European Journal of Drug Metabolism and Pharmacokinetics (2023) 48:467–494
https://doi.org/10.1007/s13318-023-00846-4

REVIEW ARTICLE

Dried Blood Spots—A Platform for Therapeutic Drug Monitoring (TDM) and Drug/Disease Response Monitoring (DRM)

Nur Nabihah Binte Zailani[1] · **Paul Chi-Lui Ho**[1,2]

Accepted: 9 July 2023 / Published online: 26 July 2023

Abstract

This review provides an overview on the current applications of dried blood spots (DBS) as matrices for therapeutic drug (TDM) and drug or disease response monitoring (DRM). Compared with conventional methods using plasma/serum, DBS offers several advantages, including minimally invasiveness, a small blood volume requirement, reduced biohazardous risk, and improved sample stability. Numerous assays utilising DBS for TDM have been reported in the literature over the past decade, covering a wide range of therapeutic drugs. Several factors can affect the accuracy and reliability of the DBS sampling method, including haematocrit (HCT), blood volume, sampling paper and chromatographic effects. It is crucial to evaluate the correlation between DBS concentrations and conventional plasma/serum concentrations, as the latter has traditionally been used for clinical decision. The feasibility of using DBS sampling method as an option for home-based TDM is also discussed. Furthermore, DBS has also been used as a matrix for monitoring the drug or disease responses (DRM) through various approaches such as genotyping, viral load measurement, assessment of inflammatory factors, and more recently, metabolic profiling. Although this research is still in the development stage, advancements in technology are expected to lead to the identification of surrogate biomarkers for drug treatment in DBS and a better understanding of the correlation between DBS drug levels and drug responses. This will make DBS a valuable matrix for TDM and DRM, facilitating the achievement of pharmacokinetic and pharmacodynamic correlations and enabling personalised therapy.

Key Points

Dried blood spots have been studied extensively for therapeutic drug monitoring.

There are relatively less studies on applying dried blood spots on drug response monitoring.

With advances in analytical technology and identification of markers as drug responses utilising dried blood spots as matrices, dried blood spots can be used as a platform for pharmacokinetic and pharmacodynamic monitoring to achieve the aims of personalised therapy.

✉ Paul Chi-Lui Ho
paul.ho@monash.edu; paul.ho@nus.edu.sg

[1] Department of Pharmacy, National University of Singapore, 18 Science Drive 4, Singapore 117543, Singapore

[2] School of Pharmacy, Monash University Malaysia, Level 5, Building 2, 47500 Bandar Sunway, Selangor Darul Ehsan, Malaysia

1 Introduction

Therapeutic drug monitoring (TDM) is a valuable tool in clinical care, involving the measurement of therapeutic drug concentrations in blood samples to ensure optimal dosing and therapeutic outcomes. It is particularly important for drugs with high variability between individuals, narrow therapeutic ranges, and potential for serious side effects [1]. TDM can also help assess patient adherence to prescribed regimens, providing valuable insights into treatment effectiveness [2].

Traditionally, TDM assays have been performed using plasma or serum samples obtained through venous blood sampling. However, this approach has limitations. It requires visits to healthcare facilities, which can be burdensome for patients [3–5], and invasive blood sampling may be challenging for certain populations, such as children and critically ill patients [6, 7].

Dried blood spots (DBS) have emerged as a patient-friendly alternative for TDM. DBS sampling offers several advantages, including minimally invasiveness and the use of small blood volumes [8]. It has lower contamination and

biohazardous risk compared with venous blood sampling. Moreover, DBS samples exhibit better stability, making storage and transport more convenient [8]. One of the significant benefits of DBS sampling is the potential for home-based TDM. Using a finger prick, patients can collect blood samples themselves, reducing the need for frequent hospital visits. With proper training, patients can perform the sampling procedure at home, improving convenience, especially for those who face challenges in travelling to healthcare facilities [9, 10]. After sample collection, patients can simply send the DBS samples via regular mail to the laboratory for analysis. This streamlined process enhances convenience, particularly for individuals with limited mobility or difficulty accessing healthcare facilities. Overall, DBS sampling for TDM offers a patient-centred approach that improves accessibility and simplifies the monitoring of drug concentrations, ultimately contributing to more effective and personalised treatment.

Despite the advantages, there are still uncertainties on whether whole blood is a comparable matrix to plasma/serum for the purpose of TDM. The differences in the respective matrices along with the physicochemical properties of the target analytes (therapeutic drugs) necessitate the need for an extensive validation to render DBS a reliable sampling method that can replace plasma/serum sampling [11]. Few studies directly use DBS concentrations [12, 13] while most apply conversion factors [14–17] to estimate plasma/serum concentrations. Nevertheless, some studies do not approve DBS sampling to replace conventional sampling [18, 19].

Another prominent challenge of DBS sampling is the influence of haematocrit (HCT) on the accuracy of DBS assays. HCT affects the spreading of blood on the sampling paper which in turn impacts factors such as the spot size, drying time and homogeneity [8]. Various studies have implemented correction factors for HCT [14, 20] and many do not recommend the use of DBS sampling for individuals with extreme HCT [4, 13]. Nonetheless, the influence of HCT on DBS assay needs to be further investigated.

Despite having a potential for application in routine clinical practice, the widespread use of DBS sampling for TDM remains limited and requires further assessment to be clinically validated. Factors that can influence the accuracy of DBS assay need to be thoroughly examined in method development and validation. This review aims to discuss the current application of DBS as matrices for TDM, focussing on existing DBS assays and their respective method validation. In addition, DBS has proven to be valuable in disease response monitoring (DRM), especially for conditions that require frequent monitoring biomarkers. For example, in the field of infectious diseases, DBS can be used to detect and monitor viral loads or antibodies in individuals infected with human immunodeficiency virus (HIV), hepatitis C or other viral pathogens [21–27]. With the applications of multi-omic analytical techniques [28, 29], DBS can be explored as a platform for both TDM and DRM. This review explores the benefits and challenges of DBS sampling, in addition to gaining insight into the future direction of DBS for TDM and both disease and drug response monitoring (DRM).

2 Methods

This paper was prepared through a review of available literature found using keywords such as "dried blood spots" or "DBS", "drug response" or "disease response", "therapeutic drug monitoring" or "TDM". Several websites were used for this search, including Pubmed, Embase, and Science Citation Index. We focussed on articles published in the last 10 years, and search was limited to publications in the English language.

3 DBS Sampling

To better understand the topic, a brief background of DBS sampling is described in this section. DBS sampling was first introduced by Robert Guthrie in 1963 for the screening of newborns, specifically in the measurement of phenylalanine to detect phenylketonuria [30]. Since then, the use of DBS has been increasing and its spectrum of analytes is continuously expanding due to advances in analytical techniques [31]. As DBS sampling only uses a small volume of blood, a more sensitive and specific analytical method is required for the measurement of analyte in DBS [8].

This sampling method is prominent in the screening and detection of numerous biological markers including amino acids, enzymes, hormones, vitamins and antibodies. Other important applications include quantitation and genotyping of viruses, toxicokinetic and pharmacokinetics studies, TDM, forensics and more [32]. In this paper, we will be looking into the use of DBS for TDM and DRM that will give another dimension of DBS in clinical application.

The general DBS sampling procedure is composed of three main stages. The first stage involves blood sample collection, in which capillary blood is obtained from the patient through finger or heel prick by single-use automatic lancet. This is followed by spotting of the blood sample, approximately 10–50 µl, onto the sampling paper. Then, the samples are dried for a minimum of 2 h before being transported to laboratories for sample preparation and analysis. During sample preparation, sample area of appropriate size, usually around 3–6 mm, is punched before extraction of the analyte. Finally, the samples are subjected to the respective assay for analysis. This process is briefly illustrated in Fig. 1.

4 Current Application of DBS for TDM

Over the last decade, assays using DBS for TDM have been reported in literatures for a wide range of therapeutic drugs [8, 33, 34]. Some classes of therapeutic drugs for which DBS assay has been reported include antiepileptics, immunosuppressants, antimicrobials and antihypertensives. Though minimal, assays have also been published on antipsychotics and antidiabetics. Tables 1, 2, 3, 4, 5 and 6 provide a summary of the reported DBS assay for each class, respectively.

The concentration of therapeutic drug in DBS is measured by various quantitative analytical methods, mainly LC–MS/MS, HPLC–UV and GC–MS. Out of all of them, LC–MS/MS was observed to be the most preferred analytical method of choice, as it is applied in almost half of the listed studies. The choice of biological matrix used for DBS sampling includes whole blood from venous sampling and capillary blood from finger prick. Studies that utilise capillary blood from finger prick mostly spotted few drops of blood directly onto the sampling paper. Only one study, however, collects capillary blood into an appropriate blood tube, after which it is spotted onto the sampling paper using a pipette [35]. This procedure is also applied to studies that use venous whole blood to produce the blood spot. The punch size, blood volume and sampling paper used are also presented in Tables 1, 2, 3, 4, 5 and 6. The stability of various therapeutic drugs in DBS samples was also demonstrated in most of the studies, where various temperatures in which the drug is considered stable were specified accordingly.

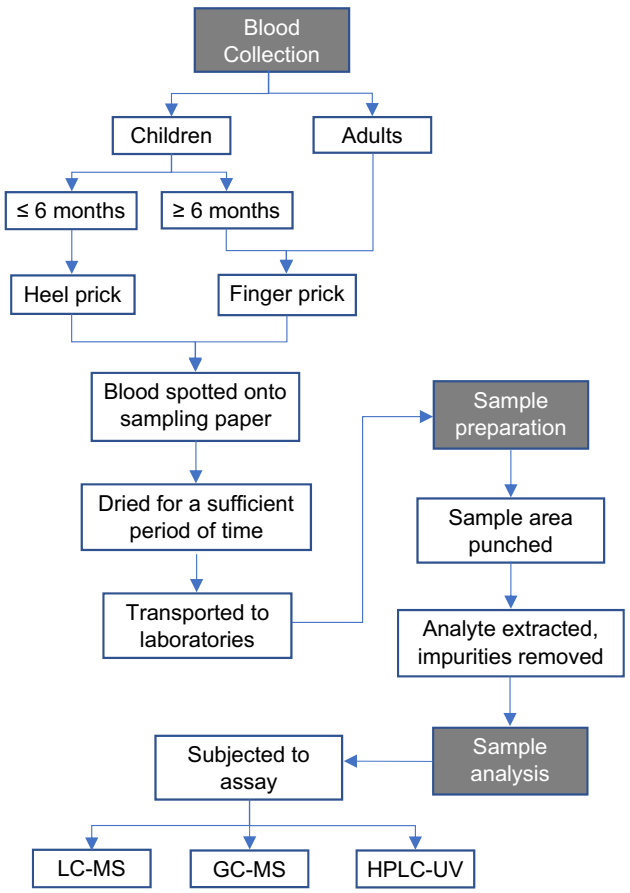

Fig. 1 Flowchart of general dried blood spot sampling procedure

5 Benefits of DBS Sampling for TDM

In contrast to venous blood sampling, DBS sampling offers a more patient-friendly experience due to its minimally invasive procedure. This is especially preferred for paediatrics, as venous blood sampling is often challenging in such population [36]. Additionally, the low volume of blood required in DBS sampling allows for practical blood collection from populations such as the paediatrics, the elderly and critically ill patients [37]. The simple procedure of DBS sampling also enables patients to perform sampling at home. The feasibility of self-sampling will be further discussed in Sect. 7.

Furthermore, the adsorption of blood onto sampling paper makes DBS samples less reactive as compared with any liquid specimen [37]. As a result, DBS often displays good stability in ambient conditions for several days or months. This allows for the possibility of DBS to be transported conveniently from the site of sample collection to the analysing laboratory, as long as necessary precautions are taken into consideration [37]. This is opposed to venous blood sampling, as liquid venous samples need to be transported under specific conditions. As such, this particular advantage of DBS is attractive for TDM in remote sites and resource-limited areas [14, 38]. Additionally, DBS has lower biohazardous risk, which can protect laboratory personnel from various infectious agents present in the blood samples during sample preparation [39].

6 Relationship Between DBS Concentration and Plasma/Serum Concentration

To aid in the interpretation of therapeutic drug concentration, reference ranges have been established on the basis of plasma/serum concentration, as these biological matrices are the gold standard for the measurement of most therapeutic drugs. Since DBS involves the measurement of drug concentration in whole (capillary) blood, it is thus necessary to assess the relationship between DBS concentration and plasma/serum concentration before correlating the values to the established reference range.

The difference between whole blood and plasma/serum concentration essentially depends on the blood-to-plasma concentration ratio of respective therapeutic drugs. To better

Table 1 Overview of reported assay using dried blood spot (DBS) for antiepileptics

Drug name	DBS blood	Punch size	Sample volume	DBS paper type	Analytical method	Analytical range	Comparison of Cdbs with Cp/Cs	HCT effect	Stability	Method validation	References
Carbamazepine	Spiked whole blood	6 mm	30 µl	Schleicher and Schuell 903 Guthrie card	HPLC–UV	1–17.5 µg/mL	–	Acceptable within 30–55%	Stable at −80 °C and 25 °C for 6 weeks	ICH	[119]
	EDTA venous blood	6 mm (15 µl)	2 drops (30 µl)	Whatman 903 Protein Saver card	GC–MS	0.5–120 µg/mL	Good correlation ($r = 0.8381$) Cp = (0.89 × Cdbs) + 1.00 µg/mL	–	Stable at − 20 °C and 25 °C for 10 days	FDA	[42]
	Blood	3.2 mm (3 µl)	20 µl	Whatman 903 Protein Saver card	LC–MS/MS	1–40 mg/L	Good correlation ($r^2 = 0.964$) Cp = Cdbs*(100/100–HCT)	Acceptable within 30–50%	Stable at −20 °C, 4 °C, 37 °C for 1 month	FDA and ICH	[20]
	Capillary blood (direct from finger prick)	3 mm	3–5 drops	Whatman 903 Protein Saver card	LC–MS/MS	0.25–40 µg/mL	Strong correlation ($r^2 = 0.892$) 18% higher Cdbs than Cp Cp = Cdbs × 0.84	Acceptable within 30–55%	–	EMA	[13]
	EDTA venous blood	–	20 µl	Whatman Cellulose Filter paper	HPLC/MS	2–20 µg/ml	Good correlation ($r = 0.958$) Cs = Cdbs × 0.83 + 1.09	–	Stable at 25 °C for 28 days	FDA	[14]
	Heparin venous blood	4.7 mm	5 drops (30 µl)	Whatman 903 Filter paper and Whatman 903 Protein Saver Card	LC–MS/MS	2.5–80 µmol/L	Good correlation ($r^2 = 0.9274$) 21% higher in Cdbs	Acceptable within 35–50%	Stable at 25 °C for 12 months and at − 80 °C for 3 months	EMA	[4]
	Capillary blood (direct from finger prick)	3 mm	3–5 drops	Whatman 903 Protein Saver card	LC–MS/MS	0.59–18.9 µg/mL	Good correlation ($r = 0.949$) Cp = Cdbs × 0.85	–	Stable at 25 °C for 12 months and at −80 °C for 18 months	EMA	[9]

Table 1 (continued)

Drug name	DBS blood	Punch size	Sample volume	DBS paper type	Analytical method	Analytical range	Comparison of Cdbs with Cp/Cs	HCT effect	Stability	Method validation	References
Lamotrigine	Spiked whole blood	6 mm	30 µl	Schleicher and Schuell 903 Guthrie card	HPLC–UV	1–17.5 µg/mL	–	Acceptable within 30–55%	Stable at −80 °C and 25 °C for 6 weeks	ICH	[119]
	Capillary blood (direct from finger prick)	3 mm	3–5 drops	Whatman 903 Protein Saver card	LC–MS/MS	0.25–40 µg/mL	Good correlation ($r^2 = 0.978$) No conversion needed	Acceptable within 30–55%	–	EMA	[13]
	Heparin venous blood	4.7 mm	5 drops (30 µl)	Whatman 903 Filter paper and Whatman 903 Protein Saver Card	LC–MS/MS	2.8–80 µmol/L	Good correlation ($r^2 = 0.9532$) 15% higher in Cdbs	Acceptable within 35–50%	Stable at 25 °C for 12 months and at −80 °C for 3 months	EMA	[4]
	Capillary blood (direct from finger prick)	3 mm	3–5 drops	Whatman 903 Protein Saver card	LC–MS/MS	0.64–20.5 µg/mL	Good correlation ($r = 0.963$) No conversion needed	–	Stable at 25 °C for 12 months and at −80 °C for 18 months	EMA	[9]
Levetiracetam	Spiked whole blood	6 mm	30 µl	Schleicher and Schuell 903 Guthrie card	HPLC–UV	1–17.5 µg/mL	–	Acceptable within 30–55%	Stable at −80 °C and 25 °C for 6 weeks	ICH	[119]
	Heparin venous blood	4.7 mm	5 drops (30 µl)	Whatman 903 Filter paper and Whatman 903 Protein Saver Card	LC–MS/MS	5–400 µmol/L	Good correlation ($r^2 = 0.9927$) Cdbs comparable to Cp	Acceptable within 35–50%	Stable at 25 °C for 12 months and at −80 °C for 3 months	EMA	[4]
	Capillary blood (direct from finger prick)	3 mm	3–5 drops	Whatman 903 Protein Saver card	LC–MS/MS	0.83–67.7 µg/mL	Good correlation ($r = 0.950$) No conversion needed	–	Stable at 25 °C for 12 months and at −80 °C for 18 months	EMA	[9]

Table 1 (continued)

Drug name	DBS blood	Punch size	Sample volume	DBS paper type	Analytical method	Analytical range	Comparison of Cdbs with Cp/Cs	HCT effect	Stability	Method validation	References
Phenobarbital	Spiked whole blood	6 mm	30 µl	Schleicher and Schuell 903 Guthrie card	HPLC–UV	1–17.5 µg/mL	–	Acceptable within 30–55%	Stable at −80 °C and 25 °C for 6 weeks	ICH	[119]
Phenytoin	EDTA venous blood	6 mm (15 µl)	2 drops (30 µl)	Whatman 903 Protein Saver card	GC–MS	0.5–120 µg/mL	Good correlation ($r = 0.9305$) Cp = [1.11 × Cdbs/(1 − 0.71 × HCT)] – 1.00 µg/mL	–	Stable at −20 °C and 25 °C for 10 days	FDA	[42]
	Spiked whole blood	3.2 mm (3 µl)	20 µl	Whatman 903 Filter paper	LC–MS/MS	0–100 mg/L	Good correlation ($r^2 = 0.982$)	–	Stable at −20 °C, 4 °C, 25 °C and 37 °C for 1 month	ICH	[16]
Rufinamide	Blood	3.2 mm (3.3 µl)	20 µl	Whatman 903 Filter paper	LC–MS/MS	0.48–47.60 mg/L	Good correlation ($r^2 = 0.996$) Cp = Cdbs × (100/100–HCT)	–	Stable at −20 °C, 4 °C and 25 °C, for 1 month	–	[43]
Topiramate	Blood	3.2 mm (3.3 µl)	20 µl	Whatman 903 Filter paper	LC–MS/MS	0.5–50 mg/L	Good correlation ($r^2 = 0.9985$) For adults: Cp = Cdbs × 1.79 For neonates: Cp = Cdbs × 2.22	For adults: acceptable within 36–52% For neonates: acceptable within 50–60%	–	–	[120]

Table 1 (continued)

Drug name	DBS blood	Punch size	Sample volume	DBS paper type	Analytical method	Analytical range	Comparison of Cdbs with Cp/Cs	HCT effect	Stability	Method validation	References
Valproic acid	EDTA venous blood	6 mm (15 µl)	2 drops (30 µl)	Whatman 903 Protein Saver card	GC–MS	0.5–120 µg/mL	Good correlation (r = 0.8531) Cp = [0.92 × Cdbs/(1 − 0.96 × HCT)] – 12.48 µg/mL	–	Stable at −20 °C and 25 °C for 10 days	FDA	[42]
	Capillary blood (direct from finger prick)	6 mm (12 µl)	–	Whatman 903 Filter paper	GC–MS	5–250 µg/mL	Good correlation (r = 0.9948) Cs = Cdbs × 1.883	Acceptable within 30–50%	Stable at 45 °C for 21 days	–	[17]
	Capillary blood (direct from finger prick)	3 mm	3–5 drops	Whatman 903 Protein Saver card	LC–MS/MS	5–300 µg/mL	Good correlation (r = 0.899) 35% lower Cdbs than Cp Cp = Cdbs × 1.58	Acceptable within 30–55%	–	EMA	[13]
	Heparin venous blood	4.7 mm	5 drops (30 µl)	Whatman 903 Filter paper and Whatman 903 Protein Saver Card	LC–MS/MS	20–1000 µmol/L	Good correlation (r^2 = 0.9488) 35% lower in Cdbs	Acceptable within 35–50%	Stable at 25 °C for 12 months and at −80 °C for 3 months	EMA	[4]
	Capillary blood (direct from finger prick)	3 mm	3–5 drops	Whatman 903 Protein Saver card	LC–MS/MS	2.90–145 µg/mL	Good correlation (r = 0.902) Cp = Cdbs × 1.65	–	Stable at 25 °C for 12 months and at −80 °C for 18 months	EMA	[9]

ICH International Council for Harmonisation, *FDA* US Food and Drug Adminstration, *EMA* European Medical Agency, *HCT* haematocrit, *HPLC* high performance liquid chromatography, *MS* mass spectrometery, LC liquid chromatography, *UV* ultraviolet, *Cp* plasma concentration, *Cs* serum concentration

Table 2 Overview of reported assay using dried blood spot (DBS) for immunosuppressants

Drug name	DBS blood	Punch size	Sample volume	DBS paper type	Analytical method	Analytical range	Comparison of Cdbs with Cv/Cp	HCT effect	Stability	Method validation	Reference
Cyclosporin	Venous blood	6 mm	25 µl	Whatman 903 card	UPLC–MS/MS	0–1500 µg/L	Good correlation ($r^2 = 0.99$)	–	Stable at 25 °C for 2 weeks	FDA	[48]
	EDTA venous blood	8 mm	50 µl	Whatman 31 ET CHR paper	LC–MS/MS	20.0–2000 µg/L	Good correlation ($r^2 = 0.9775$) Systematic difference observed	Correction needed	Stable at 22 °C for 1 week	FDA	[50]
	Venous blood	8 mm	50 µl	Whatman 903 Protein Saver Card	LC–MS/MS	30–1000 ng/ml	Good correlation ($r^2 = 0.97$)	Acceptable within 30–35%	Stable at −20 °C, 4 °C and 25 °C for 1 month	–	[47]
	Capillary blood (direct from finger prick)	8 mm	30 µl (10 mm)	Whatman 903 Filter paper	LC–MS/MS	–	No bias observed	–	–	–	[12]
	Capillary blood (direct from finger prick)	6 mm	25 µl	Whatman 903 card	UPLC–MS/MS	–	Good correlation	–	Stable at 25 °C for 2 weeks	–	[121]
	Capillary blood (direct from finger prick)	–	2 drops (10 mm)	Whatman FTA DMPK–C card	LC–MS/MS	–	Good correlation ($r^2 = 0.93$) Can be used interchangeably	–	–	–	[15]
Everolimus	Capillary blood (direct from finger prick)	7.5 mm	To fill 8 mm circle	Whatman 903 Protein Saver Card	LC–MS/MS	2–30 µg/l	Cdbs higher than Cv but not significant	–	Stable at 32 °C for 34 days, and at 60 °C for 3 days	FDA	[80]
	EDTA venous blood	8 mm	50 µl	Whatman 31 ET CHR paper	LC–MS/MS	1.00–50.0 µg/L	Good correlation ($r^2 = 0.9698$) Systematic difference observed	Correction needed	Stable at 22 °C for 1 week	FDA	[50]
	Capillary blood (direct from finger prick)	–	2 drops	Whatman FTA DMPK-C sampling paper	LC–MS/MS	1.0–50.0 µg/L	Good correlation ($r = 0.97$) No correction factor needed	–	–	–	[19]

Table 2 (continued)

Drug name	DBS blood	Punch size	Sample volume	DBS paper type	Analytical method	Analytical range	Comparison of Cdbs with Cv/Cp	HCT effect	Stability	Method validation	Reference
Mycophenolic acid	Spiked EDTA blood	8 mm	50 µl	Whatman 903 Protein Saver Card	HPLC–MS	0.74–23.4 mg/L	–	Acceptable within 25–40%	Stable at 4 °C for 26 days	FDA	[122]
	Capillary blood (direct from finger prick)	6 mm	–	Whatman 903 sampling paper	HPLC–DAD	–	Good correlation after HCT correction ($r = 0.9862$) Cp = Cdbs/[1 − (Hct/100)]	Correction needed	–	–	[123]
	Capillary blood (direct from finger prick)	7.5 mm	4 drops	Whatman 903 Filter paper	LC–MS/MS	0.5–40 mg/L	Cv = Cdbs × 0.130	Acceptable within 25–50%	Stable at 4 °C and 25 °C for long periods	FDA and EMA	[46]
	Spiked venous blood	3 mm	30 µl	Agilent Dried Matrix Spotting cards	LC/ESI/MS	0.1–30 µg/mL	HCT corrected Cdbs comparable to Cp Cp= Cdbs/(1 − HCT)	Correction needed	Stable at 25 °C for 10 days	FDA and EMA	[124]
Sirolimus	EDTA venous blood	8 mm	50 µl	Whatman 31 ET CHR paper	LC–MS/MS	1.00–50.0 µg/L	Good correlation ($r^2 = 0.8708$) No systematic difference observed	Correction needed	Stable at 22 °C for 1 week	FDA	[50]
	Venous blood	8 mm	50 µl	Whatman 903 Protein Saver Card	LC–MS/MS	1.2–40 ng/ml	Good correlation ($r^2 = 0.95$)	Acceptable within 30–35%	Stable at −20 °C, 4 °C and 25 °C for 1 month	–	[47]
	Capillary blood (direct from finger prick)	–	2 drops	Whatman FTA DMPK-C sampling paper	LC–MS/MS	1.0–50.0 µg/L	Good correlation ($r = 0.93$) No correction factor needed	–	–	–	[19]
	EDTA venous blood	8 mm	50 µl	Whatman 31 ET CHR paper	LC–MS/MS	1.00–50.0 µg/L	Good correlation ($r^2 = 0.9698$) Systematic difference observed	Correction needed	Stable at 22 °C for 1 week	FDA	[50]
	Capillary blood (direct from finger prick)	–	2 drops	Whatman FTA DMPK-C sampling paper	LC–MS/MS	1.0–50.0 µg/L	Good correlation ($r = 0.97$) No correction factor needed	–	–	–	[19]

Table 2 (continued)

Drug name	DBS blood	Punch size	Sample volume	DBS paper type	Analytical method	Analytical range	Comparison of Cdbs with Cv/Cp	HCT effect	Stability	Method validation	Reference
Tacrolimus	Capillary blood (direct from finger prick)	7.5 mm	To fill 8 mm circle (30 µl)	Whatman Schleicher and Schuell Filter paper	HPLC–MS/MS	1–30 µg/l	No significant bias observed	Acceptable within normal HCT range	Stable at 4 °C for 1 month	FDA	[125]
	Capillary blood (direct from finger prick)	7.5 mm	To fill 8 mm circle (30 µl)	Whatman Schleicher and Schuell Filter paper	HPLC–MS/MS	–	Good correlation ($r^2 = 0.96$) Cdbs 7.8% higher than Cv	–	Stable for storage and long distance	FDA	[51]
	Venous blood	6 mm	25 µl	Whatman 903 card	UPLC–MS/MS	0–50 µg/L	Good correlation ($r^2 = 0.92$)	–	Stable at 25 °C for 2 weeks	FDA	[48]
	EDTA venous blood	8 mm	50 µl	Whatman 31 ET CHR paper	LC–MS/MS	1.00–50.0 µg/L	Good correlation ($r^2 = 0.9607$) Systematic difference observed	Correction needed	Stable at 22 °C for 1 week	FDA	[50]
	Venous blood	8 mm	50 µl	Whatman 903 Protein Saver Card	LC–MS/MS	1.2–40 ng/ml	Good correlation ($r^2 = 0.99$)	Acceptable within 30–35%	Stable at −20 °C, 4 °C and 25 °C for 1 month	–	[47]
	Capillary blood (direct from finger prick)	6 mm	To fill circle	Whatman FTA-DMPK-A filter paper card	LC–MS/MS	1–50 ng/ml	Good correlation ($r^2 = 0.742$)	–	–	–	[49]
	Capillary blood (direct from finger prick)	6 mm	25 µl	Whatman 903 card	UPLC–MS/MS	–	Good correlation	–	Stable at 25 °C for 2 weeks	–	[121]
	Capillary blood (direct from finger prick)	7.5 mm	4 drops	Whatman 903 Filter paper	LC–MS/MS	2.5–50 µg/L	Cv = Cdbs × 0.763	Acceptable within 25–50%	Stable at 4 °C and 25 °C for long periods	FDA and EMA	[46]
	Capillary blood (direct from finger prick)	–	2 drops (10 mm)	Whatman FTA DMPK-C card	LC–MS/MS	–	Good correlation ($r^2 = 0.93$) Can be used interchangeably	–	–	–	[15]

FDA US Food and Drug Adminstration, *EMA* European Medical Agency, *HCT* haematocrit, *HPLC* high performance liquid chromatography, *DAD* diodearray detection, *ESI* electrospray ionization, *MS* mass spectrometery, *LC* liquid chromatography, *UPLC* ultra performance liquid chromatography, *Cv* venous concentration, *Cp* plasma concentration

Table 3 Overview of reported assay using dried blood spot (DBS) for antimicrobials

Drug name	DBS blood	Punch size	Sample volume	DBS paper type	Analytical method	Analytical range	Comparison of Cdbs with Cp	HCT effect	Stability	Method validation	Reference
Tuberculosis management											
Clarithromycin	Capillary blood (direct from finger prick)	8 mm	1 drop	Whatman 31 ET CHR paper sheets	LC–MS/MS	0.05–10 mg/L	Good correlation ($r^2 = 0.9752$)	Acceptable within 20–50%	Stable at 25 °C for 60 days, 37 °C for 30 days and 50 °C for 15 days	FDA	[63]
	Blood	3.2 mm	100 ul	Whatman 903 Filter paper	UPLC–MS/MS	0.5–10 µg/mL	Rho factor 0.938	–	–	–	[126]
Ethambutol	Capillary blood (direct from finger prick)	8 mm	1 drop	–	–	0.2–6.5 mg/L	Cp = Cdbs × 0.51	–	–	–	[127]
Linezolid	Venous blood and capillary blood (direct from finger prick)	8 mm	50 µl	Whatman 31 ET CHR paper	LC–MS/MS	0.05–30 mg/L	Significant proportional bias observed for both ratio of venous DBS/plasma higher than capillary DBS/plasma	Acceptable within 20–50%	Stable at 37 °C for 2 months and at 50 °C for 1 week	FDA	[52]
	Spiked blood	32 mm (3.3 µl)	20 µl	Whatman 903 Filter paper	LC–MS/MS	1–100 mg/L	Good correlation (R2 > 0.9) Cp = Cdbs × (100 − HCT)/100	Correction needed	Stable at −20 °C, 4 °C, 25 °C and 37 °C for 1 month	FDA	[128]
	Blood	3.2 mm	100 µl	Whatman 903 Filter paper	UPLC–MS/MS	1–20 µg/mL	Rho factor 0.811	–	–	–	[126]
Moxifloxacin	Venous blood and capillary blood (direct from finger prick)	8 mm	50 µl for venous blood	Whatman 31 ET CHR, Whatman 903 and Whatman no. 3 card	LC–MS/MS	0.05–6.00 mg/L	Good correlation (capillary DBS, $r^2 = 0.996$, venous DBS, $r^2 = 0.973$) Systemic differences between observed capillary DBS and venous DBS comparable	Correction needed	Stable at 25 °C for 4 weeks	–	[53]
	Blood	3.2 mm	100 ul	Whatman 903 Filter paper	UPLC–MS/MS	1–20 µg/mL	Rho factor 0.897	–	–	–	[126]

Table 3 (continued)

Drug name	DBS blood	Punch size	Sample volume	DBS paper type	Analytical method	Analytical range	Comparison of Cdbs with Cp	HCT effect	Stability	Method validation	Reference
Rifampicin	Capillary blood (direct from finger prick)	7 mm (18 µl)	–	Whatman BFC-180 card	HPLC–UV	1.5–20 µg/ml	Good correlation ($r^2 = 0.9206$)	–	Stable for 9 °C hours after preparation	ICH and FDA	[129]
	Capillary blood (direct from finger prick)	8 mm	1 drop	Whatman 31 ET CHR paper sheets	LC–MS/MS	0.25–30 mg/L	Good correlation ($r^2 = 0.9076$)	Bias observed at 20–50%	Stable at 25 °C for 60 days, at 37 °C for 10 days and at 50 °C for 3 days	FDA	[63]
	Capillary blood (direct from finger prick and from heparin tube)	6 mm	–	Whatman 903 Protein Saver card	LC–MS/MS	0.1–10 mg/L	Cp correlates well with Cdbs taken directly from finger prick ($r = 0.97$) Cdbs taken directly from finger prick correlates well with Cdbs collected in heparin tube ($r = 0.97$)	Acceptable within 28–66%	Stable at 25 °C for 10 days and at 4 °C for 1 month	–	[130]
	Capillary blood (direct from finger prick)	8 mm	1 drop	–	–	–	Cp = Cdbs × 0.75	–	–	–	[127]
Rifapentine	EDTA venous blood	6 mm	20 µl	Whatman Protein Saver Card	LC–MS/MS	50–80,000 ng/ml	Bias observed	Correction needed	Stable at 25 °C for 11 weeks	FDA	[131]
Other antibacterials											
Ampicillin	EDTA venous blood	–	30 µl	Whatman FTA DMPK-C White card	HPLC–MS/MS	0.05–50.0 µg/mL	Good correlation ($r^2 = 0.902$) Cp = (Cdbs − 3.223)/0.51	–	–	FDA	[132]
Ceftriaxone	Venous blood and capillary blood (direct from finger prick and from heparin tube)	–	–	Whatman 903 Protein Saver Card	LC–MS/MS	1–200 mg/L	Good correlation ($r = 0.95$) after adjustment with HCT and partitioning ratio (for all)	Minimal effect	Stable at 35 °C for 14 h, at 4 °C for 30 days and at −20 °C for 21 weeks	–	[133]

Table 3 (continued)

Drug name	DBS blood	Punch size	Sample volume	DBS paper type	Analytical method	Analytical range	Comparison of Cdbs with Cp	HCT effect	Stability	Method validation	Reference
Ciprofloxacin	Capillary blood (direct from finger prick and from heparin tube)	6 mm	–	Whatman 903 Protein Saver card	LC–MS/MS	0.1–10 mg/L	Cp correlates well with Cdbs taken directly from finger prick ($r = 0.96$) Cdbs taken directly from finger prick correlates well with Cdbs collected in heparin tube ($r = 0.97$)	Acceptable within 28–66%	Stable at 25 °C for 10 days and 4 °C for 1 month	–	[130]
Ertapenem	Spiked blood	3.2 mm	20 µl	Whatman 903 Filter paper	UPLC–MS/MS	0.5–100 mg/L	Good correlation ($r^2 = 0.9852$)	Correction needed	Stable at −20 °C for 1 month	–	[134]
Metronidazole	EDTA whole blood	3 mm	30 µl	Whatman Type C DMPK card	HPLC–MS/MS	0.05–50 mg/L	Cdbs 15% lower than Cp Good correlation ($r^2 = 0.95$)	–	–	FDA	[135]
Piperacillin	EDTA whole blood	6 mm	30 µl	Whatman DMPK FTA Type C card	HPLC–MS/MS	0.15–150 mg/L	Cdbs lower than Cp	–	–	–	[136]
Tazobactam	EDTA whole blood	6 mm	30 µl	Whatman DMPK FTA Type C card	HPLC–MS/MS	0.15–150 mg/L	Cdbs lower than Cp	–	–	–	[136]
Antiparasitics											
Benznidazole	Spiked blood	13 mm	50 µl	Whatman 903 Filter paper	HPLC–MS/MS	50–20,000 ng/mL	Comparable correlation ($r^2 = 0.8295$)	Acceptable within 20–70%	Stable at 25 °C for 1 year	FDA	[137]
	Capillary blood (direct from finger prick)	2 quarter inch	100 µl	Whatman Protein Saver 903	LC–MS/MS	9.8–5000 ng/mL	–	–	Stable at −20 °C for 20 months	–	[138]
Ivermectin	Capillary blood	–	–	Whatman 903 Filter paper card	LC–MS/MS	0.5–250 ng/mL	Good correlation ($r^2 = 0.97$) Cdbs 30% lower than Cp	Minimal effect	–	ICH	[139]
	Capillary blood (from heparin tube)	Whole DBS (20 µl)	20 µl	Whatman 903 Filter paper card	LC–MS/MS	1–100 ng/mL	Cdbs 4.8% than Cp Need to use HCT and partitioning of drug to estimate Cp	Correction needed	Stable at 25 °C for 1 month	EMA	[105]

Table 3 (continued)

Drug name	DBS blood	Punch size	Sample volume	DBS paper type	Analytical method	Analytical range	Comparison of Cdbs with Cp	HCT effect	Stability	Method validation	Reference
Antifungals											
Caspofungin	Whole blood	6 mm	50 µl	Whatman 903 Filter paper card	LC-MS/MS	0.2–20 µg/mL	–	Acceptable within 25–50%	Stable at 25 °C for 24 h and −80 °C for 30 days	FDA	[140]

ICH International Council for Harmonisation, *FDA* US Food and Drug Adminstration, *EMA* European Medical Agency, *HCT* haematocrit, *HPLC* high performance liquid chromatography, *UPLC* ultra performance liquid chromatography, *MS* mass spectrometery, *LC* liquid chromatography, *UV* ultraviolet, *Cp* plasma concentration

understand the issue, it is useful to first study the relationship between total whole blood, total plasma and unbound concentration as demonstrated in Fig. 2. It is seen from the figure that therapeutic drugs can partition into the blood cell, in which the permeability of cell membrane and the affinity of drugs to bind to constituents in plasma or blood cell can influence the extent of distribution. At equilibrium, the distribution of a drug in the blood are interpreted using three equations [8] as follows:

$$C_{\mathrm{p}} = \frac{C_{\mathrm{u}}}{f_{\mathrm{u}}} \tag{1}$$

$$C_{\mathrm{b}} = \left[\frac{1 - \mathrm{HCT}}{f_{\mathrm{u}}} + \mathrm{HCT} \times \rho\right] \times C_{\mathrm{u}} \tag{2}$$

$$\frac{C_{\mathrm{b}}}{C_{\mathrm{p}}} = (1 - \mathrm{HCT}) + \mathrm{HCT} \times \rho \times f_{\mathrm{u}} \tag{3}$$

where C_{p} is plasma concentration, C_{u} is unbound concentration, C_{b} is blood concentration, f_{u} is unbound fraction in plasma, HCT is haematocrit and ρ is blood cell partitioning. Equation (3) represents blood-to-plasma concentration ratio and is obtained by dividing Equation (2) by Equation (1).

On the basis of the equations above, we can directly infer that blood-to-plasma concentration ratio depends on f_{u}, ρ and HCT. According to Rowland and Emmons, variability in f_{u} will be a concern for estimating plasma concentration from DBS concentration for therapeutic drugs with blood-to-plasma ratio of around 0.55, which indicates greater fraction of drug present in plasma. Conversely, for therapeutic drugs with blood-to-plasma ratio of more than 2, which indicates high affinity for red blood cells (RBC), the variability in ρ becomes a greater concern instead. If small variability is observed for both f_{u} and ρ, then there is minimal concern if DBS is used [11]. In essence, it is important to conduct validation studies to translate DBS to plasma/serum concentration accurately and confidently. Some guidelines such as the EMA guideline on bioanalytical method validation [40] and the CLSI guideline for Method Comparison and Bias Estimation Using Patient Samples [41] specify the need for method comparison to determine the agreement between DBS and plasma/serum concentrations.

Referring to Table 1 (antiepileptics) as an example, various studies have demonstrated different approaches to determine estimated plasma/serum concentration from DBS concentration for antiepileptic drugs. One approach used by Linder et al. [9] and Rhoden et al. [17] is known as the ratio approach where DBS concentration is simply multiplied by the average ratio of plasma/serum concentration to DBS concentration. Another approach adopted by Kong et al. is known as the theoretical approach where blood-to-plasma

Table 4 Overview of reported assay using dried blood spot (DBS) for antihypertensives

Drug name	DBS blood	Punch size	Sample volume	DBS paper type	Analytical method	Analytical range	Comparison of Cdbs with Cp	HCT effect	Stability	Method validation	References
Beta blockers											
Atenolol	Spiked whole blood	5 mm (15 µl)	30 µl	Whatman 903 Filter paper	LC–HRMS	25–1500 ng/ml	–	–	Stable at 25 °C for 2 months	–	[61]
	Spiked blood	8 mm (20 µl)	30 µl	Whatman 903 Filter paper	LC–HRMS	10–1500 ng/ml	–	Acceptable within 35–55%	Stable at 25 °C for 10 weeks	FDA and EMA	[141]
Bisoprolol	Spiked whole blood	8 mm (20 µl)	30 µl	Whatman 903 Filter paper	LC–HRMS	0.1–100 ng/ml	–	–	Stable at 25 °C for 12 weeks	FDA	[2]
	Spiked blood	8 mm (20 µl)	30 µl	Whatman 903 Filter paper	LC–HRMS	0.1–100 ng/ml	–	Acceptable within 35–55%	Stable at 25 °C for 10 weeks	FDA and EMA	[141]
ACE inhibitors											
Enalapril	Capillary blood (direct from finger prick)	6 mm	1 drop	Whatman Protein Saver 903 card	UHPLC–MS/MS.	1.45–580 µg/L	No conversion needed	Minimal effect	Stable at 25 °C for 11 days	FDA and EMA	[142]
Lisinopril	Spiked blood	8 mm (20 µl)	30 µl	Whatman 903 Filter paper	LC–HRMS	0.1–100 ng/ml	–	Acceptable within 35–55%	Stable at 25 °C for 10 weeks	FDA and EMA	[141]
Perindopril	Capillary blood (direct from finger prick)	6 mm	1 drop	Whatman Protein Saver 903 card	UHPLC–MS/MS.	1.21–484 µg/L	Cdbs higher than Cp Cp = [(Cdbs–5.782) × (1/0.745)]	Minimal effect	Stable at 25 °C for 11 days	FDA and EMA	[142]
Ramipril	Spiked whole blood	8 mm (20 µl)	30 µl	Whatman 903 Filter paper	LC–HRMS	0.5–100 ng/ml	–	–	Stable at 25 °C for 12 weeks	FDA	[2]
	Spiked blood	8 mm (20 µl)	30 µl	Whatman 903 Filter paper	LC–HRMS	0.1–100 ng/ml	–	Acceptable within 35–55%	Stable at 25 °C for 10 weeks	FDA and EMA	[141]
Calcium channel blockers											
Amlodipine	Spiked blood	8 mm	15 µl	Guthrie Ahlstorm 226 card	HPLC–MS/MS	0.5–30 ng/mL	Good correlation ($r^2 > 0.99$)	Minimal effect	Stable at −20 °C for 3 months	FDA	[62]
	Capillary blood (direct from finger prick)	6 mm	1 drop	Whatman Protein Saver 903 card	UHPLC–MS/MS	10.92–437 µg/L	Cdbs higher than Cp Cp = Cdbs × (1/1.443)	Minimal effect	Stable at 25 °C for 11 days	FDA and EMA	[142]

Table 4 (continued)

Drug name	DBS blood	Punch size	Sample volume	DBS paper type	Analytical method	Analytical range	Comparison of Cdbs with Cp	HCT effect	Stability	Method validation	References
Nifedipine	Capillary blood (direct from finger prick)	6 mm	1 drop	Whatman Protein Saver 903 card	UHPLC–MS/MS.	23.26–2326 µg/L	Cdbs higher than Cp Cp = [(Cdbs − 29.250) × (1/0.622)]	Minimal effect	–	FDA and EMA	[142]
Angiotensin II Receptor Blockers											
Losartan	Spiked blood	8 mm (20 µl)	30 µl	Whatman 903 Filter paper	LC–HRMS	5–1000 ng/ml	–	Acceptable within 35–55%	Stable at 25 °C for 10 weeks	FDA and EMA	[141]
	Capillary blood (direct from finger prick)	6 mm	1 drop	Whatman Protein Saver 903 card	UHPLC–MS/MS	3.86–1930 µg/L	Cdbs lower than Cp Cp = [(Cdbs − 29.660) × (1/0.359)]	Minimal effect	Stable at 25 °C for 11 days	FDA and EMA	[142]
Valsartan	Spiked blood	8 mm (20 µl)	30 µl	Whatman 903 Filter paper	LC–HRMS	50–4000 ng/ml	–	Acceptable within 35–55%	Stable at 25 °C for 10 weeks	FDA and EMA	[141]
	Capillary blood (direct from finger prick)	6 mm	1 drop	Whatman Protein Saver 903 card	UHPLC–MS/MS	28.54–2854 µg/L	Cdbs lower than Cp Cp = Cdbs × (1/0.553)	Minimal effect	–	FDA and EMA	[142]

FDA US Food and Drug Adminstration, *EMA* European Medical Agency, *HCT* haematocrit, *HPLC* high performance liquid chromatography, *UHPLC* ultra high performance liquid chromatography, *HRMS* high resolution mass spectrometer, *ESI* electrospray ionization, *MS* mass spectrometery, *LC* liquid chromatography, *Cp* plasma concentration

Table 5 Overview of reported assay using dried blood spot (DBS) for antipsychotics

Drug name	DBS blood	Punch size	Sample volume	DBS paper type	Analytical method	Analytical range	Comparison of Cdbs with Cp	HCT effect	Stability	Method validation	References
Aripiprazole	Capillary blood (direct from finger prick)	6 mm	1 drop	Whatman 903 Protein Saver Card	UHPLC–MS/MS	10–1000 µg/L	Cdbs lower than Cp	Acceptable within 30–45%	Stable at 25 °C for 10 days, at 4 °C and −80 °C for 1 month	FDA and EMA	[57]
	Capillary blood (direct from finger prick)	6 mm	1–4 drops	Whatman 903 Protein Saver Card	UHPLC–MS/MS	10–1000 µg/L	Cp = (Cdbs/(1 − HCT)/1.26) + 33.28	–	–	FDA and EMA	[18]
Pipamperone	Capillary blood (direct from finger prick)	6 mm	1 drop	Whatman 903 Protein Saver Card	UHPLC–MS/MS	10–1000 µg/L	Cdbs lower than Cp	Acceptable within 30–45%	Stable at 25 °C for 10 days, at 4 °C and −80 °C for 1 month	FDA and EMA	[57]
	Capillary blood (direct from finger prick)	6 mm	1–4 drops	Whatman 903 Protein Saver Card	UHPLC–MS/MS	10–1000 µg/L	Cp = Cdbs/0.16–2.88	–	–	FDA and EMA	[18]
Risperidone	Capillary blood (from heparin tube)	Whole DBS	50 µl	Whatman 903 Filter paper	LC–MS/MS	2.5–300 µg/L	Good correlation ($r^{2.} > 0.99$)	–	Stable at 8 °C and 25 °C for 3 months	FDA and EMA	[35]
	Capillary blood (direct from finger prick)	6 mm	1 drop	Whatman 903 Protein Saver Card	UHPLC–MS/MS	2–200 µg/L	Cdbs lower than Cp	Acceptable within 30–45%	Stable at 25 °C for 10 days, at 4 °C and −80 °C for 1 month	FDA and EMA	[57]
	Capillary blood (direct from finger prick)	6 mm	1–4 drops	Whatman 903 Protein Saver Card	UHPLC–MS/MS	2–200 µg/L	Cp = Cdbs/(1 − HCT)	–	–	FDA and EMA	[18]

FDA US Food and Drug Adminstration, *EMA* European Medical Agency, *HCT* haematocrit, *UHPLC* ultra high performance liquid chromatography, *MS* mass spectrometery, *LC* liquid chromatography, *Cp* plasma concentration

Table 6 Overview of reported assay using dried blood spot (DBS) for antidiabetics

Drug name	DBS blood	Punch size	Sample volume	DBS paper type	Analytical method	Analytical range	Comparison of Cdbs with Cplasma/Cserum	HCT effect	Stability	Method validation	References
Metformin	Capillary blood	6 mm	30 µl	Guthrie card	HPLC–UV	300–5000 ng/ml	–	–	Stable at –70 °C for 2 months	–	[143]
	Spiked EDTA blood	–	40 µl	Whatman GB 004 Blotting paper	HPLC–UV	0.2–5 µg/mL	–	–	Stable at 25 °C for 1 month	–	[102]
	Capillary blood	–	40 µl	Whatman GB 004 Blotting paper	HPLC–UV	0.2–5 µg/mL	–	–	–	–	[103]
Sitagliptin	Spiked EDTA blood	–	40 µl	Whatman GB 004 Blotting paper	LC–ESI–MS/MS	3–500 ng/mL	–	–	Stable at 25 °C for 1 month	–	[102]
	Capillary blood	–	40 µl	Whatman GB 004 Blotting paper	LC–ESI–MS/MS	3–500 ng/mL	–	–	–	–	[103]

HCT haematocrit, *HPLC* high performance liquid chromatography, *ESI* electrospray ionization, *MS* mass spectrometery, *LC* liquid chromatography

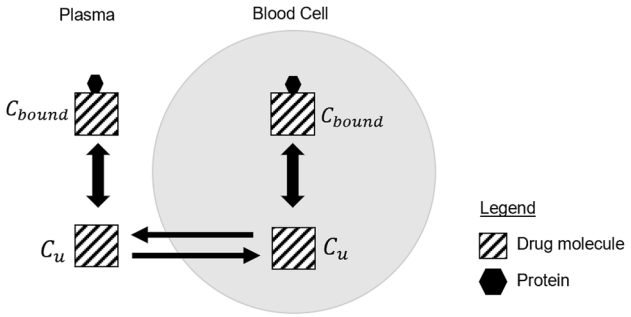

Fig. 2 Partitioning of drug molecule into blood cell. C_u and C_{bound} refers to unbound and bound concentration, respectively

ratio (denoted as K in equation) and HCT are considered in estimating plasma concentration through the following equation: $C_{plasma} = C_{DBS}/[1 - HCT(1 - K)]$. This equation is then further adjusted on the basis of linear regression to obtain the final equation [42]. Linder et al. compared the two different approaches and reported minimal difference [13]. Furthermore, La Marca et al. [43] and Shokry et al. [20] only corrected for HCT in the estimation of plasma/serum concentration from DBS concentration.

To see how blood-to-plasma concentration ratio plays a role in the relationship between DBS concentration and plasma/serum concentration, we will look at two examples of antiepileptic drugs: carbamazepine and valproic acid. Carbamazepine has a blood-to-plasma ratio of 1.06 [42], which implies a similar fraction of the drug partitions between RBC and plasma. Various studies displayed good correlation of DBS concentration to plasma concentration, although some found that DBS concentration of carbamazepine was slightly higher than plasma concentration, hence utilising conversion factors ranging from 0.83 to 0.89 [9, 14, 42]. Furthermore, plasma concentration of valproic acid was found to be significantly higher than DBS concentration, and thus conversion factors ranging from 0.92 to 1.88 were applied [4, 9, 13, 17, 42]. Valproic acid has a blood-to-plasma ratio of 0.64 [44], which indicates low partitioning into the RBC. Therefore, the RBC in DBS may act as a diluent and ultimately results in lower DBS concentration measured. Additionally, Kong et al. highlights that the lipophilicity of valproic acid, which can lead to the dissolvement and detachment from RBC during centrifugation, can be another reason for the higher plasma concentration measured [42].

In contrast to many therapeutic drugs, the routine assay of immunosuppressants are mostly performed in venous whole blood due to their high affinity to RBC [11]. Although both capillary and venous blood are essentially whole blood, minor differences in drug concentration may be seen between the two [45] due to the presence of arterial component as well as intracellular and interstitial fluid in capillary blood [34]. On the basis of the recently reported DBS assay for tacrolimus as an example, only one study

[46] introduced a conversion factor as higher concentration in DBS was observed. Although other studies have reported similar findings [15, 47–51], they concluded that the difference between the two methods is still within acceptable limits. Noticeably, since capillary and venous blood are more similar in terms of matrix components, fewer studies reported the use of conversion factor for immunosuppressants as compared with antiepileptics.

Nevertheless, the examples discussed above prove that case-by-case-based evaluation is necessary to determine the validity of using DBS for TDM. Depending on the physicochemical properties of each therapeutic drug, as well as the physiological difference between biological matrices, the relationship between DBS concentration and plasma/serum concentration, or venous concentration in the case of immunosuppressants, can differ significantly. Although the use of conversion factors or equations seems to suggest that DBS can be a possible alternative to plasma/serum, further validation still has to be performed for it to be widely accepted in clinical practice.

Additionally, it is also important to note that some studies use venous blood spotted DBS to perform the method validation. Considering that the true matrix of DBS sampling is capillary blood, there are concerns in which the results obtained from such studies may not hold true in the clinical application of DBS. Vu et al. compared between venous blood spotted DBS (Vdbs) and capillary blood spotted DBS (CAdbs) for linezolid and moxifloxacin in separate studies [52, 53]. They found that Vdbs gives a higher drug concentration than CAdbs for linezolid, whereas Vdbs and CAdbs concentration for moxifloxacin is comparable. As such, for studies that use venous blood spotted DBS, additional analysis has to be performed before applying the method to clinical practice. Perhaps standardising and establishing a set of reference range solely on the basis of DBS capillary blood can be considered to help in the interpretation of DBS concentration without correlating to plasma/serum concentration. Until such ranges are established, the conversion to estimated plasma/serum concentration can be applied for the use of DBS for TDM.

7 Factors that Can Affect DBS Sampling Method

There are four key factors that can affect DBS sampling method: HCT, blood spot volume, sampling paper and chromatographic effect.

7.1 HCT

On the basis of the previous section, it is apparent that HCT also plays a significant role in the translation of DBS concentration to plasma/serum concentration. HCT refers to the volume or fraction of blood occupied by the RBC and it is known to be a prominent parameter that can influence the validity of DBS method results [8]. Normal values of HCT range from 40% to 50% for men and 35–45% for women, while paediatrics have higher HCT values ranging from 42% to 65% [37]. Patients with certain diseases such as anaemia may deviate from the normal HCT values.

HCT contributes to blood viscosity, which in turn affects the spreading of blood on the sampling paper. As a result, the spot size, drying time and homogeneity may vary with different HCT values [8]. High HCT values would result in poorer distribution of blood and thus smaller but denser blood spots produced. This would then lead to a higher concentration of therapeutic drug measured. The opposite applies to low HCT values, where lower concentration may be measured instead [54]. Consequently, the effect of HCT highlights the need to introduce strategies that can minimise its influence on the accuracy of DBS sampling method.

One way to tackle HCT effect is to avoid it entirely by analysing the entire DBS instead of punching a specific spot size [8]. The rationale behind this strategy is that the variation in spreading and homogeneity caused by varying HCT can be eliminated when the entire spot is analysed. Zheng et al. investigated this technique for the analysis of apixaban in DBS [55]. The results obtained showed that whole spot approach can increase the accuracy for all HCT levels, and is hence effective in avoiding HCT effect. Similarly, Li et al. performed perforated dried blood spot microsampling, which essentially also involves the extraction of the entire blood spot, and concluded the same [56]. Nonetheless, this strategy is only possible if a fixed and known blood volume is spotted onto the sampling paper. Since DBS in TDM are targeting the use of capillary blood from finger prick, this strategy may not be suitable, especially when patient self-sampling is utilised.

As such, a more popular strategy practised involves the preparation of calibration standards at an HCT level that corresponds to the expected HCT level of the study samples [8]. In addition, the effect of HCT is evaluated through a range of HCT levels, and depending on the results, HCT correction may or may not be required. This was performed in a handful of studies where they establish the range in which minimal effect of HCT was seen and whether HCT correction is required (as presented in Tables 1, 2, 3, 4, 5 and 6). This strategy, however, would mean that it is essential to know the HCT value of each sample before analysis [57], and hence appears to be a prominent disadvantage for DBS sampling. As HCT values are usually measured in liquid whole blood [58], it is thus challenging to determine the HCT level of a sample if home sampling is intended. Surely, an ideal approach is if HCT can be determined from

DBS sample itself. Rufail et al. investigated this possibility through the detection of potassium in DBS since it was postulated that potassium corresponds well with RBC fraction [59]. Nevertheless, further assessment of this method needs to be performed before its use in routine clinical practice.

Above all, assessing the influence of HCT in DBS sampling method should be made mandatory for method development and validation. Since DBS uses whole (capillary) blood, the effect of HCT cannot be ignored. Referring to Tables 1, 2, 3, 4, 5 and 6, it is noticeable that HCT effect was not evaluated in some studies. In addition, capillary blood was shown to have a higher HCT value than venous blood [54]. Therefore, for studies that use venous blood to determine sample HCT [18], caution in extrapolating the HCT value to capillary blood is to be considered if capillary blood is used for DBS sampling.

7.2 Blood Spot Volume

As DBS uses capillary blood from finger prick, it is not possible to control the amount of blood spotted onto the sampling paper. This would lead to variation in spot size between patients. Blood spot size was said to be proportional to blood volume on cellulose-based cards such as Whatman 903 [60]. This would mean that a fixed spot size punched would contain the same about of blood, regardless of the amount blood volume spotted initially [37]. However, extreme differences in blood volume (> 20 µl) can still result in different concentrations of the analytes [37].

The effect of blood volume is typically investigated by spotting increasing volume of blood onto the sampling paper after which the analyte concentration is measured. For example, Lawson et al. investigated the effect of blood volume for the quantitation of atenolol by preparing 20 µl, 30 µl and 40 µl blood spots at three different concentrations [61]. A fixed spot size was then punched and evaluated. They concluded that the effect of blood volume is considered acceptable within the 20–40 µl range. However, this assumes that blood spot size increases linearly with blood volume. The same may not be observed for patients with HCT level outside of the normal range. Additionally, as the assumption applies for cellulose-based cards, non-cellulose-based cards may need to undergo further assessment on the effect of blood volume.

7.3 Sampling Paper

Various types of sampling paper differ by its physical characteristic such as thickness, pore size and particle retention [60]. Referring to Tables 1, 2, 3, 4, 5 and 6, the most used filter papers include Whatman FTA, Whatman 903 and Whatman 31 ET CHR. The Whatman FTA cards are designed for nucleic acid analysis [37]. There are various types of Whatman FTA cards: Whatman FTA Elute, Whatman FTA DMPK-A, Whatman FTA DMPK-B and Whatman FTA DMPK-C. Whatman FTA Elute, DMPK-A and DMPK-B are treated with chemicals that can lyse cells and denature proteins, whereas DMPK C is untreated filter paper [37]. As a result, Whatman DMPK-C is more commonly used in DBS method as it would not interfere with the measurement of the drug concentration. Only one study used DMPK-A card for the validation of DBS sampling [49]. The CLSI guideline recommends the use of untreated filter papers, namely Whatman 903 and Ahlstrom 226 [31]. However, it is apparent that Whatman 903 is generally more preferred, as only one study [62] used Ahlstrom 226. The use of alternative non-DBS-specific papers such as Whatman 31 ET CHR and Whatman no. 3 were also seen in some studies [50, 52, 53, 63].

Comparison test for various sampling paper types were conducted in some studies. Koster et al. compared Whatman 31 ET CHR and Whatman FTA DMPK-C cards to conclude that no significant differences between the two were found in the analysis of immunosuppressants [50]. However, it was observed that most studies performed comparison tests between cellulose- and non-cellulose-based filter paper. One popular example of a non-cellulose-based filter paper is Agilent Bond Elut DMS, which was claimed to perform better and minimise the influence of HCT [8]. For instance, Enderle et al. compared Agilent Bond Elut DMS card with Whatman FTA DMPK-C card and revealed that Agilent Bond Elut DMS card has lower sensitivity and a larger volume of blood was needed to create the same spot size [64]. The same was also concluded by Meister et al. [65] and Lawson et al. [61]. As such, Agilent Bond Elut DMS card may not be the ideal sampling paper for DBS, and certain consideration should be taken if used. Additionally, Meister et al. also compared Whatman FTA DMPK-C card with Whatman 903 card. As both are cellulose-based cards, the two filter papers are comparable. Interestingly, Lawson et al. reported that the concentration measured from Whatman 903 was lower than the other two sampling papers (Ahlstrom 226 and Agilent Bond Elut DMS card). As the thickness of paper increases from Whatman 903 to Agilent Bond Elut DMS card, this suggest that paper thickness can impact the results of DBS sampling method. All in all, different sampling papers have different properties, and thus testing for the most appropriate type of sampling paper to measure a certain analyte is a good strategy to optimise DBS sampling method.

7.4 Chromatographic Effects

In addition to the factors discussed above, chromatographic effect also plays a part in DBS sampling method. Chromatographic effect refers to the interaction of blood and analyte with the materials of the sampling paper [54]. This may

lead to different concentration measured between central and peripheral areas within a spot. Certainly, this effect relates closely to HCT, blood volume and type of sampling paper used. Investigation of this effect can be performed by measuring concentration from different locations of the same blood spot. Among the reported DBS sampling method for TDM, only Ippolito et al. investigated this effect on the assay of lumefantrine and revealed minimal impact [66]. Since chromatographic effect may vary depending on the physicochemical properties of the analyte, method validation for each therapeutic drug should include the assessment of chromatographic effect.

8 Volumetric Absorptive Microsampling Approach

Initially, dried blood spot (DBS) sampling was a minimally invasive strategy primarily employed for newborn screening, where small volumes of blood were sampled and analysed using automated or manual hole punches with fixed area sub-punches. However, this approach introduced a bias related to haematocrit (HCT) as described earlier. The viscosity of blood changes with the concentration of red blood cells, causing variations in the total area of a DBS from the same blood volume. Specifically, increased HCT concentrations result in smaller DBS areas, whereas low HCT concentration lead to larger DBS areas. To overcome challenges related to the haematocrit effect, spot inhomogeneity and sample volume bias, a volumetric absorptive microsampling (VAMS) approach was developed [67]. The VAMS technique utilises a specialised device consisting of a spherical hydrophilic tip that is securely attached to a plastic holder. The hydrophilic tip is designed with small pores that enable controlled sample collection, ensuring a consistent and fixed volume of sample is absorbed by the device. The innovative sampling method has been successfully employed for blood collection and TDM in individuals receiving various drugs, such as paracetamol, thiamine, therapeutic monoclonal antibody and hydroxychloroquine, among others [68–71]. VAMS has also been explored for metabolomics studies [72, 73]. A recent study compared conventional DBS with the VAMS approach for tacrolimus and mycophenolic acid determination [74]. The results revealed that HCT levels did not have a significant impact on the quantification of the analysis of tacrolimus and mycophenolic acid. Both VAMS and DBS demonstrated favourable analytical performance for both tacrolimus and mycophenolic acid analysis. However, DBS exhibited superior characteristics compared with VAMS in terms of sample quality, cost and clinical performance. These findings suggest that DBS remained the preferred approach for TDM in subjects with HCT levels within the normal range.

9 Guideline for Development and Validation of DBS-Based Methods for TDM

The general guideline for development and validation of DBS-based methods for TDM aligns with the guidelines established by the Food and Drug Administration (FDA) and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) [75, 76]. For specific details on the validation of DBS-based bioanalytical methods, the article by Capiau et al. provides relevant guidance [77].

To establish the validity of a DBS-based method, a comparison with the conventional plasma- or blood-sample-based method is essential. Differences and relationship between the two methods are assessed using Passing–Bablok regression analysis [77]. Predictive performance is evaluated by calculating the median percentage predictive error (MPPE) and the median absolute percentage predictive error (MAPE). The acceptance criteria for the fit performance are that MPPE and MAPE values should be below 15%. The correlation between the methods is determined using the intraclass correlation coefficient (ICC) [78].

Agreement between the methods is analysed using the Bland–Altman plot [77], which allows for the evaluation of the agreement results. The analytical requirements state that the difference between the two methods should be $\leq 20\%$ for at least 67% of the paired samples [75, 76].

The impact of haematocrit (HCT) levels on the results is assessed through linear regression analysis [77]. The concentration difference between the two methods (%) is expressed as a function of HCT levels, and the slope of this regression is examined to determine if it significantly differs from 0. This is determined by calculating its 95% confidence interval (CI) and conducting a *t*-test.

10 Feasibility of Home Sampling

TDM can be a burden to some families as frequent travels to medical facilities are required for blood sample collection. Moreover, for cases in which patients have to be present at a medical facility at a specific time, such as for timed trough drug concentration level [38], inconvenience may be imposed. The collection of capillary blood from finger prick is simple to perform [8], such that any individual who has received proper education and training on the sampling procedure can adequately perform the procedure. Despite it being a promising option, feasibility of home-based TDM using DBS still requires further evaluation.

One major concern with regard to home sampling is the ability of patients or guardians to produce blood spots of acceptable quality that can allow for accurate measurement of the therapeutic drug concentration. Linder et al. conducted a comparison on the quality between samples created by guardians and samples created by trained nurses [9]. Prior to sample collection, guardians watched an instructional video with the same sampling kit to be used for the actual blood collection. Interestingly, it was found that smaller number of samples (8.3%) collected by guardians were rejected as compared with samples collected by nurses (8.0%). The study also concluded that guardians managed to sample satisfactory blood spot of appropriate size and volume. Additionally, Leichtle et al. reported that after training for self-sampling, 91% of the patients were able to sample blood without any help and 83% of the blood spots were adequate [79]. The same was also found by Sakhi et al., in which 93% of blood spots sampled by patients were valid, although there are a small number of people who highlighted issues with regard to lack of blood (5.79%) and difficulties in sampling (1.29%) [10]. Nevertheless, there is strong evidence that blood spots of acceptable quality can be produced by patients or guardians as long as proper training is given. Hence, home-based TDM using DBS is highly feasible.

Aside from that, another concern involves the stability of DBS in envelopes and essentially through the whole mailing process, as these samples have to be transported to the laboratory. As long as there is protection from moisture and humidity, such as using low gas permeable bag, the DBS samples can be transported through normal mail [54]. The stability of DBS samples has been investigated by various studies as presented in Tables 1, 2, 3, 4, 5 and 6. Heijden et al. demonstrated that everolimus is stable for 3 days in the mailbox at 60 °C and 32 days at 4 °C [80]. Hoogtanders et al., meanwhile, investigated the stability of DBS in postal transport by measuring drug concentration immediately after sampling and in a duplicate sample sent by the patient from home by mail [81]. There is agreement between the two methods, indicating stability of DBS during mail delivery. Ultimately, on the basis of the variation of temperature reported stable for DBS, there is a possibility that different therapeutic drugs may have different stabilities. Hence, it is of utmost importance to evaluate the stability of each drug, especially through the mailing process. Until stability is proven, home-based TDM may not be feasible.

It is also important to understand any logistical and psychosocial barriers that may impact the effectiveness of home-based TDM. This includes issues such as difficulty in sending the DBS samples to the analysing laboratory within the specified time frame [82].

11 DBS as a Matrix for Monitoring Drug Responses

DBS was first proposed to be used for neonatal screening for congenital disorders [30]. In the last decades, there are emerging numbers of studies reporting the use of DBS as a matrix for gauging drug responses and disease status.

11.1 DBS as a Matrix to Monitor Inflammatory Responses

It has been employed for measurement of inflammatory markers and neurotrophins using a multiplexed sandwich immunoassay on the basis of flowmetric Luminex® xMAP technology [83]. The assay methodology was validated and improved in further studies [84–86]. A high-frequency longitudinal tracking of inflammatory markers over periods of up to 9 years using DBS as matrix was carried out [87]. In their study, proteins and peptides of the inflammatory markers on the DBS were detected and quantified by a stable isotope standard and captured by anti-peptide antibodies mass spectrometric method (SISCAPA) [88]. The acute phase response proteins of the inflammatory markers related well to major infections, vaccination, surgery, extreme exercise and Crohn's disease [87]. The study shed light that DBS can be a novel approach to track multi-markers of inflammation and could be used in clinical trials of anti-inflammatory biologics, antibiotics and cancer therapeutics to relate the pharmacodynamic response to the drug treatment and/or to the disease states. Aabye et al. reported a simple method to quantitate IP-10 in DBS and DPS (dried plasma spots), a small proinflammatory molecule and an established marker for infection with *M. tuberculosis* [83, 89, 90]. The authors proposed that this approach can be combined with DBS-based therapeutic drug monitoring in clinical practice [89]. This approach can also be used to monitor other inflammatory conditions including viral and bacterial infections, immune dysfunction and tumour development [90] and their responses to treatment. In a study by Tonby et al., they detected a significant decline of IP-10 in both plasma and DPS in response to anti-tuberculosis (anti-TB) therapy [91].

11.2 DBS as a Matrix to Monitor Viral Load

DBS has been employed extensively and successfully for viral load testing, especially for monitoring HIV infection [22, 92, 93]. Patients with HIV require lifelong treatment, and viral load monitoring is required to monitor treatment compliance and efficiency over a lifetime period. Lippman et al. used DBS as a platform to monitor HIV viral load, levels of antiretroviral therapy (ART) drugs, and presence of drug resistance mutation (DRM), the latter through

genotyping [94]. Patients with high viral loads (≥ 5000 copies/mL) but negative ART results had higher prevalence of DRM. These patients likely took ART inconsistently or had interrupted treatment.

DBS has also been used to detect and quantify the respective hepatitis B virus (HBV) [26, 95–97] and hepatitis C virus (HCV) [25, 27, 98, 99]. These studies indicated that DBS can provide a very accurate detection of the viruses and be utilised as point-of-care testing for patients. HCV infection is curable in most patients. The timely diagnosis of all infected patients will be paramount for the treatment of these patients.

11.3 DBS as a Matrix to Monitor Other Diseases

Gaucher disease (GD) is an inherited metabolic disorder caused by impaired catabolism of the glycosphingolipid, glucosylceramide [100]. The deacetylated derivative, glucosylsphingosine (GluSph), is a biomarker for GD, which is also a pharmacodynamic biomarker in response to enzyme replacement therapy. A robust assay was developed for the detection and quantification of Glusph on DBS.

In a preclinical study by Kong et al., they demonstrated that DBS approach can be used to monitor an anti-epileptic drug levels of valproate and the immediate and delayed metabolic changes after treatment, indicating that DBS is a powerful tool to monitor pharmacokinetic/pharmacodynamic changes [101].

Scherf-Clavel et al. used DBS for analysis of anti-diabetic drugs metformin, sitagliptin and blood creatinine levels simultaneously [102, 103]. The DBS creatinine levels were converted to the conventional plasma creatinine levels by multiplying with a correction factor. The calculated plasma creatinine levels were then used to estimate the creatinine clearance. With their approach, they were able to identify how the co-administered drugs, namely the diuretics, NSAIDs and renin-angiotensin system blockers decrease renal function and their influence on the elimination of metformin.

12 Future Direction

Moving forward, the prospect of DBS is focussed on automating the sampling process. For example, fully automated extraction system for DBS analysis has been demonstrated by Duthaler et al. and Velghe et al. using DBS-MS 500 autosampler [104, 105]. The system involves the use of a robotic arm to move samples between different workstations and incorporates built-in cameras to allow for blood spot examination, after which the extraction head can adjust to the centre of each spot. Inadequate blood spot can be recognised by the software and hence excluded from the analysis [105]. Subsequent process in DBS analysis, such as the addition of internal standards and the extractions of analytes, were all performed automatically by the system.

Sample preparation for DBS can be laborious and thus the development of automated DBS analysis is expected to expand further. However, a drawback to the automated system is its high cost, which will impose an additional toll to the relatively low cost of DBS sampling [106]. This would be impractical for the application of DBS sampling especially in resource-limited settings. Nevertheless, more research has to be done to investigate feasibility of DBS automation. In actual clinical practice, DBS samples can vary in terms of shape, volume and HCT level, and thus it is important to ensure that the automation system are able to tackle such deviations. Perhaps development of advanced devices to minimise such variation during sample collection can be considered in the future as well.

The application of DBS for pharmacokinetic and pharmacodynamic monitoring is most successful in viral load testing. It has been applied extensively in longitudinal studies. DBS is a convenient and economical approach for monitoring viral infections and their treatment in continents with large populations where people are scattered wide across the continents. With measurement of the viral loads and antiviral drug levels, clinicians will be able to examine therapy efficiency and patient compliance to treatment. However, to date DBS is less commonly applied to monitor drug responses in other diseases. With establishment of more suitable biomarkers in DBS for diseases and their treatment responses, DBS could become a conventional platform for therapeutic drug levels and drug response monitoring.

13 Conclusions

Clearly, the use of DBS for TDM as an alternative to conventional plasma/serum seems to be very promising. At present, there are already many assays reported and validated for various therapeutic drugs. It is apparent that the main hurdle in the implementation of DBS sampling for TDM is due to plasma/serum being the golden standard for many years. In addition, various factors discussed that can affect DBS sampling method also pose a challenge in the widespread implementation of DBS sampling in clinical practice. It is thus recommended that extensive validation addressing all the various factors should be made mandatory to ensure reliability of the method. Nevertheless, the benefits of DBS over conventional methods are so valuable that it is worthwhile to advance the method as a viable choice for TDM and DRM.

DBS sampling has made significant contributions to improving patient outcomes in various areas of healthcare. One notable application is in newborn screening programs

worldwide, whereas DBS sampling is routinely used for the early detection of congenital disorders such as phenylketonuria, sickle cell disease and hypothyroidism [30, 107–110].

In the field of HIV testing and viral load monitoring, DBS offers several advantages. It allows for easy sample collection and transportation, particularly in resource-limited settings, making it a valuable tool for expanding access to testing and monitoring services [21, 22, 93, 111, 112].

DBS has also been instrumental in TDM for patients receiving antiepileptic drugs [9], immunosuppressants [74], direct oral anticoagulants [113] and antiretroviral agents [114, 115]. By using DBS samples, healthcare professionals can conveniently monitor drug levels in patients and optimise treatment regimens.

Furthermore, DBS has shown promise for genetic testing, especially in the context of inherited disorders [116, 117]. DBS enables the extraction of DNA from the dried blood spots, allowing for subsequent analysis of specific genes or genetic markers. Overall, DBS has played a crucial role in enhancing patient care and outcomes, facilitating early diagnosis, monitoring treatment and carrying out genetic testing across various healthcare domains.

The small sample volume (30 µl) inherent to DBS presents challenges in detection and analysis. Due to the limited amount of samples available on a DBS card, sensitive detection methods are required to accurately measure and quantify analytes of interest. Analytical techniques with high sensitivity and specificity such as mass spectrometers, are commonly employed for DBS analysis. To date, mass spectrometers with improved sensitivity and specificity can measure analytes, ranging from small molecules to biomacromolecules. Therefore, mass spectrometers can be applied in the respective areas, such as therapeutic drug monitoring, pharmacogenomics, pharmacomicrobiomics, pharmacoepigenomics and immuopeptidomics [118]. In the context of TDM and DRM, mass spectrometers can be particularly valuable when combined with surface analysis techniques for the analysis of DBS [37]. By utilising DBS as matrices, these applications facilitate the achievement of pharmacokinetic and pharmacodynamic correlations, contributing to the realisation of personalised therapy.

Declarations

Funding Open Access funding enabled and organized by CAUL and its Member Institutions.

Conflicts of Interest Nur Nabihah Binte Zailani and Paul Chi-Lui Ho declare that they have no potential conflicts of interest that might be relevant to the content of this manuscript.

Author Contributions Nur Nabihah Binte Zailani and Paul Chi-Lui Ho conceptualised the manuscript. Nur Nabihah Binte Zailani performed the literature search and wrote the manuscript. Paul Chi-Lui Ho revised the manuscript.

Data Availability Statement Data sharing is not applicable to this article as no datasets were generated or analysed.

Ethics Approval Not Applicable.

Consent to Participate Not Applicable.

Consent for Publication Not Applicable.

Code Availability Not Applicable.

References

1. Kang JS, Lee MH. Overview of therapeutic drug monitoring. Korean J Intern Med. 2009;24(1):1–10.
2. Lawson G, Cocks E, Tanna S. Bisoprolol, ramipril and simvastatin determination in dried blood spot samples using LC-HRMS for assessing medication adherence. J Pharm Biomed Anal. 2013;81–82:99–107.
3. Zwart TC, et al. Therapeutic drug monitoring of tacrolimus and mycophenolic acid in outpatient renal transplant recipients using a volumetric dried blood spot sampling device. Br J Clin Pharmacol. 2018;84(12):2889–902.
4. Linder C, et al. Carbamazepine, lamotrigine, levetiracetam and valproic acid in dried blood spots with liquid chromatography tandem mass spectrometry; method development and validation. J Chromatogr B Analyt Technol Biomed Life Sci. 2018;1072:116–22.
5. Martial LC, et al. Dried blood spot sampling in psychiatry: perspectives for improving therapeutic drug monitoring. Eur Neuropsychopharmacol. 2017;27(3):205–16.
6. Avataneo V, et al. LC-MS application for therapeutic drug monitoring in alternative matrices. J Pharm Biomed Anal. 2019;166:40–51.
7. Velghe S, De Troyer R, Stove C. Dried blood spots in therapeutic drug monitoring and toxicology. Expert Opin Drug Metab Toxicol. 2018;14(1):1–3.
8. Wilhelm AJ, den Burger JC, Swart EL. Therapeutic drug monitoring by dried blood spot: progress to date and future directions. Clin Pharmacokinet. 2014;53(11):961–73.
9. Linder C, et al. Dried blood spot self-sampling by guardians of children with epilepsy is feasible: comparison with plasma for multiple antiepileptic drugs. Ther Drug Monit. 2019;41(4):509–18.
10. Sakhi AK, et al. Feasibility of self-sampled dried blood spot and saliva samples sent by mail in a population-based study. BMC Cancer. 2015;15:265.

11. Emmons G, Rowland M. Pharmacokinetic considerations as to when to use dried blood spot sampling. Bioanalysis. 2010;2(11):1791–6.
12. Wilhelm AJ, et al. Clinical validation of dried blood spot sampling in therapeutic drug monitoring of ciclosporin A in allogeneic stem cell transplant recipients: direct comparison between capillary and venous sampling. Ther Drug Monit. 2013;35(1):92–5.
13. Linder C, et al. Comparison between dried blood spot and plasma sampling for therapeutic drug monitoring of antiepileptic drugs in children with epilepsy: a step towards home sampling. Clin Biochem. 2017;50(7–8):418–24.
14. Das S, et al. Determination of serum carbamazepine concentration using dried blood spot specimens for resource-limited settings. Hosp Pract. 2017;45(2):46–50.
15. Veenhof H, et al. Clinical validation of simultaneous analysis of tacrolimus, cyclosporine A, and creatinine in dried blood spots in kidney transplant patients. Transplantation. 2017;101(7):1727–33.
16. Villanelli F, et al. Dried blood spot assay for the quantification of phenytoin using liquid chromatography-mass spectrometry. Clin Chim Acta. 2015;440:31–5.
17. Rhoden L, et al. Simple procedure for determination of valproic acid in dried blood spots by gas chromatography-mass spectrometry. J Pharm Biomed Anal. 2014;96:207–12.
18. Kloosterboer SM, et al. Dried blood spot analysis for therapeutic drug monitoring of antipsychotics: drawbacks of its clinical application. Ther Drug Monit. 2018;40(3):344–50.
19. Veenhof H, et al. Clinical application of a dried blood spot assay for sirolimus and everolimus in transplant patients. Clin Chem Lab Med. 2019;57(12):1854–62.
20. Shokry E, et al. Therapeutic drug monitoring of carbamazepine and its metabolite in children from dried blood spots using liquid chromatography and tandem mass spectrometry. J Pharm Biomed Anal. 2015;109:164–70.
21. Vojnov L, et al. The performance of using dried blood spot specimens for HIV-1 viral load testing: a systematic review and meta-analysis. Plos Med. 2022;19(8):1.
22. Hrapcak S, et al. HIV viral load scale-up among children and adolescents: trends in viral load suppression, sample type and processing in 7 PEPFAR countries, 2015–2018. Pediatr Infect Dis J. 2023;42(4):E102–4.
23. Jennings L, et al. Tenofovir diphosphate in dried blood spots predicts future viremia in persons with HIV taking antiretroviral therapy in South Africa. AIDS. 2022;36(7):933–40.
24. Khamduang W, et al. HIV RNA measurement in dried blood spots of HIV-infected patients in Thailand using Abbott m2000 system. PLoS ONE. 2020;15(1):1.
25. Catlett B, et al. Diagnostic accuracy of assays using point-of-care testing or dried blood spot samples for the determination of hepatitis C virus RNA: a systematic review(). J Infect Dis. 2022;226(6):1005–21.
26. Garg R, et al. Evaluation of blood samples collected by dried blood spots (DBS) method for hepatitis B virus DNA quantitation and its stability under real life conditions. J Clin Virol Plus. 2022;2(4):1.
27. Gomez L, et al. Diagnostic test accuracy of the cobas 6800 system for detection of hepatitis C virus viraemia levels from dried blood spots. Enferm Infect Microbiol Clin. 2020;38(6):267–74.
28. Kerkhofs M, et al. Cross-omics: integrating genomics with metabolomics in clinical diagnostics. Metabolites. 2020;10(5):1.
29. Zhuang YJ, et al. Multi-omics analysis from archival neonatal dried blood spots: limitations and opportunities. Clin Chem Lab Med. 2022;60(9):1318–41.
30. Scriver CC. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants, by Robert Guthrie and Ada Susi, Pediatrics, 1963;32:318–343. Pediatrics. 1998;102(1 Pt 2):236–7.
31. Zakaria R, et al. Advantages and challenges of dried blood spot analysis by mass spectrometry across the total testing process. Ejifcc. 2016;27(4):288–317.
32. Gupta K, Mahajan R. Applications and diagnostic potential of dried blood spots. Int J Appl Basic Med Res. 2018;8(1):1–2.
33. Edelbroek PM, van der Heijden J, Stolk LM. Dried blood spot methods in therapeutic drug monitoring: methods, assays, and pitfalls. Ther Drug Monit. 2009;31(3):327–36.
34. Antunes MV, Charão MF, Linden R. Dried blood spots analysis with mass spectrometry: potentials and pitfalls in therapeutic drug monitoring. Clin Biochem. 2016;49(13–14):1035–46.
35. Weber J, et al. Validation of a dried blood spot method for therapeutic drug monitoring of citalopram, mirtazapine and risperidone and its active metabolite 9-hydroxyrisperidone using HPLC-MS. J Pharm Biomed Anal. 2017;140:347–54.
36. Aykanat Girgin B, Göl I. Reducing pain and fear in children during venipuncture: a randomized controlled study. Pain Manag Nurs. 2020;21(3):276–82.
37. Wagner M, et al. The use of mass spectrometry to analyze dried blood spots. Mass Spectrom Rev. 2016;35(3):361–438.
38. Dickerson JA, et al. Tacrolimus and sirolimus in capillary dried blood spots allows for remote monitoring. Pediatr Transplant. 2015;19(1):101–6.
39. Enderle Y, Foerster K, Burhenne J. Clinical feasibility of dried blood spots: analytics, validation, and applications. J Pharm Biomed Anal. 2016;130:231–43.
40. (EMA), E.M.A., *Guideline on Bioanalytical Method Validation*, in *EMEA/CHMP/EWP/199217/2009*. 2011: London, United Kingdom.
41. (CLSI), C.a.L.S.I., *Measurement Procedure Comparison and Bias Estimation Using Patients Samples*, in *EP09-A3*. 2013: Pennsylvania.
42. Kong ST, et al. Clinical validation and implications of dried blood spot sampling of carbamazepine, valproic acid and phenytoin in patients with epilepsy. PLoS ONE. 2014;9(9): e108190.
43. la Marca G, et al. Rapid assay of rufinamide in dried blood spots by a new liquid chromatography-tandem mass spectrometric method. J Pharm Biomed Anal. 2011;54(1):192–7.
44. Milosheska D, Grabnar I, Vovk T. Dried blood spots for monitoring and individualization of antiepileptic drug treatment. Eur J Pharm Sci. 2015;75:25–39.
45. Chiou WL. The phenomenon and rationale of marked dependence of drug concentration on blood sampling site: Implications in pharmacokinetics, pharmacodynamics, toxicology and therapeutics (Part I). Clin Pharmacokinet. 1989;17(3):175–99.
46. Martial LC, et al. Dried blood spot sampling for tacrolimus and mycophenolic acid in children: analytical and clinical validation. Ther Drug Monit. 2017;39(4):412–21.
47. Sadilkova K, et al. Clinical validation and implementation of a multiplexed immunosuppressant assay in dried blood spots by LC-MS/MS. Clin Chim Acta. 2013;421:152–6.
48. Hinchliffe E, Adaway JE, Keevil BG. Simultaneous measurement of cyclosporin A and tacrolimus from dried blood spots by ultra high performance liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2012;883–884:102–7.
49. Koop DR, et al. Analysis of tacrolimus and creatinine from a single dried blood spot using liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2013;926:54–61.

50. Koster RA, et al. Fast LC-MS/MS analysis of tacrolimus, sirolimus, everolimus and cyclosporin A in dried blood spots and the influence of the hematocrit and immunosuppressant concentration on recovery. Talanta. 2013;115:47–54.
51. Cheung CY, et al. Dried blood spot measurement: application in tacrolimus monitoring using limited sampling strategy and abbreviated AUC estimation. Transpl Int. 2008;21(2):140–5.
52. Vu DH, et al. Dried blood spot analysis for therapeutic drug monitoring of linezolid in patients with multidrug-resistant tuberculosis. Antimicrob Agents Chemother. 2012;56(11):5758–63.
53. Vu DH, et al. Determination of moxifloxacin in dried blood spots using LC-MS/MS and the impact of the hematocrit and blood volume. J Chromatogr B Analyt Technol Biomed Life Sci. 2011;879(15–16):1063–70.
54. Li W, Tse FL. Dried blood spot sampling in combination with LC-MS/MS for quantitative analysis of small molecules. Biomed Chromatogr. 2010;24(1):49–65.
55. Zheng N, et al. "Center punch" and "whole spot" bioanalysis of apixaban in human dried blood spot samples by UHPLC-MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci. 2015;988:66–74.
56. Li F, et al. Perforated dried blood spots: a novel format for accurate microsampling. Bioanalysis. 2011;3(20):2321–33.
57. Tron C, et al. Dried blood spots combined with ultra-high-performance liquid chromatography-mass spectrometry for the quantification of the antipsychotics risperidone, aripiprazole, pipamperone, and their major metabolites. Ther Drug Monit. 2017;39(4):429–40.
58. Billett HH. Clinical Methods: The History, Physical, and Laboratory Examinations. in Hemoglobin and Hematocrit, H.W. Walker HK, Hurst JW, Editor. 1990, Butterworth: Boston.
59. Rufail ML, McCloskey LJ, Stickle DF. Estimation of hematocrit in filter paper dried bloodspots by potassium measurement: advantage of use of perimeter ring samples over circular center sub-punch samples. Clin Chem Lab Med. 2017;55(1):53–7.
60. Klak A, Pauwels S, Vermeersch P. Preanalytical considerations in therapeutic drug monitoring of immunosuppressants with dried blood spots. Diagnosis (Berl). 2019;6(1):57–68.
61. Lawson G, Cocks E, Tanna S. Quantitative determination of atenolol in dried blood spot samples by LC-HRMS: a potential method for assessing medication adherence. J Chromatogr B Analyt Technol Biomed Life Sci. 2012;897:72–9.
62. Chen G, et al. Quantification of amlodipine in dried blood spot samples by high performance liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2018;1072:252–8.
63. Vu DH, et al. Simultaneous determination of rifampicin, clarithromycin and their metabolites in dried blood spots using LC-MS/MS. Talanta. 2014;121:9–17.
64. Enderle Y, et al. Dried blood spot technique for the monitoring of ambrisentan, bosentan, sildenafil, and tadalafil in patients with pulmonary arterial hypertension. Anal Chem. 2015;87(24):12112–20.
65. Meister I, et al. Development and validation of an enantioselective LC-MS/MS method for the analysis of the anthelmintic drug praziquantel and its main metabolite in human plasma, blood and dried blood spots. J Pharm Biomed Anal. 2016;118:81–8.
66. Ippolito MM, et al. Semi-quantitative measurement of the antimalarial lumefantrine from untreated dried blood spots using LC-MS/MS. J Pharm Biomed Anal. 2018;155:241–6.
67. Denniff P, Spooner N. Volumetric absorptive microsampling: a dried sample collection technique for quantitative bioanalysis. Anal Chem. 2014;86(16):8489–95.
68. Boffel L, et al. Application of a volumetric absorptive microsampling (VAMS)-based method for the determination of paracetamol and four of its metabolites as a tool for pharmacokinetic studies in obese and non-obese patients. Clin Pharmacokinet. 2022;61(12):1719–33.
69. Gao X, et al. Volumetric absorptive microsampling (VAMS(R)) in therapeutic protein quantification by LC-MS/MS: Investigation of anticoagulant impact on assay performance and recommendations for best practices in method development. J Pharm Biomed Anal. 2021;196: 113895.
70. Qu Y, et al. Capillary blood collected on volumetric absorptive microsampling (VAMS) device for monitoring hydroxychloroquine in rheumatoid arthritis patients. J Pharm Biomed Anal. 2017;140:334–41.
71. Verstraete J, Stove C. Volumetric absorptive microsampling (VAMS) as a reliable tool to assess thiamine status in dried blood microsamples: a comparative study. Am J Clin Nutr. 2021;114(3):1200–7.
72. Kok MGM, et al. Targeted metabolomics of whole blood using volumetric absorptive microsampling. Talanta. 2019;197:49–58.
73. Volani C, et al. Pre-analytic evaluation of volumetric absorptive microsampling and integration in a mass spectrometry-based metabolomics workflow. Anal Bioanal Chem. 2017;409(26):6263–76.
74. Paniagua-Gonzalez L, et al. Comparison of conventional dried blood spots and volumetric absorptive microsampling for tacrolimus and mycophenolic acid determination. J Pharm Biomed Anal. 2022;208: 114443.
75. ICH guideline M10 on bioanalytical method validation and study sample analysis, in EMA/CHMP/ICH/172948/2019, I.C.f.H.o.T.R.f.P.f.H.U. (ICH), Editor.
76. Bioanalytical method validation, guidance for industry, 2018, in http://www.regulations.gov/document?D=FDA-2013-D-1020-0039, D.o.H.a.H.s. US, Food and Drug Administration (FDA), Editor.
77. Capiau S, et al. Official international association for therapeutic drug monitoring and clinical toxicology guideline: development and validation of dried blood spot-based methods for therapeutic drug monitoring. Ther Drug Monit. 2019;41(4):409–30.
78. Paniagua-Gonzalez L, et al. Volumetric absorptive microsampling (VAMS) for assaying immunosuppressants from venous whole blood by LC-MS/MS using a novel atmospheric pressure ionization probe (UniSpray). J Pharm Biomed Anal. 2020;189: 113422.
79. Leichtle AB, et al. Potential of dried blood self-sampling for cyclosporine c(2) monitoring in transplant outpatients. J Transplant. 2010;2010: 201918.
80. van der Heijden J, et al. Therapeutic drug monitoring of everolimus using the dried blood spot method in combination with liquid chromatography-mass spectrometry. J Pharm Biomed Anal. 2009;50(4):664–70.
81. Hoogtanders K, et al. Dried blood spot measurement of tacrolimus is promising for patient monitoring. Transplantation. 2007;83(2):237–8.
82. Al-Uzri A, et al. Longitudinal study on the use of dried blood spots for home monitoring in children after kidney transplantation. Pediatr Transplant. 2017;21(6):1.
83. Skogstrand K, 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):1854–66.
84. Skogstrand K, et al. Effects of blood sample handling procedures on measurable inflammatory markers in plasma, serum and dried blood spot samples. J Immunol Methods. 2008;336(1):78–84.

85. Huang Y, McDade TW. Measurement of inflammatory cytokines in dried blood spot samples by multiplex immunoassay. Am J Hum Biol. 2009;21(2):257–257.
86. McDade TW, et al. A highly sensitive multiplex immunoassay for inflammatory cytokines in dried blood spots. Am J Hum Biol. 2021;33(6):1.
87. Anderson L, et al. Precision multiparameter tracking of inflammation on timescales of hours to years using serial dried blood spots. Bioanalysis. 2020;12(13):937–55.
88. Anderson NL, et al. Mass spectrometric quantitation of peptides and proteins using stable isotope standards and capture by anti-peptide antibodies (SISCAPA). J Proteome Res. 2004;3(2):235–44.
89. Aabye MG, et al. A simple method to quantitate IP-10 in dried blood and plasma spots. PLoS ONE. 2012;7(6):1.
90. Drabe CH, Blauenfeldt T, Ruhwald M. ELISA-based assay for IP-10 detection from filter paper samples. In: Cytokine Bioassays: Methods and Protocols, I. Vancurova, Editor. 2014. pp. 27–37.
91. Tonby K, et al. IP-10 measured by dry plasma spots as biomarker for therapy responses in mycobacterium tuberculosis infection. Sci Rep. 2015;5:1.
92. Nguyen TA, et al. Feasibility of dried blood spots for HIV viral load monitoring in decentralized area in North Vietnam in a test-and-treat era, the MOVIDA project. PLoS ONE. 2020;15(4):1.
93. Teran RA, et al. Longitudinal viral load monitoring using home-collected dried blood spot specimens of MSM living with HIV: results from a feasibility pilot study. AIDS Behav. 2021;25(3):661–6.
94. Lippman SA, et al. The role of drug resistance in poor viral suppression in rural South Africa: findings from a population-based study. BMC Infect Dis. 2020;20(1):1.
95. Jackson K, et al. Real-world application of the Xpert (R) HBV viral load assay on serum and dried blood spots. J Med Virol. 2021;93(6):3707–13.
96. Poiteau L, et al. Evaluation of the Xpert HBV viral load for hepatitis B virus molecular testing. J Clin Virol. 2020;129:1.
97. Roger S, et al. Evaluation of the Aptima (TM) HBV Quant Dx assay for semi-quantitative HBV viral load from dried blood spots. J Clin Virol. 2020;129:1.
98. Prinsenberg T, et al. Dried blood spot self-sampling at home is a feasible technique for hepatitis C RNA detection. PLoS ONE. 2020;15(4):1.
99. Tran TH, et al. Dried blood spots perform well to identify patients with active HCV infection in Vietnam. J Viral Hepatitis. 2020;27(5):514–9.
100. Saville JT, et al. Expanding the clinical utility of glucosylsphingosine for Gaucher disease. J Inherit Metab Dis. 2020;43(3):558–63.
101. Kong ST, et al. Dried blood spots as matrix for evaluation of valproate levels and the immediate and delayed metabolomic changes induced by single valproate dose treatment. Int J Mol Sci. 2022;23(13):1.
102. Scherf-Clavel M, Högger P. Analysis of metformin, sitagliptin and creatinine in human dried blood spots. J Chromatogr B Analyt Technol Biomed Life Sci. 2015;997:218–28.
103. Scherf-Clavel M, et al. Dried blood spot testing for estimation of renal function and analysis of metformin and sitagliptin concentrations in diabetic patients: a cross-sectional study. Eur J Clin Pharmacol. 2019;75(6):809–16.
104. Velghe S, Deprez S, Stove CP. Fully automated therapeutic drug monitoring of anti-epileptic drugs making use of dried blood spots. J Chromatogr A. 2019;1601:95–103.
105. Duthaler U, et al. Development and validation of an LC-MS/MS method for the analysis of ivermectin in plasma, whole blood, and dried blood spots using a fully automatic extraction system. J Pharm Biomed Anal. 2019;172:18–25.
106. Snijdewind IJ, et al. Current and future applications of dried blood spots in viral disease management. Antiviral Res. 2012;93(3):309–21.
107. Lampret BR, et al. Expanded newborn screening program in Slovenia using tandem mass spectrometry and confirmatory next generation sequencing genetic testing. Zdr Varst. 2020;59(4):256–63.
108. Moat SJ, George RS, Carling RS. Use of dried blood spot specimens to monitor patients with inherited metabolic disorders. Int J Neonatal Screen. 2020;6(2):26.
109. Mason K, et al. Newborn screening for sickle cell disease: Jamaican experience. West Indian Med J. 2015;65(1):18–26.
110. Ndeezi G, et al. Burden of sickle cell trait and disease in the Uganda Sickle Surveillance Study (US3): a cross-sectional study. Lancet Glob Health. 2016;4(3):e195-200.
111. Newman H, Hardie D. HIV-1 viral load testing in resource-limited settings: challenges and solutions for specimen integrity. Rev Med Virol. 2021;31(2):1.
112. Nguyen LBL, et al. Performances of dried blood spots and point-of-care devices to identify virological failure in HIV-infected patients: a systematic review and meta-analysis. AIDS Patient Care STDS. 2023;37(2):66–83.
113. Foerster KI, et al. Dried-blood-spot technique to monitor direct oral anticoagulants: clinical validation of a UPLC-MS/MS-based assay. Anal Chem. 2018;90(15):9395–402.
114. Calcagno A, et al. Dried plasma/blood spots for monitoring antiretroviral treatment efficacy and pharmacokinetics: a cross-sectional study in rural Burundi. Br J Clin Pharmacol. 2015;79(5):801–8.
115. Kromdijk W, et al. Therapeutic drug monitoring of antiretroviral drugs at home using dried blood spots: a proof-of-concept study. Antivir Ther. 2013;18(6):821–5.
116. Dantonio P, et al. Proficiency testing of human leukocyte antigen-DR and human leukocyte antigen-DQ genetic risk assessment for type 1 diabetes using dried blood spots. J Diabetes Sci Technol. 2010;4(4):929–41.
117. Shearer AE, et al. Comprehensive genetic testing for deafness from fresh and archived dried blood spots. Otolaryngol Head Neck Surg. 2018;159(6):1058–60.
118. Cui JJ, et al. Mass spectrometry-based personalized drug therapy. Mass Spectrom Rev. 2020;39(5–6):523–52.
119. Shah NM, et al. A simple bioanalytical method for the quantification of antiepileptic drugs in dried blood spots. J Chromatogr B Analyt Technol Biomed Life Sci. 2013;923–924:65–73.
120. la Marca G, et al. Rapid assay of topiramate in dried blood spots by a new liquid chromatography-tandem mass spectrometric method. J Pharm Biomed Anal. 2008;48(5):1392–6.
121. Hinchliffe E, et al. Therapeutic drug monitoring of ciclosporin A and tacrolimus in heart lung transplant patients using dried blood spots. Ann Clin Biochem. 2014;51(Pt 1):106–9.
122. Wilhelm AJ, et al. Analysis of mycophenolic acid in dried blood spots using reversed phase high performance liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci. 2009;877(30):3916–9.
123. Arpini J, et al. Clinical evaluation of a dried blood spot method for determination of mycophenolic acid in renal transplant patients. Clin Biochem. 2013;46(18):1905–8.
124. Iboshi H, et al. Development of a liquid chromatography-tandem mass spectrometric method for quantification of mycophenolic acid and its glucuronides in dried blood spot samples. Ther Drug Monit. 2017;39(6):648–53.
125. Hoogtanders K, et al. Therapeutic drug monitoring of tacrolimus with the dried blood spot method. J Pharm Biomed Anal. 2007;44(3):658–64.

126. Lee K, et al. Multiplex assay of second-line anti-tuberculosis drugs in dried blood spots using ultra-performance liquid chromatography-tandem mass spectrometry. Ann Lab Med. 2016;36(5):489–93.
127. Martial LC, et al. Evaluation of dried blood spot sampling for pharmacokinetic research and therapeutic drug monitoring of anti-tuberculosis drugs in children. Int J Antimicrob Agents. 2018;52(1):109–13.
128. la Marca G, et al. Rapid and sensitive LC-MS/MS method for the analysis of antibiotic linezolid on dried blood spot. J Pharm Biomed Anal. 2012;67–68:86–91.
129. Allanson AL, et al. Determination of rifampicin in human plasma and blood spots by high performance liquid chromatography with UV detection: a potential method for therapeutic drug monitoring. J Pharm Biomed Anal. 2007;44(4):963–9.
130. Knippenberg B, et al. Validation and application of a dried blood spot assay for biofilm-active antibiotics commonly used for treatment of prosthetic implant infections. Antimicrob Agents Chemother. 2016;60(8):4940–55.
131. Parsons TL, et al. Quantification of rifapentine, a potent antituberculosis drug, from dried blood spot samples using liquid chromatographic-tandem mass spectrometric analysis. Antimicrob Agents Chemother. 2014;58(11):6747–57.
132. Le J, et al. Comparative analysis of ampicillin plasma and dried blood spot pharmacokinetics in neonates. Ther Drug Monit. 2018;40(1):103–8.
133. Page-Sharp M, et al. Validation and application of a dried blood spot ceftriaxone assay. Antimicrob Agents Chemother. 2016;60(1):14–23.
134. la Marca G, et al. Development of an UPLC-MS/MS method for the determination of antibiotic ertapenem on dried blood spots. J Pharm Biomed Anal. 2012;61:108–13.
135. Cohen-Wolkowiez M, et al. Determining population and developmental pharmacokinetics of metronidazole using plasma and dried blood spot samples from premature infants. Pediatr Infect Dis J. 2013;32(9):956–61.
136. Cohen-Wolkowiez M, et al. Developmental pharmacokinetics of piperacillin and tazobactam using plasma and dried blood spots from infants. Antimicrob Agents Chemother. 2014;58(5):2856–65.
137. Galindo Bedor DC, et al. Dried blood spot technique-based liquid chromatography-tandem mass spectrometry method as a simple alternative for benznidazole pharmacokinetic assessment. Antimicrob Agents Chemother. 2018;62(12):1.
138. Momper JD, et al. Determination of benznidazole in human dried blood spots by liquid chromatography-mass spectrometry to monitor adherence to trypanosoma cruzi infection treatment in infants and children. Am J Trop Med Hyg. 2019;101(1):116–22.
139. Duthaler U, et al. Population pharmacokinetics of oral ivermectin in venous plasma and dried blood spots in healthy volunteers. Br J Clin Pharmacol. 2019;85(3):626–33.
140. Cheng X, et al. Development and validation of a liquid chromatography/tandem mass spectrometry method for determination of caspofungin in dried blood spots. Rapid Commun Mass Spectrom. 2018;32(13):1068–74.
141. Bernieh D, Lawson G, Tanna S. Quantitative LC-HRMS determination of selected cardiovascular drugs, in dried blood spots, as an indicator of adherence to medication. J Pharm Biomed Anal. 2017;142:232–43.
142. Peeters LEJ, et al. Clinical validation of a dried blood spot assay for 8 antihypertensive drugs and 4 active metabolites. Ther Drug Monit. 2020;42(3):460–7.
143. Aburuz S, Millership J, McElnay J. Dried blood spot liquid chromatography assay for therapeutic drug monitoring of metformin. J Chromatogr B Analyt Technol Biomed Life Sci. 2006;832(2):202–7.