ChiKD CHILDHOOD KIDNEY DISEASES

Review Article

Child Kidney Dis 2024;28(1):16-26
pISSN 2384-0242 · eISSN 2384-0250
https://doi.org/10.3339/ckd.23.023

Advances in the use of dried blood spots on filter paper to monitor kidney disease

Carla Nicola[1], Vandréa de Souza[1]

[1]Graduate Health Sciences Program, Universidade de Caxias do Sul, Caxias do Sul, Brazil

Patients with kidney disease require frequent blood tests to monitor their kidney function, which is particularly difficult for young children and the elderly. For these people, the standard method is to evaluate serum creatinine or cystatin C or drug levels through venous sampling, but more recently, evaluation using dried blood spots has been used. This narrative review reports information from the literature on the use of dried blood spots to quantify the main markers used to detect kidney diseases. The ScienceDirect and PubMed databases were searched using the keywords: "dried blood on filter paper," "markers of renal function," "renal function," "creatinine," "cystatin C," "urea," "iohexol," and "iotalamate." Studies using animal samples were excluded, and only relevant articles in English or Spanish were considered. Creatinine was the most assessed biomarker in studies using dried blood spots to monitor kidney function, showing good performance in samples whose hematocrit levels were within normal reference values. According to the included studies, dried blood spots are a practical monitoring alternative for kidney disease. Validation parameters, such as sample and card type, volume, storage, internal patterns, and the effects of hematocrit are crucial to improving the reliability of these results.

Keywords: Creatinine; Cystatin C; Dried blood spot testing; Iohexol; Urea

Introduction

Kidney disease is a public health problem, with more than 750 million people diagnosed worldwide. In 2019, 1.3 million people lost their lives due to kidney failure, and nearly 1.7 million die from acute kidney injury every year [1-3]. Chronic kidney disease (CKD) is a challenge because it manifests with unspecific or no clinical symptoms; symptoms are detected only at more advanced stages [4,5]. The most frequent complications of this disease are cardiovascular disorders, mineral and bone imbalance, and progression of CKD [6].

Kidney diseases can be recognized by identifying an imbalance in markers such as amino acids, lipids, and nucleotides. These compounds can suggest that there is a problem, expediting proper treatment and thus reducing complications [7-9]. The main indicators of kidney injury are albuminuria (albumin to creatinine ratio ≥30 mg/g), urinary sediment abnormalities characteristic of tubular disease, electrolytic disorders, and reduced renal function (glomerular filtration rate [GFR] <60 mL/min/1.73 m^2) [10-12].

Indicators of kidney injury are mainly detected through urine and venous blood samples. These samples must be refrigerated due to the instability of the compounds, which can undergo enzymatic degradation [13,14]. However, dried blood spots (DBS) have gained relevance and may especially benefit populations at risk of CKD [13,15]. DBS is advantageous for infants and el-

Received: November 24, 2023; Revised: January 23, 2024; Accepted: February 9, 2024

Correspondence to
Carla Nicola
Graduate Health Sciences Program, Universidade de Caxias do Sul, Francisco Getúlio Vargas, 1130 Caxias do Sul 95070-560, Brazil
E-mail: carla1982n@hotmail.com

https://doi.org/10.3339/ckd.23.023

derly patients, especially because it requires less blood volume than conventional tests [16,17]. Despite the observed practical advantages, these assays involve methodological concerns that should be discussed, such as homogeneity of the sample point, hematocrit, and sample recovery [18,19].

Hence, this narrative review covers potential applications of DBS including GFR estimation, drug level monitoring, and its advantages and limitations, as well as precautions when applying it in clinical practice.

Markers of kidney function

The GFR, which describes the volume of plasma filtered from the glomerular capillaries into Bowman's capsules per unit of time [20], is considered a sensitive and specific indicator of abnormal kidney function [21]. The gold standard for GFR measurement is determining the clearance of compounds filtered exclusively by the glomeruli. Exogenous markers, such as iohexol, inulin, and iothalamate, meet this criterion, but they are used only in specific situations (e.g., drug adjustment or kidney protocols) due to their cost and complexity [10].

In most circumstances, GFR is estimated using compounds eliminated by the kidneys (creatinine and cystatin C) based on mathematical equations to correct for biological variations [22,23]. Creatinine levels vary according to age, sex, metabolism, muscle mass, and nutritional status. Cystatin C seems to be less dependent on biological factors, but its levels may increase with glucocorticoid use and show poor agreement during pregnancy due to placental production [8,24,25].

Principles and applications of DBS methods

The first officially established tests to use dried whole blood samples on filter paper in the pre-analytical phase of laboratory testing were performed in 1963, with the discovery of an effective low-cost neonatal screening test to identify phenylketonuria [26]. The successful screening of this and other inborn errors of metabolism using DBS has led to its adaptation for a myriad of analytical parameters, such as drug monitoring, protein studies, and infectious disease management [14,27,28]. Table 1 summarizes the main applications of DBS in kidney diseases.

The filter paper method has advantages over conventional venipuncture, since blood collection is easy to perform, less invasive, and relatively painless [29,30]. The paper filter method minimizes the volume of blood taken from patients and may be performed without specialized structures [29]. Furthermore, it is better suited for clinical research and patients who must undergo numerous blood tests or who have damaged veins, as well as for infants and older people [29,31,32].

Table 1. Potential applications of dried blood spots in kidney disease

- Screening and monitoring GFR decline in high-risk patients for CKD progression.
- Drug monitoring or adjustment in patients using nephrotoxic drugs or having underlying kidney disease.
- Patients at high risk for CKD who need multiple blood sampling at home (e.g., underlying diabetes mellitus and infants or elderly patients).

GFR, glomerular filtration rate; CKD, chronic kidney disease.

Determining biochemical parameters from blood samples requires a well-established quality control system [33]. Factors such as sample collection procedure, sample volume, spot quality, filter paper type, drying and storage methods, hematocrit, and the incorporation of internal standards are important parameters for good DBS performance and vary depending on the analyte [34-37].

Relevant factors in DBS methods

Sample collection

In the classic filter paper system, a few drops of whole blood (5–50 μL) are collected on a card by finger prick with a lancet [29]. At this stage, certain precautions are essential, such as thorough disinfection, discarding the first drop of blood, which may contain tissue fluid, completely filling in the card's outlined circle, and drying the sample at room temperature [14,38]. In viability testing of home-collected DBS samples for creatinine analysis, blood adherence to the cards was high, but only 80% of the spots showed accurate saturation and were suitable for analysis [39].

Capillary blood collected by finger prick is a mixture of arterial blood, venous blood, and interstitial fluids. Biomarker concentrations in capillary blood collected in DBS should be different from those found in venous blood [35]. Lower concentrations of cystatin C were found in blood collected by finger prick than in venous blood [40]. GFR measured by iohexol clearance has proven reliable in venous samples and capillary blood spots, although the capillary method overestimated venous GFR by 7.2% [41]. Conversely, both venous sampling and finger stick sampling at 2-time points after iohexol infusion resulted

in an acceptably accurate GFR measurement [42]. Variability in creatinine levels between capillary and venous blood samples was compared using the gold standard method, isotope dilution mass spectrometry, which reinforced the importance of using correction factors derived from validation studies to align the values obtained through each method [43].

Filter paper

The filter paper type may affect the homogeneity and behavior of blood spreading, as well as compound stability and recovery [35,44]. The main types of filter paper are made of cellulose (Whatman, GE Healthcare and Ahlstrom, Perkin-Elmer) or glass microfiber (Agilent Bond Elut DMS and Sartorius) [29,38].

Cellulose-based cards may contain additives, such as enzyme inhibitors or denaturing agents [35,38]. Whatman FTA DMPK-A cards are impregnated with radical inhibitors [sodium dodecyl sulfate, tris(hydroxymethyl) aminomethane] and can promote cell lysis and denature proteins on contact. Similarly, Whatman FTA DMPK-B cards are impregnated with chaotropic agents (guanidinium thiocyanate). Cotton-based cards, such as Whatman FTA DMPK-C, are not impregnated with stabilizing materials and are suitable for protein analysis, as are Whatman 903 and Ahlstrom 226 [33].

Due to the range of available filter cards, the European Bioanalysis Forum recommends fully validating DBS sampling methods for specific paper types [45,46]. Recommended validation parameters include drying conditions, storage stability, the effects of sample recovery, linearity, accuracy, and precision [46].

Hematocrit

Hematocrit variability is the main factor affecting the quality of DBS results [47]. Hematocrit reflects the relative volume of red blood cells and affects blood viscosity. High hematocrit results in low absorption into the card [31]. Human reference values vary according to biological parameters such as age, sex, nutritional status, race, pathological conditions, and pregnancy, in addition to extrinsic factors, such as altitude and smoking [47]. Mathematical equations to correct these variations have been determined based on the patient's baseline value or reference values for men and women [14]. Using computer systems to apply specific correction factors based on demographic data may help correct the impact of hematocrit on DBS measurements and achieve accurate analytical results. However, for precision, many sources of random errors (pipettes, volumetric flasks, detector, extraction procedure) must be accounted for [47].

The effect of hematocrit depends on the analyte of interest, and different results may be obtained according to its physical and chemical properties [48,49]. This effect can be measured either directly or indirectly through endogenous compounds such as sphingomyelin and potassium [47,50]. Incorporating internal standards, in association with accurate volume sampling, whole-spot extraction, and automated direct elution techniques has been shown to minimize the effect of hematocrit and thus improve reliability [51,52].

In studies involving individuals with abnormal hematocrit levels, DBS sampling proved unsuitable for iothalamate analysis [53]. Low hematocrit also significantly influenced creatinine analysis (deviation of 15%), and correction with endogenous compounds (potassium) was suggested [50]. Conversely, some studies reported that hematocrit's effects on precision were within acceptable limits [32,54,55].

Applicability of the DBS technique in nephrology

Measurement of endogenous markers

Using DBS to quantify endogenous markers of kidney function has mainly occurred in the last decade (Table 2) [13,34,40,43,56-63]. A strong correlation was found between conventionally obtained venous blood samples and those collected through DBS [43,57,58]. Using the reference method, creatinine quantification in DBS samples showed good accuracy [58]. Nevertheless, only Dalton et al. [43] compared creatinine levels in whole capillary DBS samples (n=66) using isotope dilution mass spectrometry.

One observed advantage of DBS is the stability of compounds. Creatinine showed 7-day stability at 32 °C in blood collected on Whatman FTA DMPK-C cards [32]. Quraishi et al. [56] also reported that creatine is stable for up to 90 days between 4 °C and 37 °C in serum samples stored on filter discs. Similarly, DBS urea concentrations were stable for up to 120 days at 4 °C and for 90 days at 37 °C [63]. However, cystatin C values decreased when shipping times exceeded 8 days (n=3,149) [34].

Measurement of exogenous markers

To determine the GFR through the clearance of exogenous compounds, blood must be collected several times over specific periods [64]. The filter paper method could simplify this process and be more tolerable in special populations, such as children [42]. Table 3 shows the main studies that have as-

https://doi.org/10.3339/ckd.23.023

https://doi.org/10.3339/ckd.23.023

Table 2. Studies involving measurement of endogenous biomarkers of kidney function through DBS samples

Author	Collection method	Sample size	Analytical technique	Storage and quality control	Sample (range or mean±SD)	Assessment of agreement/performance
Creatinine						
Quraishi [56]	VB Whatman	60	Colorimetric assay	37 °C and 4 °C for 15–90 day	Creatinine range: 0.5–3.3 mg/dL Serum creatinine: 1.99±0.64 mg/dL DBS creatinine: 1.92±0.55 mg/dL	R=0.94, ICC=0.93
Abraham [57]	VB Whatman n3	15	Enzymatic assay	4 °C for 7 day Matrix effect	DBS: 1.39±0.46 mg/dL Serum: 1.35±0.50 mg/dL	R=0.91, ICC=0.92
Silva [13]	VB, CB	106	Colorimetric (Jaffé) assay	Not reported	Adult: 57±12 yr	R=0.48 Mean difference BA (LA): 0 (0.68 to −0.55) Diagnostic cutoff GFR <60 mL/min/1.73 m^2 CKD-EPI: DBS sensitivity 94%, DBS specificity 55%, precision 90%
Nakano [58]	VB	100	MS/MS	Not reported	Pediatric: 7.9 yr Serum creatinine: 0.4 mg/dL Creatinine range: 0.12–1.2 mg/dL Calibration curve: linearity (0.039–5.0 mg/dL) Accuracy: 81.6%–104.9% CV: 0.1%–5.8%	Creatinine: 0.12–1.2 mg/dL R=0.86 Mean difference BA (LA): 0 (−0.087 to +0.09) Creatinine: 0.12–0.8 mg/dL R=0.72 / DBS=0.565×creatinine BA (LA): 0 (−0.081 to 0.091)
Bachini [59]	CB Whatman 903	9	FIA-MS	Not reported	Olympic athletes Serum creatinine: 813.6±102.4 µmol/L (9.20±1.16 mg/dL) DBS creatinine: 812.4±108.1 µmol/L (9.19±1.22 mg/dL)	CV=10.7%, ICC=0.57
Dalton [43]	VB, CB Whatman 903	66	ID-LCMS Colorimetric enzymatic assay	−80 °C Standard 914a	Adult: 24–88 yr Venous DBS creatinine: 0.85±1.10 mg/dL Capillary DBS creatinine: 0.83±1.19 mg/dL	Sensitivity: 100% Specificity: 62.7%–94.9%
Sham [60]	VB	3	LC-MS/MS PSI-MS/MS	2–8 °C	Creatinine: 2.5–20 µg/mL	Precision ≤6.3%, recovery 88%–94%, R^2>0.99
Cystatin C						
Vogl [40]	VB, CB Whatman 903	141	ELISA Nephelometry	−70 °C Hematocrit[a)]	ELISA Intra-assay CV: 5.4%, Inter-assay CV: 7.4% Nephelometry Intra-assay CV: 4.2%, Inter-assay CV: 6.9%	R=0.94 Cystatin C: 0.51–1.02 mg/L DBS sensitivity 94%, DBS specificity 55% Misclassified CKD stage: 31% Correlation venous vs. capillary blood: R=0.97
Crimmins [61]	VB Whatman 903	82	ELISA	−70 °C	Adult: >50 yr Mean cystatin C: 0.75 (0.41–1.39)	R=0.78 Regression: DBS=0.355+0.7×cystatin C Mean difference BA (LA): −0.2 (−0.45 to 0.1)
Crimmins [34]	VB Whatman 903	3,149	ELISA	≥32.2 °C, time before freezing (0–2, 3, 4–5, 6–7, and >8 day) Volume[b)]	Adult: >50 yr Mean cystatin C: 1.2 (0.5–9.2)	R^2=0.78 Regression: DBS=0.43+0.84×cystatin C

(Continued to the next page)

Table 2. Continued

Author	Collection method	Sample size	Analytical technique	Storage and quality control	Sample (range or mean±SD)	Assessment of agreement/performance
Urea						
Plumbe [62]	VB, CB	20	Enzymatic assay	Analysis: <7 day Hematocrit[a)]	CV: 6%	Venipuncture: R=0.99 Regression: DBS=1.07×urea−0.6 Capillary sample: R=0.99 Regression: DBS=1.07×urea+0.1
Quraishi [63]	VB Whatman	75	Enzymatic assay	120 day (4 °C) or 90 day (37 °C) Hematocrit[c)]	Intra-assay CV=4.2%, Inter-assay CV=6.3%	R=0.97, ICC=0.96

DBS, dried blood spots; SD, standard deviation; VB, venous blood; R, Pearson correlation coefficient; ICC, intraclass correlation coefficient; CB, capillary blood; BA (LA), Bland-Altman and limits of agreement; GFR, glomerular filtration rate; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; MS/MS, tandem mass spectrometry; CV, coefficient of variation; FIA-MS, flow injection analysis-mass spectrometry; ID-LCMS, isotope dilution-liquid chromatography/mass spectrometry; LC, liquid chromatography; PSI, paper spray ionization; ELISA, enzyme-linked immunosorbent assay.
[a)]Lowest influence or undefined variations in the assessed parameters. [b)]Presence or [c)]absence of statistical differences in biomarker concentrations according to variations in the assessed parameters.

sessed methods of measuring exogenous markers of kidney function through DBS [41,42,53,65-69]. As found in a previous study, there was strong agreement between DBS and venous GFR, with acceptable bias, precision, and accuracy, especially in patients with GFR <60 mL/min/1.73 m^2 [41]. Linear regression analyses also found good agreement between 82 serum and DBS samples regarding iohexol concentration [65].

Serum medication levels

Simultaneous assessment of kidney function indicators and medications in filter paper collection systems is a promising method for controlling the clearance or toxicity of drugs or their metabolites [70]. Forms of nephrotoxicity include tubular epithelial cell injury (antimicrobials, chemotherapeutic drugs, and venous contrast agents), interstitial nephritis (antibiotics, anti-inflammatory drugs, proton pump inhibitors, and immune-checkpoint inhibitors), and the formation of intratubular crystals (acyclovir, indinavir, antimicrobials, methotrexate, and sulfadiazine) [71].

Risk factors, such as advanced age, cardiovascular disease, diabetes, and liver disease, contribute to the development of kidney dysfunction after nephrotoxic drug use [72]. Combined therapies with diuretics, non-steroidal anti-inflammatory drugs, and renin angiotensin system inhibitors may potentiate nephrotoxicity in this group of patients [73]. Since drugs can also accumulate when kidney function is reduced (digoxin, metformin, and lithium), periodical kidney function assessment is needed in these patients [74,75].

Good correlations have been observed between serum and DBS samples for creatinine and immunosuppressant quantification by liquid chromatography-tandem mass spectrometry (Table 4) [32,39,54,74,76-80]. Simultaneous analysis of creatinine and diabetes medications (metformin and sitagliptin) has also shown good accuracy and precision in DBS samples [74,76]. Cystatin C-based measures of renal function improved ceftriaxone clearance prediction in 26 elderly patients [81]. Conversely, vancomycin clearance levels could not be accurately predicted through DBS [54].

Kidney transplant patients also require constant kidney function assessment, in addition to effective dose management of immunosuppressant drugs (cyclosporine, tacrolimus, and mycophenolate) [80,82]. The side effects of these drugs can lead to treatment nonadherence, as shown by Almardini et al. [83], who reported 36% nonadherence to mycophenolate in a group of children. The economic cost and social implications of

https://doi.org/10.3339/ckd.23.023

Table 3. Studies involving measurement of exogenous markers of kidney function through DBS samples

Author	Collection method	Sample size	Analytical technique	Storage and quality control	Study population	Assessment of agreement/performance
Iohexol						
Niculescu-Duvaz [65]	VB, CB (3 points) Schleicher & Schuell Grade 903	82	HPLC	−20 °C Hematocrit[a] Recovery[b]	Mean age: 41 yr	R^2=0.953
Mafham [66]	VB, CB (3 points) Schleicher & Schuell Grade 903	81	HPLC	Analysis: <4 hr Hematocrit[a]	Mean age: 53±17 yr GFR 15–124 mL/min/1.73 m^2	Bias ±1.96×SD (mL/min/1.73 m^2) 3-spot iohexol clearance: 1.1±15.1 2-spot iohexol clearance: 0.6±14.9 1-spot iohexol clearance: 4.5±21.2
Maahs [67]	VB, CB (5 points) Whatman 903 Protein Saver	15	HPLC	Analysis: <4 hr Hematocrit[a]	Patients with type 1 diabetes Mean age: 29±12 yr Iohexol IV (1,500 mg)	5-point blood spot GFR: 84.1±15.4 mL/min/1.73 m^2 (R=0.89), mean BA difference=0.16 2-point blood spot GFR: 83.4±15.4 mL/min/1.73 m^2 (R=0.89), mean BA difference=0.81
Salvador [41]	VB, CB (7 points) Whatman 903 Protein Saver	32	HPLC	Hematocrit[a]	Age: <6 yr Iohexol IV (647 mg/mL)	Median (range) reference GFR 65 (6–122) mL/min/1.73 m^2; 2, 3, and 4-point blood spot GFR: R=0.947, R=0.945, and R=0.937, respectively Diagnostic accuracy for 2-point blood spot: 87.5% and 96.9±15% (P15) and 96.9±30% (P30) of the reference GFR respectively GFR <60 mL/min/1.73 m^2, P15 and P30 accuracy 100%
Wang [68]	VB, CB (3 points)	45	Not reported	Not reported	Pediatric patients with chronic kidney disease	R=0.958 Bias 4.26±9.06 mL/min/1.73 m^2
Luis-Lima [69]	VB, CB (7 points) Whatman 903	203	HPLC	Volume[c]	Mean age: 57.3±15.3 yr Mean GFR: 63.6±34.8 mL/min	Capillary blood on card: total deviation index=26% Blood pipetted on card: total deviation index=13% *In vivo* studies: deviation index=9.5%
Staples [42]	VB, CB (4 points) Schleicher & Schuell Grade 903	41	HPLC	Analysis: <5 hr Hematocrit[d]	Age: 1–21 yr Iohexol IV (647 mg/mL) Mean creatinine: 1.13±0.45 mg/dL	Correlation between the DBS and 2-point venous GFR: R=0.95 2-point GFR±10% 4-point GFR: 94% DBS GFR±10% 2-point GFR: 80%
Iothalamate						
Hagan [53]	VB (6 points) Whatman 903 Protein Saver	10	HPLC	Analysis: <5 hr Hematocrit[c]	Mean age: 65.2±13.4 yr Mean GFR: 33.4±10.1 mL/min/1.73 m^2	Regression: slope of 0.95 (95% CI, 0.82–1.17) BA: bias (LA) 2 mL/min (−6 to 10 mL/min) Precision (% coefficient of variation): 3.2%–13.3% Accuracy (% error): 1.3%–3.7%

DBS, dried blood spots; VB, venous blood; CB, capillary blood; HPLC, high-performance liquid chromatography; SD, standard deviation; IV, intravenous; GFR, glomerular filtration rate; BA, Bland-Altman; R, Pearson correlation coefficient; CI, confidence interval; LA, limits of agreement. [a]Concentration corrected according to a mathematical equation. [b]Absence or [c]presence of different statistics in marker oncentrations according to variations in the assessed parameters. [d]Lowest influence or undefined variations in the assessed parameters.

organ rejection due to treatment nonadherence among transplant recipients make it essential to search for a simpler and less invasive method of drug therapy monitoring [78].

Final considerations

Although the early detection of kidney disease through simple and accurate identification of biomarkers is essential, it has been explored by few studies. The studies in this review found DBS to be a promising alternative for quantifying the main biomarkers of kidney diseases, but sources of variability should be considered separately for each analyte. Practical applications should follow strict validation protocols that contain information about sample type, card type, volume, temperature,

Table 4. Studies that simultaneously measured creatinine and medication clearance through DBS samples

Author	Assessed medication	Collection method	Sample size	Analytical technique	Storage and quality control	Study population (yr)	Calibration and performance
Scherf-Clavel [74,76]	Metformin and sitagliptin	VB, CB	70	LC-MS/MS, enzymatic assay	Volume[a]	Mean±SD: 67±11	Limit of quantification Cr: 0.15 mg/dL, Cf capillary vs. plasma=0.916±0.088 R=0.944, mean BA deviation=0.001 mg/dL
Mathew [77]	Tacrolimus	VB, CB Whatman 903	131	LC-MS/MS	Time: 5 day Temperature: ambient Hematocrit[b]	Range: 30–49	Imprecision <12% and limits of clinical acceptance within 15% against the venous samples
Koop [78]	Tacrolimus	VB, CB FTA DMPK-A	21	LC-MS/MS	Time: 4 wk Temperature: ambient	Mean±SD: 14±4.6	Limit of quantification Cr 0.01 mg/dL, accuracy 7.94% Intra- and inter-day precision: 3.48%–4.11%
Al-Uzri [39]	Tacrolimus	VB, CB	30 Subjects 216 cards	LC-MS/MS, colorimetric assay, RIA	Time: 4 wk up to 1 mo on a dissected card Temperature: ambient	Mean±SD: 13.6±5.4 Range: 2–21	Correlation between DBS vs. intravenous samples: tacrolimus: R^2=0.81 Cr: R^2=0.95
Francke [79]	Tacrolimus and cyclosporin	VB, CB	176	LC-MS/MS	Hematocrit[c]	Mean: 62	R=0.953
Veenhof [80]	Tacrolimus and cyclosporin	VB, CB Whatman DMPK-C	172 Subjects 210 cards	LC-MS/MS, enzymatic creatinine assay	1–7 day at room temperature after: −20 °C Hematocrit[b]	Mean±SD: 55±14	Correlation between DBS vs. intravenous samples Mean serum Cr: 149 µmol/L (n=199), R^2=0.97, y=0.73x–1.55 BA bias of −2.1 µmol/L (95% CI, −3.7 to −0.5) BA=[Cr serum µmol/L]=[DBS]/0.73 Mean serum tacrolimus 7.1 µg/L (n=106), R^2=0.93, y=1.0x–0.23, BA bias of −0.28 µg/L (95% CI, −0.45 to −0.12) Mean serum cyclosporine A 109 µg/L (n=61), R^2=0.93, y=0.99x–1.86
Koster [32]	Tacrolimus, sirolimus, everolimus, and cyclosporin	VB, FTA DMPK-C	50	LC-MS/MS, enzymatic assay	32 °C for 1 wk, −20 °C for 29 wk Volume[b] Hematocrit[b]	Not available	Range for Cr: 7-point calibration curve (120–480 µmol/L), 1-point calibration curve (116–7,000 µmol/L), 8-point calibration curve (1–400 µmol/L) Precision and accuracy (all validations): maximum CV of 14.0% and maximum bias of −5.9%
Scribel [54]	Vancomycin	VB, CB Whatman 903	29 Subjects 54 Samples	LC-MS/MS	22 °C and 45 °C for 2 wk Hematocrit[b]	Age: >18 yr	Cr validation: accuracy (99.6%–102.6%), intra-assay precision=2.6%–5.6%, inter-assay precision=3.5%–6.1% DBS and serum comparison: accuracy (94.4%–102.6%), intra-assay precision=2.1%–5.6%, inter-assay precision=3.5%–7.0% Cr serum to DBS concentration ratio: 0.8–1.28; R=0.96 Correlation between DBS vs. intravenous samples: Vancomycin: R^2=0.89 (n=54) DBS capillary blood Vancomycin: R^2=0.93 (n=19) DBS venous blood Cr: R^2=0.95 (n=54)

DBS, dried blood spots; VB, venous blood; CB, capillary blood; LC, liquid chromatography; MS/MS, tandem mass spectrometry; SD, standard devation; Cr, creatinine; Cf, correction factor; R, Pearson correlation coefficient; BA, Bland-Altman; RIA, radioimmunoassay; CI, confidence interval; CV, coefficient of variation.
[a]Absence of differences in marker concentrations according to variations in the assessed parameters. [b]Lowest influence on the assessed parameters. [c]Concentration corrected according to a mathematical equation.

https://doi.org/10.3339/ckd.23.023

humidity, and hematocrit parameters. Moreover, the assessment should include control subjects to ensure quality. Finally, future research should include expressive samples of patients at different stages of kidney disease and report information on clinical parameters.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

Funding

None.

Author contributions

All the work was done by CN and VS.

References

1. Bikbov B, Perico N, Remuzzi G; on behalf of the GBD Genitourinary Diseases Expert Group. Disparities in chronic kidney disease prevalence among males and females in 195 countries: analysis of the global burden of disease 2016 study. Nephron 2018;139:313-8.
2. Luyckx VA, Tonelli M, Stanifer JW. The global burden of kidney disease and the sustainable development goals. Bull World Health Organ 2018;96:414-422D.
3. World Health Organization (WHO). The top 10 causes of death [Internet]. WHO; 2020 [cited 2023 Nov 24]. Available from: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death
4. Byrne C, Cove-Smith A. Clinical assessment of renal disease. Medicine 2019;47:475-81.
5. Mihai S, Codrici E, Popescu ID, Enciu AM, Albulescu L, Necula LG, et al. Inflammation-related mechanisms in chronic kidney disease prediction, progression, and outcome. J Immunol Res 2018;2018:2180373.
6. Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, et al. Chronic kidney disease: global dimension and perspectives. Lancet 2013;382:260-72.
7. Biljak VR, Honovic L, Matica J, Kresic B, Vojak SS. The role of laboratory testing in detection and classification of chronic kidney disease: national recommendations. Biochem Med (Zagreb) 2017;27:153-76.
8. Wang YN, Ma SX, Chen YY, Chen L, Liu BL, Liu QQ, et al. Chronic kidney disease: biomarker diagnosis to therapeutic targets. Clin Chim Acta 2019;499:54-63.
9. Rysz J, Gluba-Brzozka A, Franczyk B, Jablonowski Z, Cialkowska-Rysz A. Novel biomarkers in the diagnosis of chronic kidney disease and the prediction of its outcome. Int J Mol Sci 2017;18:1702.
10. Lamb E. Assessment of kidney function in adults. Medicine 2015;43:368-73.
11. Levin A, Stevens PE, Bilous RW, Coresh J, De Francisco AL, De Jong PE, et al. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int Suppl 2013;3:1-150.
12. Soares AA. Ferramentas para detecção da doença renal: valores de referência da taxa de filtração glomerular e desempenho das equações de estimativa com creatinina e cistatina C séricas em indivíduos saudáveis [doctor's thesis]. Postgraduate Program in Medical Sciences: Endocrinology, Universidade Federal do Rio Grande do Sul (UFRGS); 2013.
13. Silva AC, Gomez JF, Lugon JR, Graciano ML. Creatinine measurement on dry blood spot sample for chronic kidney disease screening. J Bras Nefrol 2016;38:15-21.
14. Enderle Y, Foerster K, Burhenne J. Clinical feasibility of dried blood spots: analytics, validation, and applications. J Pharm Biomed Anal 2016;130:231-43.
15. Rowland M, Emmons GT. Use of dried blood spots in drug development: pharmacokinetic considerations. AAPS J 2010;12:290-3.
16. Lakshmy R. Analysis of the use of dried blood spot measurements in disease screening. J Diabetes Sci Technol 2008;2:242-3.
17. Gupta K, Mahajan R. Applications and diagnostic potential of dried blood spots. Int J Appl Basic Med Res 2018;8:1-2.
18. Sharma A, Jaiswal S, Shukla M, Lal J. Dried blood spots: concepts, present status, and future perspectives in bioanalysis. Drug Test Anal 2014;6:399-414.
19. Velghe S, Delahaye L, Stove CP. Is the hematocrit still an issue in quantitative dried blood spot analysis? J Pharm Biomed Anal 2019;163:188-96.
20. Shahbaz H, Gupta M. Creatinine clearance. In: StatPearls [Internet]. StatPearls Publishing; 2024. Available from: https:// pubmed.ncbi.nlm.nih.gov/31334948
21. Sodre FL, Costa JCB, Lima JCC. Evaluation of renal function and damage: a laboratorial challenge. J Bras Patol Med Lab 2007;43:329-37.
22. Bruck K, Jager KJ, Dounousi E, Kainz A, Nitsch D, Arnlov J, et al. Methodology used in studies reporting chronic kidney disease prevalence: a systematic literature review. Nephrol Dial Transplant 2015;30 Suppl 4(Suppl 4):iv6-16.
23. George JA, Gounden V. Novel glomerular filtration markers. Adv Clin

Chem 2019;88:91-119.
24. Hocher B, Adamski J. Metabolomics for clinical use and research in chronic kidney disease. Nat Rev Nephrol 2017;13:269-84.
25. Bjornstad P, Anderson PL, Maahs DM. Measuring glomerular filtration rate by iohexol clearance on filter paper is feasible in adolescents with type 1 diabetes in the ambulatory setting. Acta Diabetol 2016;53:331-3.
26. Guthrie R, Susi A. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. Pediatrics 1963;32:338-43.
27. Meesters RJ, Hooff GP. State-of-the-art dried blood spot analysis: an overview of recent advances and future trends. Bioanalysis 2013;5:2187-208.
28. Castro AC, Borges LG, Souza Rda S, Grudzinski M, D'Azevedo PA. Evaluation of the human immunodeficiency virus type 1 and 2 antibodies detection in dried whole blood spots (DBS) samples. Rev Inst Med Trop Sao Paulo 2008;50:151-6.
29. Lehmann S, Delaby C, Vialaret J, Ducos J, Hirtz C. Current and future use of "dried blood spot" analyses in clinical chemistry. Clin Chem Lab Med 2013;51:1897-909.
30. Edelbroek PM, van der Heijden J, Stolk LM. Dried blood spot methods in therapeutic drug monitoring: methods, assays, and pitfalls. Ther Drug Monit 2009;31:327-36.
31. Koster RA, Alffenaar JW, Botma R, Greijdanus B, Touw DJ, Uges DR, et al. What is the right blood hematocrit preparation procedure for standards and quality control samples for dried blood spot analysis? Bioanalysis 2015;7:345-51.
32. Koster RA, Greijdanus B, Alffenaar JW, Touw DJ. Dried blood spot analysis of creatinine with LC-MS/MS in addition to immunosuppressants analysis. Anal Bioanal Chem 2015;407:1585-94.
33. Lim MD. Dried blood spots for global health diagnostics and surveillance: opportunities and challenges. Am J Trop Med Hyg 2018;99:256-65.
34. Crimmins EM, Zhang YS, Kim JK, Frochen S, Kang H, Shim H, et al. Dried blood spots: effects of less than optimal collection, shipping time, heat, and humidity. Am J Hum Biol 2020;32:e23390.
35. Capiau S, Veenhof H, Koster RA, Bergqvist Y, Boettcher M, Halmingh O, 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;41:409-30.
36. Denniff P, Woodford L, Spooner N. Effect of ambient humidity on the rate at which blood spots dry and the size of the spot produced. Bioanalysis 2013;5:1863-71.
37. Prentice P, Turner C, Wong MC, Dalton RN. Stability of metabolites in dried blood spots stored at different temperatures over a 2-year period. Bioanalysis 2013;5:1507-14.
38. Malsagova K, Kopylov A, Stepanov A, Butkova T, Izotov A, Kaysheva A. Dried blood spot in laboratory: directions and prospects. Diagnostics (Basel) 2020;10:248.
39. Al-Uzri A, Freeman KA, Wade J, Clark K, Bleyle LA, Munar M, et al. Longitudinal study on the use of dried blood spots for home monitoring in children after kidney transplantation. Pediatr Transplant 2017;21:e12983.
40. Vogl PT. Measurement of cystatin C in dried blood spot specimens [master's thesis]. University of Washington; 2013.
41. Salvador CL, Tondel C, Morkrid L, Bjerre A, Brun A, Bolann B, et al. Glomerular filtration rate measured by iohexol clearance: a comparison of venous samples and capillary blood spots. Scand J Clin Lab Invest 2015;75:710-6.
42. Staples A, Wong C, Schwartz GJ. Iohexol-measured glomerular filtration rate in children and adolescents with chronic kidney disease: a pilot study comparing venous and finger stick methods. Pediatr Nephrol 2019;34:459-64.
43. Dalton RN, Isbell TS, Ferguson R, Fiore L, Malic A, DuBois JA. Creatinine standardization: a key consideration in evaluating whole blood creatinine monitoring systems for CKD screening. Anal Bioanal Chem 2022;414:3279-89.
44. Koster RA, Botma R, Greijdanus B, Uges DR, Kosterink JG, Touw DJ, et al. The performance of five different dried blood spot cards for the analysis of six immunosuppressants. Bioanalysis 2015;7:1225-35.
45. Timmerman P, White S, Cobb Z, de Vries R, Thomas E, van Baar B, et al. Update of the EBF recommendation for the use of DBS in regulated bioanalysis integrating the conclusions from the EBF DBS-microsampling consortium. Bioanalysis 2013;5:2129-36.
46. Timmerman P, White S, Globig S, Ludtke S, Brunet L, Smeraglia J. EBF recommendation on the validation of bioanalytical methods for dried blood spots. Bioanalysis 2011;3:1567-75.
47. Daousani C, Karalis V, Malenovic A, Dotsikas Y. Hematocrit effect on dried blood spots in adults: a computational study and theoretical considerations. Scand J Clin Lab Invest 2019;79:325-33.
48. Wilhelm AJ, den Burger JC, Swart EL. Therapeutic drug monitoring by dried blood spot: progress to date and future directions. Clin Pharmacokinet 2014;53:961-73.
49. Koster RA, Alffenaar JW, Botma R, Greijdanus B, Uges DR, Kosterink JG, et al. The relation of the number of hydrogen-bond acceptors with recoveries of immunosuppressants in DBS analysis. Bioanalysis 2015;7:1717-22.
50. den Burger JC, Wilhelm AJ, Chahbouni AC, Vos RM, Sinjewel A, Swart EL. Haematocrit corrected analysis of creatinine in dried blood spots

https://doi.org/10.3339/ckd.23.023

through potassium measurement. Anal Bioanal Chem 2015;407:621-7.
51. Abu-Rabie P, Denniff P, Spooner N, Chowdhry BZ, Pullen FS. Investigation of different approaches to incorporating internal standard in DBS quantitative bioanalytical workflows and their effect on nullifying hematocrit-based assay bias. Anal Chem 2015;87:4996-5003.
52. Reyes-Garces N, Alam MN, Pawliszyn J. The effect of hematocrit on solid-phase microextraction. Anal Chim Acta 2018;1001:40-50.
53. Hagan AS, Jones DR, Agarwal R. Use of dried plasma spots for the quantification of iothalamate in clinical studies. Clin J Am Soc Nephrol 2013;8:909-14.
54. Scribel L, Zavascki AP, Matos D, Silveira F, Peralta T, Goncalves Landgraf N, et al. Vancomycin and creatinine determination in dried blood spots: analytical validation and clinical assessment. J Chromatogr B Analyt Technol Biomed Life Sci 2020;1137:121897.
55. Anibaletto Dos Santos AL, Cezimbra da Silva AC, Feltraco Lizot LL, Schneider A, Meireles YF, Hahn RZ, et al. Development and validation of an assay for the measurement of gentamicin concentrations in dried blood spots using UHPLC-MS/MS. J Pharm Biomed Anal 2022;208:114448.
56. Quraishi R, Lakshmy R, Mukhopadhyay AK, Jailkhani BL. Creatinine measurement and stability in dried serum. J Diabetes Sci Technol 2012;6:988-9.
57. Abraham RA, Kapil U, Aggarwal SK, Pandey RM, Sharma M, Ramakrishnan L. Measurement of creatinine from dried blood spot by enzymatic method. Int J Adv Res Chem Sci 2015;2:42-6.
58. Nakano M, Uemura O, Honda M, Ito T, Nakajima Y, Saitoh S. Development of tandem mass spectrometry-based creatinine measurement using dried blood spot for newborn mass screening. Pediatr Res 2017;82:237-43.
59. Bachini FI, Pereira D, Santos R, Hausen M, Pereira G, Vieira C, et al. Creatine and creatinine quantification in olympic athletes: dried blood spot analysis pilot study. Biol Sport 2022;39:745-9.
60. Sham TT, Badu-Tawiah AK, McWilliam SJ, Maher S. Assessment of creatinine concentration in whole blood spheroids using paper spray ionization-tandem mass spectrometry. Sci Rep 2022;12:14308.
61. Crimmins E, Kim JK, McCreath H, Faul J, Weir D, Seeman T. Validation of blood-based assays using dried blood spots for use in large population studies. Biodemography Soc Biol 2014;60:38-48.
62. Plumbe RM, Worth HG. Dried blood spot test estimation of urea. Ann Clin Biochem 1985;22(Pt 4):408-11.
63. Quraishi R, Lakshmy R, Mukhopadhyay AK, Jailkhani BL. Analysis of the stability of urea in dried blood spots collected and stored on filter paper. Ann Lab Med 2013;33:190-2.
64. Selistre LS, Cochat P, Rech D, Parant F, Souza VCD, Dubourg L. Associação entre taxa de filtração glomerular (medida por cromatografia liquida de alto desempenho com iohexol) e oxalato plasmático. J Bras Nephrol 2018;40:73-6.
65. Niculescu-Duvaz I, D'Mello L, Maan Z, Barron JL, Newman DJ, Dockrell ME, et al. Development of an outpatient finger-prick glomerular filtration rate procedure suitable for epidemiological studies. Kidney Int 2006;69:1272-5.
66. Mafham MM, Niculescu-Duvaz I, Barron J, Emberson JR, Dockrell ME, Landray MJ, et al. A practical method of measuring glomerular filtration rate by iohexol clearance using dried capillary blood spots. Nephron Clin Pract 2007;106:c104-12.
67. Maahs DM, Bushman L, Kerr B, Ellis SL, Pyle L, McFann K, et al. A practical method to measure GFR in people with type 1 diabetes. J Diabetes Complications 2014;28:667-73.
68. Wang BB, Wu Y, Qin Y, Gong MC, Shi XM, Jing HL, et al. Application of plasma clearance of iohexol in evaluating renal function in Chinese children with chronic kidney disease. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 2015;37:171-8.
69. Luis-Lima S, Gaspari F, Negrin-Mena N, Carrara F, Diaz-Martin L, Jimenez-Sosa A, et al. Iohexol plasma clearance simplified by dried blood spot testing. Nephrol Dial Transplant 2018;33:1597-603.
70. Wu H, Huang J. Drug-induced nephrotoxicity: pathogenic mechanisms, biomarkers and prevention strategies. Curr Drug Metab 2018;19:559-67.
71. Perazella MA, Rosner MH. Drug-induced acute kidney injury. Clin J Am Soc Nephrol 2022;17:1220-33.
72. Pinto PS, Carminatti M, Lacet T, Rodrigues DF, Nogueira LO, Bastos MG, et al. Nephrotoxic acute renal failure: prevalence, clinical course and outcome. J Bras Nephrol 2009;31:183-9.
73. Calvo DM, Saiz LC, Leache L, Celaya MC, Gutierrez-Valencia M, Alonso A, et al. Effect of the combination of diuretics, renin-angiotensin-aldosterone system inhibitors, and non-steroidal anti-inflammatory drugs or metamizole (triple whammy) on hospitalisation due to acute kidney injury: a nested case-control study. Pharmacoepidemiol Drug Saf 2023;32:898-909.
74. Scherf-Clavel M, Albert E, Zieher S, Valotis A, Hickethier T, Hogger P. 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;75:809-16.
75. Lea-Henry TN, Carland JE, Stocker SL, Sevastos J, Roberts DM. Clinical pharmacokinetics in kidney disease: fundamental principles. Clin J Am Soc Nephrol 2018;13:1085-95.
76. Scherf-Clavel M, Hogger P. Analysis of metformin, sitagliptin and creatinine in human dried blood spots. J Chromatogr B Analyt Technol Biomed Life Sci 2015;997:218-28.

77. Mathew BS, Mathew SK, Aruldhas BW, Prabha R, Gangadharan N, David VG, et al. Analytical and clinical validation of dried blood spot and volumetric absorptive microsampling for measurement of tacrolimus and creatinine after renal transplantation. Clin Biochem 2022;105-106:25-34.
78. Koop DR, Bleyle LA, Munar M, Cherala G, Al-Uzri A. 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.
79. Francke MI, van Domburg B, Bouarfa S, van de Velde D, Hellemons ME, Manintveld OC, et al. The clinical validation of a dried blood spot method for simultaneous measurement of cyclosporine A, tacrolimus, creatinine, and hematocrit. Clin Chim Acta 2022;535:131-9.
80. Veenhof H, Koster RA, Alffenaar JC, Berger SP, Bakker SJ, Touw DJ. Clinical validation of simultaneous analysis of tacrolimus, cyclosporine A, and creatinine in dried blood spots in kidney transplant patients. Transplantation 2017;101:1727-33.
81. Tan SJ, Cockcroft M, Page-Sharp M, Arendts G, Davis TM, Moore BR, et al. Population pharmacokinetic study of ceftriaxone in elderly patients, using cystatin C-based estimates of renal function to account for frailty. Antimicrob Agents Chemother 2020;64:e00874-20.
82. Cheung CY, van der Heijden J, Hoogtanders K, Christiaans M, Liu YL, Chan YH, et al. Dried blood spot measurement: application in tacrolimus monitoring using limited sampling strategy and abbreviated AUC estimation. Transpl Int 2008;21:140-5.
83. Almardini R, Taybeh EO, Alsous MM, Hawwa AF, McKeever K, Horne R, et al. A multiple methods approach to determine adherence with prescribed mycophenolate in children with kidney transplant. Br J Clin Pharmacol 2019;85:1434-42.

https://doi.org/10.3339/ckd.23.023