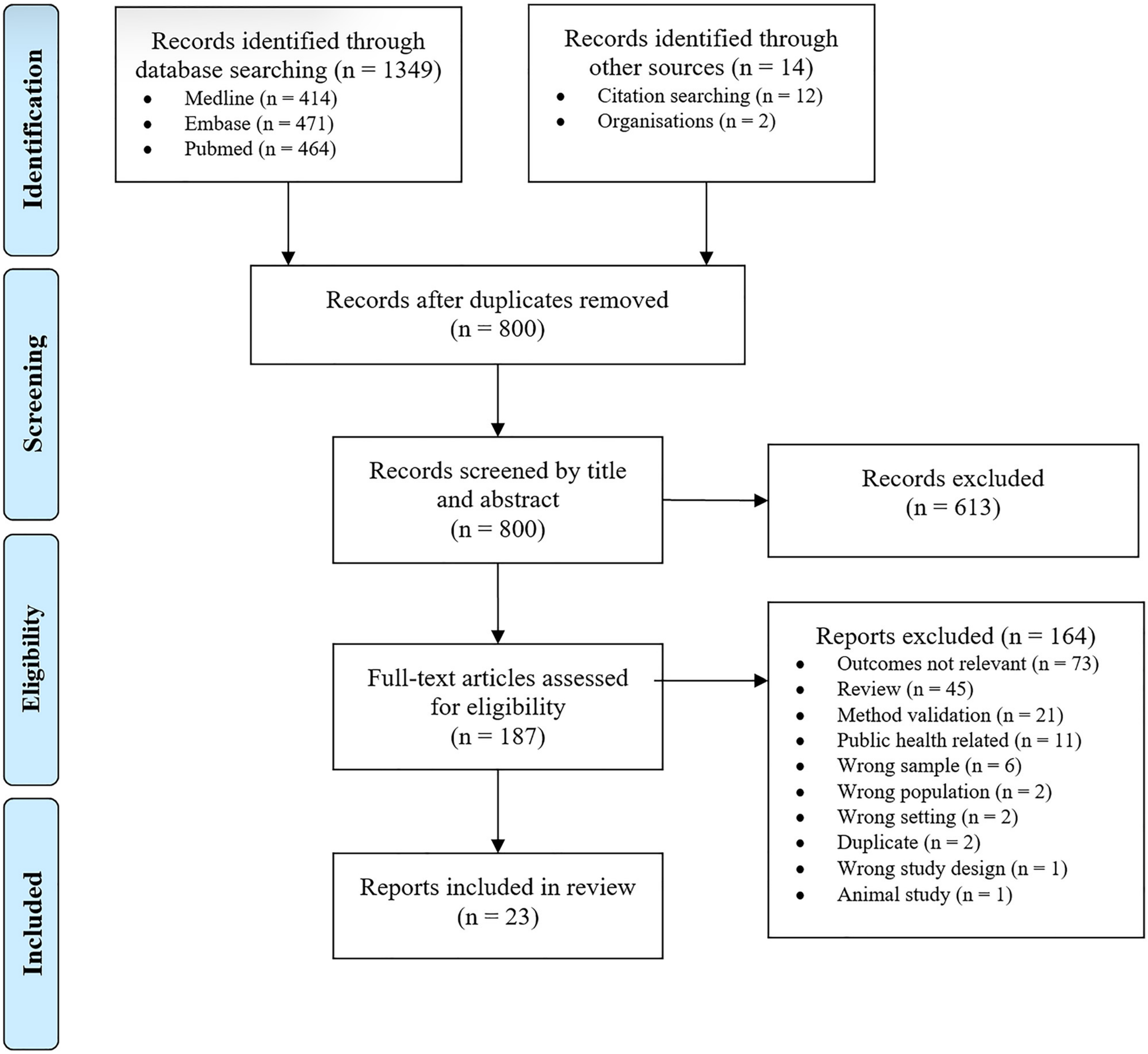

Figure 1: PRISMA flow diagram [41].
The process of study identification and selection from the systematic search is presented above.

in DBS samples, of which several may serve as potential disease biomarkers in newborn screening programs [20].

Metabolomic outcomes

LC-MS/MS is the mainstay of metabolomic analyses amongst others such as flow injection analysis, electrospray ionisation and direct injection techniques. Variable time-dependent discrepancies in metabolite stability were reported in RT and −20 °C storage for prolonged periods, where nonpolar metabolites were subject to higher fluctuations [24]. Where implemented, a storage temperature of −80 °C was the most reliable in terms of preserving metabolite composition [37–39]. The metabolomic profile of DBS samples demonstrated excellent correlation with traditional plasma samples [24, 38, 40], and may even be shown to extract a higher level of analytes [37].

Discussion

Targeted vs. untargeted omics

Omics assays can be divided into two branches – targeted and untargeted – depending on the experimental workflow

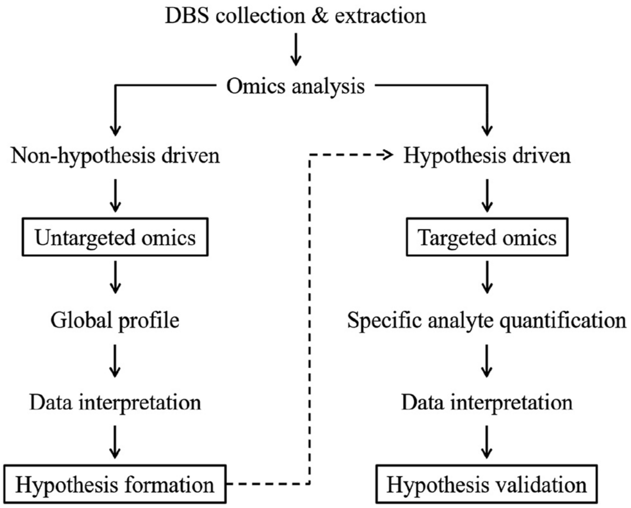

Figure 2: Schematic of untargeted vs. targeted omics workflow. By gathering global analyte profiles via untargeted omics assays, new hypotheses can be formed and employed to steer towards targeted analyses of specific analytes.

and biological information desired (Figure 2). The targeted form investigates a small number of pre-labelled analytes in a specific pathway to validate a defined hypothesis [28]. In contrast, the non-hypothesis-driven untargeted approach represents an unbiased, high-throughput analysis of all measurable analytes in a sample, often detecting 100–1000s of chemicals [28].

Clinical applications of omics

In paediatrics the term "omics" is largely associated with the diagnosis of IEMs, a group of diseases caused by defective enzyme activities in certain metabolic pathways. There is immense clinical utility in the assimilation of multi-omics data as it can link molecular changes to underlying exposures. Currently, NBS programs use targeted metabolomic platforms to determine the presence of IEM diseases [42, 43]. Examples include the measurement of the amino acid phenylalanine for Phenylketonuria or succinyl acetone for Tyrosinemia Type 1 [9]. Genomic and proteomic avenues are also used to confirm clinical hypotheses by comparing patient data to reference profiles. In the background, untargeted approaches fuel discovery of prognostic biomarkers of disease state and hence inform changes in future screening agendas. However, due to the relative novelty of these technologies, there are some concerns regarding cost, feasibility and turnover time [9]. Yet combined with biotechnological and societal development, these concerns are likely to be addressed in the future.

Opportunities and challenges of DBS analysis

DBS are now rapidly expanding the scope of clinical research as an alternative to standard venous blood sampling [12, 15, 44, 45]. As it requires only 12–15 µL of whole blood per spot, collected by nurses rather than phlebotomists, DBS are one of the most suitable blood-sampling practices for large population studies [46]. Nevertheless, there remain various challenges in DBS applications across the total testing process, especially when coupled with the multitude of omics approaches. The following section will explore the opportunities and complexities concerning the pre-analytical, analytical, and post-analytical phases of DBS sampling.

Preanalytical phase

The pre-analytical phase commences from sample collection and transportation to storage and receipt by the laboratory. Within this stage, the concerns of haematocrit, low sample volume and stability of DBS samples are highly debated in the scientific literature [47].

Influence of haematocrit

In the context of DBS analysis, there have been in-depth studies regarding the issue of haematocrit (Hct). Hct is the percentage of red blood cells per unit volume, and hence a direct reflection of blood viscosity. Variation in Hct levels impact the distribution of blood on filter paper, resulting in variable spot diameter and homogeneity [45, 48]. Despite using a fixed-size punch of DBS, high Hct samples may comprise a higher analyte concentration than that of low Hct samples [48, 49]. Hence, a specified range of Hct values is advised during the calibration of blood samples for concentration-based studies [48, 49]. Meanwhile, this is less of an issue for an assay that uses a relative ratio measure rather than discrete quantities.

Blood spot volume considerations

The minimal volume collected in DBS exacerbates the problem of finite reserves and appropriate resource allocation. Considering the possibility of unviable samples (such as through poor sampling techniques, risk of contamination and biological degradation), the number of viable specimens that reach the analytical stage may be fewer than initially intended. With only microlitres of quality biosample per spot to examine, it is of utmost importance to thoroughly plan any application in a research setting, particularly the pros and cons of sample use/loss relative to the amount/quality of data that may be generated.

Inconsistent distribution of blood spotted onto the filter cards is also a relevant concern for some downstream assays. Even with an equal Hct value, the calculated analyte concentrate from a standard punch size may be affected by unequal analyte distribution within the spotted blood [50]. The crux of this issue lies in the random error manifested by the "direct spotting" technique, which – along with Hct variation – may be solved by using a calibrated pipette to supply a fixed aliquot of blood [51]. Nevertheless, this may prove a difficult feat as the multiple tools may negate the simplicity of DBS. Newer microsampling tools (such as the hemaPEN Trajan Scientific, Ringwood Vic, Australia) are emerging, but it is unknown if they will be taken up by the market, and whether the cost to benefit ratio will be desirable [52]. As every method is accompanied by some measure of error, the question then lies in whether this error is acceptable for a given objective.

Sample stability, time and temperature

The advantages of DBS as a sample include the reasonable stability of many biomolecules once spotted onto a card, facilitating transportation and storage. However, for any specific proposed measure issues, short term vs long term stability, the temperature of storage, and the differing composition of analytes need to be considered [48].

The sample journey from collection to processing can be highly variable depending on the clinical setting and circumstances of the patient, but often takes place at room temperature (RT) [53]. It is therefore important to evaluate the stability of specific biomolecules within DBS samples at room temperature over time. A two-week study designed by Drolet et al. illustrated that carbohydrates, nucleotides and vitamins in DBS cards are most sensitive to storage at RT and 37 °C and may even be affected in as little as three days [54]. In contrast, a study conducted by Trifonova et al. assessed the stability of the metabolome at RT by measuring the level of each analyte via direct injection mass spectrometry at specified time points (7, 14, 21, and 28 days) against control samples collected on day zero [28]. No significant differences in analytical performance were noted for most clinically relevant compounds (creatine, L-glutamine, L-carnitine and glucose) [28]. Despite conflicting studies regarding RT storage, shipment of DBS generally remains at ambient conditions in most settings as the omission of the cold chain logistics enables significant cost reduction [22].

NBS are usually received within 4 days of collection. Current recommendations from Prentice et al. favour −20 °C or −80 °C for longer term storage of at least two years, although, as Trifonova et al. proposes, analyses conducted at RT within 28 days of sample collection are possible however with gradual decline in metabolite concentration [28, 39]. Several studies have demonstrated that DBS samples at −20 °C and −80 °C are stable for a minimum of 12 months for most metabolites [39, 54, 55]. The GenV recruitment phase will occur over a two-year period and it is unlikely that any analyses will be performed on samples prior to collection of the entire cohort. As such −80 °C storage appears most appropriate for maintaining the integrity of biomolecules on DBS samples within the GenV biorepository.

It is important to note however, that different analytes degrade at different rates at different temperatures. Some may begin breaking down in 1 week (e.g. creatinine), and others in 3 weeks (e.g. L-glutamine) when stored at RT [28]. As there is a certain degree of variability in metabolites, it is likely that the same applies to other analyte categories such as proteins and DNA. This should be taken into consideration during the target molecule selection process associated with applications for biobank access.

Analytical approaches and considerations

Once the DBS sample is taken out of storage, extraction and analysis can take place. Currently, mass spectrometry (MS) is the primary assay for analysis [56–58], due to its high sensitivity and specificity to detect and quantify low concentrations of analytes [47, 59]. Recent advances in bioinformatics and other analytical tools, coupled with ever increasing sensitivity of analytical platforms, are driving rapid advances in omics technology, leading to what is currently termed the "dawn of the omics" era [9]. Despite this, there is an increasing need to synthesise and evaluate these advances, prior to any translation into future clinical applications.

Genomics

DBS samples serve as a precious source of nucleic acids (DNA and RNA) for genomic and epigenomic research, albeit in very limited amounts. Approximately ~60 ng of genomic DNA (gDNA) can be extracted from a 3.2 mm DBS punch which has been a limiting factor for the application of high-throughput genetic screening [60]. However, St Julien et al. showed that ~20 ng (5 ng/μL) or more of unamplified gDNA extracted from DBS is sufficient for robust genome-wide single nucleotide polymorphism (SNP) genotyping, whilst featuring >99.99% replication frequency in duplicate extractions from the same DBS [61]. Furthermore, multiple studies, such as that of Hollegaard and colleagues in 2013, demonstrate that whole genome amplified DNA (wgaDNA) can provide a high genotypic correlation with unamplified gDNA despite using just a fraction of the sample (2 × 3.2 mm DBS punches for Hollegaard) [11, 62, 63]. WgaDNA has the added benefit of halved preparation time whilst maintaining comparable costs with conventional gDNA [61]. Hence, regardless of wgaDNA or unamplified gDNA, DBS cards provide ample material for accurate genomic analyses in addition to reserving enough sample volume to accommodate for other types of omics analyses.

Another point to highlight is the reliability of data extracted from DBS as opposed to whole blood samples. Several papers now demonstrate a high concordance between DBS and blood, whether it be single nucleotide variants (SNVs) [44] or high resolution melting curve analysis [12] in whole genome sequencing, or in DNA methylation profiles [15]. DBS are thus suitable starting material for high throughput genomic and epigenomic approaches in identifying genetic and environmental prognostic factors of disease phenotypes.

Proteomics

The quantitative analysis of endogenous proteins constituting the "proteome" has been increasingly adopted in clinical diagnostics and biomarker discovery. Proteins execute essential biological functions, therefore assessing protein malfunction can inform the aetiology of disease, as well as track disease progress and therapeutic response [59]. Numerous biomarkers of disease have been discovered via targeted analyses of the DBS matrix, including ceruloplasmin (Wilson's disease) and alpha-1-antitrypsin (alpha-1-antitrypsin deficiency) [20]. Conversely, untargeted proteomic assays can swiftly identify unique peptides and proteins of numbers up to four orders of magnitude [20]. In 2013, Martin and colleagues highlighted the potential for automated parallel analysis of proteins on a large scale by using proteases with broader specificity [20]. Whilst work is ongoing, the user-friendly interface combined with reliable output of proteomic analyses are highly favourable features that deliver consistent results.

Proteomic techniques have been under continual development, where single-target immunoassays like ELISAs have largely been replaced by multiplex technologies such as antibody arrays and MS [20]. It was not until the past decade or so that these techniques – initially designed for serum or plasma – have been approved for DBS as they demonstrate equivalent protein profiles [19, 21, 59]. The integration of DBS with antibody array technology overcomes the limitations of past immunoassay approaches by increasing output protein identification and cost effectiveness, reducing sample volume requirements, and decreasing the technical burden associated with elaborate platforms like MS [59]. However, this is still a developing field warranting additional stringent standardisation and monitoring.

Metabolomics

Compared to proteomics, metabolomic measures have been relatively well established for DBS over the last two decades [9]. Metabolomics features the identification of small molecule metabolites in the form of the metabolome – the downstream functional end-product of the genome. It represents a snapshot of the biological specimen at a particular point in time and is influenced by genetic and environmental factors. Multiple 'snapshots' of the metabolome at various intervals may be helpful to discern changes with time. The assimilation of metabolomics with genomics and proteomics can foster further understanding of the broader pathophysiological context, thereby lending more credibility to the holistic disease phenotype [37].

In targeted metabolomics, a distinctive metabolic profile is formed in a hypothesis-driven manner, facilitating the identification of disease-associated biomarkers [64]. Studies using targeted liquid and gas chromatography MS to analyse DBS samples have produced highly reliable, sensitive, and (in some cases) ground-breaking results. This includes the downregulation of sorbitol in cystic fibrosis patients [65], as well as elevated ceramide and sphingolipid compounds in paediatric acute myeloid leukaemia [66]. Such findings enable the development of novel clinical tests to inform clinical treatment decisions as well as areas of further research.

On the other hand, there is a role for untargeted metabolomics in conjunction with high resolution mass spectrometry to detect hundreds to thousands of metabolites simultaneously [57]. Courraud et al. generated chemical structural data for 1,009 metabolites via LC-MS/MS-based untargeted metabolomics from newborn DBS, and identified significant variation in their concentrations relative to gestational age, age at sampling and month of birth [67]. Given that metabolic profiles are highly influenced by external factors, these must be addressed to prevent erroneous interpretations.

Post-analytical phase

The post analytical phase involves the interpretation of the data generated. With this interpretation, consideration needs to include the pre-analytical and analytical components. Understanding the constraints of the earlier phases is essential for the robustness of the data. As an example, the paper the dried blood spots are collected on, haematocrit and sample stability are critical pre-analytical components.

The inherent properties of DBS paper prevent the separation of samples into distinct blood cell lines, yet it is important to address cellular heterogeneity in DNA methylation to avoid confounding data [15]. Fortunately, specific algorithms have been generated to adjust for this heterogeneity [68]. In studies comparing profiles from DBS and plasma samples, normalisation and standardisation processes are also imperative to remove underlying bias [23]. As such, the reliability of output data is enriched with the implementation of appropriate post-analytical algorithms.

In our view, the translation from bioinformatic output to human comprehension is a difficult yet crucial step in generating useful scientific information. The sheer amount of patient-specific data would be collected in vain if not interpreted correctly into practical measures in the clinical setting. This can be aided by incorporating omics-specific academic training into laboratory medicine courses, focusing on the extrapolation of key proteomic and metabolomic data. In doing so, high reproducibility of reliable data can be achieved at the hands of any qualified technician or biologist. In the future algorithms developed by artificial intelligence will likely also assist in clinical interpretation. However, this only becomes possible once the -omics panels are tested in large cohorts that include the short- and long-term outcomes needed to develop and refine the AI algorithms to a clinical level of precision [69, 70].

Limitations of omics review

Whilst a broad search strategy was used across three databases, more papers may exist outside of those gathered. In the absence of a meta-analysis, the evidence is less supported. Findings are only applicable to certain categories of omics as the others are less reported in the literature and thus beyond the scope of this paper (e.g., transcriptomics, exposomics). Overall, the inferences of this review are to be deliberated with due consideration.

Considerations for large population-based prospective cohort studies: GenV

The GenV biobank led from the MCRI is amassing a collection of samples from the Victorian population of births over a 2 year period [8]. This includes samples from excess NBS for all GenV families providing explicit consent to do so. This biobank will be a finite resource and as such, it is critical to determine and prioritise assays that will deliver the utmost benefit for population health, while not depleting the resource prematurely.

Based on the current literature, metabolomics is clearly established and has the requisite analytical sensitivity to be soundly utilised for DBS. Although genomics applications may be suitable for consideration of research access, one's genotype is unlikely to change throughout their lifetime, therefore it may be more appropriate to use these DBS samples to investigate biomolecules that are specifically pertinent to the newborn period. The application of DBS for proteomics however is still emerging and may be realised in due time with continued improvement in analytical sensitivity, accuracy, and robustness.

As the GenV project custodians collaborate with other services and agencies for access requests, they will need to determine the most appropriate use of this resource to translate novel pathophysiological insights into evidence-based medicine for future generations. The implementation of dedicated storage at −80 °C soon after the completion of clinical testing is a critical step to ensure the maximum stability and utility of samples for future data generation.

Conclusions

The DBS sampling method holds immense potential in the field of population-based research. It offers several advantages including simplicity, minimal volume specifications and economical transport and storage, therefore yielding profound compatibility with the GenV biobank for subsequent research. The prospect of using omics technology to assess the molecular profiles of neonatal blood samples is highly achievable and valuable to the scientific community, yet they are not necessarily equally advanced. Metabolomic approaches have been extensively substantiated in literature and can be reliably executed.

Although technology is likely to evolve as instrumentation sensitivity and specificity improve, there is sufficient evidence to validate the current use of high throughput untargeted assays, later supported by targeted analyses to pinpoint core biomarkers of neonatal disease. It would be beneficial to consolidate the output of various multi-omic techniques, however ongoing work is warranted to achieve this.

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