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ORIGINAL ARTICLE

Volumetric microsampling for simultaneous remote immunosuppressant and kidney function monitoring in outpatient kidney transplant recipients

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Aims: Immunosuppressant and kidney function monitoring are crucial for kidney transplant recipient follow-up. Microsamples enable remote sampling and minimise patient burden as compared to conventional venous sampling at the clinic. We developed a liquid chromatography–tandem mass spectrometry assay to quantify tacrolimus, mycophenolic acid (MPA), creatinine and iohexol in dried blood spot (DBS), and volumetric absorptive microsample (VAMS) samples.

Methods: The assay was successfully validated analytically for all analytes. Clinical validation was conducted by direct comparison of paired DBS, VAMS and venous reference samples from 25 kidney transplant recipients. Patients received iohexol 5–15 minutes before immunosuppressant intake and were sampled 0, 1, 2 and 3 hours thereafter, enabling tacrolimus and MPA area under the concentration–time curve (AUC) and creatinine-based and iohexol-based glomerular filtration rate (GFR) estimation. Method agreement was evaluated using Passing–Bablok regression, Bland–Altman analysis and the percentages of values within 15–30% of the reference (P_{15}–P_{30}) with a P_{20} acceptance threshold of 80%.

Results: For DBS samples, method agreement was excellent for tacrolimus trough concentrations ($n = 25$, $P_{15} = 92.0\%$) and AUCs ($n = 25$; $P_{20} = 95.8\%$) and adequate for creatinine-based GFR trend monitoring ($n = 25$; $P_{20} = 80\%$). DBS-based MPA AUC assessment showed suboptimal agreement ($n = 16$; $P_{20} = 68.8\%$), but was considered acceptable given its P_{30} of 100%. The assay performed inadequately for DBS-based iohexol GFR determination ($n = 24$; $P_{20} = 75\%$). The VAMS technique generally showed inferior performance, but can be considered for certain situations.

Conclusion: The assay was successfully validated for tacrolimus, MPA and creatinine quantification in DBS samples, enabling simultaneous remote kidney function trend

The authors confirm that the Principal Investigator for this article is Dirk Jan Moes.

monitoring and immunosuppressant therapeutic drug monitoring in kidney transplant recipients.

KEYWORDS
immunosuppressants, kidney function, kidney transplantation, microsampling, therapeutic drug monitoring

1 | INTRODUCTION

The current guidelines on the clinical management of kidney transplant recipients recommend regular evaluation of graft function and immunosuppressant exposure.[1] This includes, amongst other markers, monitoring of the serum creatinine, estimated glomerular filtration rate (eGFR), and the immunosuppressant trough concentration (C_0) and/or area under the concentration–time curve (AUC). Typically, these are determined during recurrent outpatient clinic visits, which can pose a patient burden in terms of time, travel costs and lost productivity.

Microsampling techniques relying on capillary whole blood collection via a finger prick, including dried blood spot (DBS) and volumetric absorptive microsample (VAMS) sampling, have introduced options for remote sampling and have gained interest as a patient-friendly and cost-effective[2,3] approach for monitoring kidney transplant recipients.

Various validation studies have been conducted for liquid chromatography–tandem mass spectrometry (LC–MS/MS) assays capable of quantifying **tacrolimus**, cyclosporine, **everolimus**, sirolimus and/or **mycophenolic acid** (MPA) in DBS,[2,4–33] VAMS,[34–45] or both.[46–48] Five of these assays allowed for the simultaneous quantification of 1 or more immunosuppressant and serum creatinine,[11,13,15,20,38,48] facilitating synchronised remote monitoring of kidney function and immunosuppressant exposure. However, the absence of MPA in these assays comprised an important limitation, as most kidney transplant recipients receive immunosuppressive therapy with tacrolimus, MPA and prednisolone. As therapeutic drug monitoring (TDM)-guided dose adaptation is also recommended for MPA,[49] its inclusion in such multianalyte LC–MS/MS assays is important to advance the utility of remote microsampling-based kidney transplant recipient monitoring. Furthermore, whereas the availability of such assays is important as a first step towards remote monitoring, subsequent clinical validation to demonstrate interchangeability with conventional venous sampling-based methods is crucial before considering these methods for routine clinical care. Whereas such clinical validation studies with DBS and/or VAMS samples have been performed for tacrolimus, either alone[34–36,42,46] or in combination with MPA,[2,7,47] sirolimus,[16] cyclosporine,[19,40] creatinine,[20,38,48] or creatinine and cyclosporine,[13] and for everolimus, either alone[44] or in combination with sirolimus,[4] this is not the case for MPA and creatinine, let alone tacrolimus in combination with MPA and creatinine.

Additionally, while the eGFR comprises a convenient marker for kidney function trend monitoring, it shows limited agreement with measured GFR (mGFR)-based techniques, which are considered to best resemble the true GFR.[50–52] Unfortunately, the available mGFR methods rely on extensive sampling,[50,53] which has hampered their application in routine care. Nevertheless, iohexol plasma clearance-based mGFR assessment has gained interest for clinical and research purposes,[50,51,53] and also harnesses options for (partially) remote determination utilising microsampling.[54–58]

What is already known about this subject

- Microsampling techniques relying on capillary whole blood collection allow for remote blood sampling, providing a patient-friendly and cost-effective alternative to conventional venous sample collection at the clinic.
- This has introduced options for remote blood collection for therapeutic drug monitoring purposes, including the monitoring of immunosuppressant exposure in kidney transplant recipients.
- However, the application of these alternative sampling matrices in routine clinical care is still limited, but can probably be increased by addition of a kidney function marker leading to enhanced clinical feasibility.

What this study adds

- A novel liquid chromatography tandem–mass spectrometry assay was developed and validated for the simultaneous quantification of tacrolimus, mycophenolate and creatinine in microsamples, enabling simultaneous remote immunosuppressant and kidney function monitoring.
- Our study demonstrates that these analytes can be accurately and precisely quantified in dried blood spot, and, to a lesser extent, volumetric absorptive microsampling samples, and can pose a reliable alternative to conventional venous sampling at the clinic.
- Finally, we show that microsampling-based remote tacrolimus and mycophenolate therapeutic drug monitoring is feasible within routine clinical care, and propose options to further enhance the widespread implementation of this approach.

Interestingly, the clinical potential of remote microsampling-based kidney transplant recipient monitoring can be further enhanced with pharmacometric model-based maximum a posteriori Bayesian estimation. This approach enables immunosuppressant AUC and iohexol mGFR estimation based on a limited number of aligned blood draws taken early after drug administration,[59,60] facilitating robust kidney function assessment and highly informative immunosuppressant TDM at limited patient discomfort.

Here, we developed and analytically validated a multiplex LC–MS/MS assay capable of simultaneously quantifying tacrolimus, MPA, sirolimus, everolimus, cyclosporine, creatinine and iohexol in volumetric DBS and VAMS samples. Subsequent clinical validation was performed for tacrolimus, MPA, everolimus, creatinine and iohexol based on direct comparison of paired DBS, VAMS and conventional venous whole blood samples from clinically stable kidney transplant recipients. Additionally, we evaluated the clinical feasibility of remote microsampling-based immunosuppressant TDM in the kidney transplantation setting.

2 | METHODS

2.1 | Bioanalytical method development and validation

A novel multianalyte LC–MS/MS assay capable of quantifying tacrolimus, everolimus, sirolimus, cyclosporine, MPA, creatinine and iohexol simultaneously in DBS and VAMS samples was developed as per the European Medicines Agency guideline on bioanalytical method validation.[61] The method development was based on previously validated LC–MS/MS assays for these agents.[7,13,15,57,62–65]

2.2 | Clinical validation study

2.2.1 | Study design

After the bioanalytical method development and validation, a clinical validation study was performed for tacrolimus, MPA, iohexol, creatinine and everolimus, by direct comparison of paired DBS, VAMS and venous EDTA reference samples.

2.2.2 | Patients and samples

All data for the clinical validation study originated from participants of the RRFD trial (NTR7256). The RRFD trial aimed to include 100 clinically stable kidney(–pancreas) transplant recipients >1 year after transplantation with a creatinine clearance >25 mL/min/1.73 m^2, receiving immunosuppressive therapy centred around once-daily tacrolimus (Advagraf). The current study was performed in 25 RRFD participants and was approved by the Medical Ethical Committee of Leiden University Medical Center (LUMC; P16.170); all participants gave written informed consent.

At their final RRFD visit, each patient provided 4 sequential paired DBS, VAMS and venous EDTA samples, collected by a nurse practitioner just before (C_0) and at 1, 2 and 3 hours after oral immunosuppressant intake. Iohexol (Omnipaque 300, GE Healthcare BV, Eindhoven, the Netherlands; containing 3235 mg iohexol) was administered intravenously 5–15 minutes before oral immunosuppressant intake. This schedule was selected to allow for blood draw alignment for population pharmacokinetic model-based maximum a posteriori Bayesian estimation of the tacrolimus AUC_{0-24}, MPA AUC_{0-12}, everolimus AUC_{0-12} and the iohexol mGFR.

Capillary access for DBS and VAMS sample collection was acquired via a finger prick. Before sampling, the fingertip was cleaned with water, dried and punctured using a safety lancet (Sarstedt, Nümbrecht, Germany). The first capillary blood drop was discarded. DBS samples were collected with the HemaXis DB10 device, which combines a microfluidic plate for volumetric blood sampling (10 μL) and a Whatman 903 protein saver card for blood collection (DBS System, Gland, Switzerland).[66] VAMS samples were collected with the Mitra Clamshell device (Neotyrex, Torrance, CA, USA; 20 μL).[67] Venous access for EDTA sample collection was acquired using a standard cannulation procedure. Before analysis, all DBS and VAMS samples were inspected by a laboratory technician to ensure sample quality. Any analyte concentrations exceeding the established upper limits of quantification of the assay were diluted and then re-analysed.

2.2.3 | EDTA bioanalytical reference assays

Tacrolimus, MPA and everolimus were quantified in the venous EDTA reference samples using a previously validated multianalyte LC–MS/MS assay,[7,68] and iohexol with a previously validated HPLC-UV assay.[60] Creatinine was quantified on a Cobas 8000 instrument (Roche, Almere, The Netherlands), using a standard creatininase–sarcosine enzymatic assay utilised for routine patient care at the LUMC clinical chemistry laboratory (validated concentration range: 44.5–884 μmol/L, <2% coefficient of variation [CV]).

2.3 | Clinical feasibility study

Whereas demonstration of adequate analytical performance of the developed assay comprised our main focus, its clinical potential also depends of adequate patient satisfaction with and clinical feasibility of these alternative sampling devices.

Patient satisfaction was evaluated in the clinical validation study population ($n = 25$) using the System Usability Scale (SUS), a previously validated questionnaire to assess end-user satisfaction with a given device.[69] The SUS comprises 10 question items which are scored on a 5-point Likert scale, ranging from 1 ('strongly disagree') to 5 ('strongly agree').[69] The score contribution of question items 1, 3,

5, 7 and 9 is their scale position minus 1 point, whereas items 2, 4, 6, 8 and 10 contribute 5 points minus their scale position.[69] The item scores are summed and multiplied by 2.5 to yield the total SUS score, ranging from 0–100 with higher scores indicating higher system usability.[69]

Additionally, we evaluated the clinical feasibility of the remote DBS-based immunosuppressant TDM process based on historic DBS tacrolimus and MPA TDM measurements from kidney transplant recipients, performed as part of routine clinical care at LUMC. Remote DBS-based tacrolimus and MPA TDM has been routinely applied at LUMC since its successful validation in 2018.[7] In short, patients are provided with a practical, written and visual instruction of the sampling procedure at the clinic. Patients then perform the blood collection for subsequent TDM instances remotely and send their DBS kit to our laboratory. A complete DBS kit contains 4 sequential samples and a form with the times of drug intake and sample collection. Before analysis, each kit is inspected by a laboratory technician who registers any anomalies. For this feasibility study, we extracted the laboratory data of all DBS kits received between April and September 2021, evaluated the percentage of clinically applicable DBS kits, and investigated any anomalies.

2.4 | Statistics and software

Method comparison between the DBS, VAMS and reference samples was conducted for the individual tacrolimus, MPA and everolimus concentrations, AUCs, and AUC-based daily dosing recommendations. Similarly, iohexol concentrations were compared individually and on the mGFR level. As the creatinine concentrations were quantified in 4-fold, these were averaged across the 4 DBS or VAMS samples before comparison with the EDTA creatinine concentrations and eGFRs, as calculated using the CKD-EPI formula.[70] To investigate the utility of the assay for combined tacrolimus C_0 TDM and kidney function monitoring from 1 blood sample, the variability of the creatinine values across the 4 samples was also assessed.

Additionally, the physiological differences between capillary whole blood and venous EDTA plasma or serum and, to a lesser extent, venous EDTA whole blood may dictate the need for a conversion factor to translate drug concentrations quantified in DBS or VAMS samples to their corresponding venous EDTA concentrations to ensure correct clinical interpretation. Namely, for analytes that are normally quantified in venous EDTA plasma or serum, the presence of erythrocytes in a capillary whole blood sample yields a dilution effect, typically resulting in lower concentrations than observed in the corresponding venous EDTA sample. We anticipated that this phenomenon would be most pronounced for MPA and iohexol as these analytes are typically quantified in venous EDTA plasma and display negligible erythrocyte partitioning.[71,72] In this case, the dilution effect is approximated by the ratio between the erythrocyte volume and the total blood volume in the sample, which equals the blood haematocrit. By contrast, tacrolimus,[73] sirolimus,[74] cyclosporine,[75] everolimus[76] and creatinine[77] display extensive erythrocyte partitioning and were thus not expected to require concentration conversion. Several methods can be used for the concentration conversion, including individual or mean population haematocrit-based conversion, mean concentration ratio-based conversion, and conversion based on the Passing–Bablok regression fit.[7] For MPA, we previously demonstrated that a mean concentration ratio-based conversion factor of 1/0.68 can be used to translate capillary whole blood concentrations to EDTA plasma concentrations.[7] This factor was thus also applied in the current study. For iohexol, we investigated the applicability of a similar ratio-based conversion method, in addition to individual and population haematocrit-based conversion.

For the method comparison, linearity and bias between the methods were evaluated using Passing–Bablok regression[78] and Bland–Altman plots.[79] Herein, Passing–Bablok regression allows for evaluation of the correlation between the results of both methods, taking into account the variability in both the x and y dimension.[80] Bland–Altman plots visualise the absolute, relative or ratio bias between the results of both methods over their mean concentration range, allowing for inspection of the mean bias, the variability of the bias and any trends of the bias over the concentration range.[80] Additionally, the percentages of samples falling within 15–30% (P_{15}–P_{30}) of the reference samples were evaluated. Herein the general acceptance threshold for method agreement was set at a P_{20} of 80%, whereas a more stringent P_{15} threshold of 80% was applied for tacrolimus C_0 comparison. The latter threshold was derived from a similar validation study for tacrolimus quantification in microsamples.[46] Finally, a recent guideline on the bioanalytical method development of assays intended specifically for drug quantification in microsamples recommended evaluation of any haematocrit-dependencies on the performance of the assay.[80] We thus evaluated, for each analyte and matrix, whether the ratio difference between the methods displayed any haematocrit dependency.

For the feasibility data, the total SUS scores for the DBS and VAMS devices were compared using a Wilcoxon signed rank test ($\alpha = 0.05$).[81]

Data handling, statistics and visualization were performed in R 3.6.2 (R Project for Statistical Computing, Vienna, Austria) and RStudio 1.2.5019 (RStudio Inc., Boston, MA, USA). The tacrolimus AUC_{0-24}, MPA AUC_{0-12} and everolimus AUC_{0-12} were derived using maximum a posteriori Bayesian estimation based on their concentrations just before, and at 1, 2 and 3 hours after oral intake, using validated population pharmacokinetic models for these agents[82–85] translated for application in MW/Pharm.[86] Similarly, the iohexol mGFR was estimated based on the concentrations at 5–15 minutes, and at 1, 2 and 3 hours after intravenous administration, using a validated population pharmacokinetic model for iohexol mGFR determination in kidney transplant recipients[60] in NONMEM 7.4.4 (Icon Development Solutions, Ellicott City, MD, USA).

2.5 | Nomenclature of targets and ligands

Key ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to Pharmacology.[87]

3 | RESULTS

3.1 | Bioanalytical method development and validation

The novel LC–MS/MS assay was successfully developed and validated bioanalytically. A detailed description of the technical aspects and bioanalytical validation is provided in the electronic supplementary material (ESM).

3.2 | Clinical validation study

3.2.1 | Patients and samples

Twenty-five patients participated in this study, who provided a total of 97 blood samples. Their clinical characteristics are summarised in Table 1. For 1 patient, no venous samples could be obtained at 1, 2 and 3 hours due to unsuccessful cannulation.

A full clinical validation was conducted for tacrolimus (97 concentrations; 24 AUCs), MPA (64 concentrations; 16 AUCs), creatinine (24 mean concentrations; 24 eGFRs) and iohexol (96 concentrations; 24 mGFRs), whereas the limited number of everolimus samples (12 concentrations; 3 AUCs) only allowed for an exploratory analysis. No patient samples containing sirolimus or cyclosporine could be obtained, thwarting clinical validation of the assay for these analytes.

3.2.2 | Tacrolimus

For tacrolimus, the DBS and VAMS methods showed adequate linearity with the EDTA reference method, with Passing–Bablok regression slopes of 0.93 and 1.05 for the individual concentrations (Figure 1A, C) and 1.01 and 1.12 for the AUCs (Figure 1B, D), respectively (Table 2). The DBS method displayed mean biases of −6% (range: −25 to +19%) for the individual concentrations and −6% (range: −21 to +10%) for the AUCs, with P_{20} of 95.9 and 95.8%, respectively (Figure 2A, B; Table 2). The VAMS method showed slightly inferior performance, with mean biases of +7% (range: −24 to +37%) and +6% (range: −14 to +22%), and P_{20} of 76.3 and 79.2% for the individual concentrations and AUCs, respectively (Figure 2C, D; Table 2). Daily dosing recommendation of the DBS and VAMS methods differed from the reference values on 7/24 (29.2%) and 12/24 (50.0%) of occasions (Figure S2). Mean DBS dosing recommendations were 0.13 ± 0.30 mg (range: −0.5; +1.0) higher than for the reference (relative difference: +4.46% ± 9.62%, range: −6.67 to +33.3%). Those of the VAMS method were 0.19 ± 0.46 mg (range: −1.5; +0.5) lower than the reference (relative difference: −4.77% ± 9.31%, range: −25 to +14.3%).

A sub-analysis with tacrolimus C_0 samples exclusively ($n = 25$), displaying EDTA concentrations of 2.5–7.7 μg/L, showed excellent performance for the DBS samples with P_{15} and P_{20} of 92 and 100%, respectively (Table 2). By contrast, the VAMS samples showed inferior performance for C_0 samples, with P_{15}, P_{20} and P_{30} of 68, 88 and 96%.

TABLE 1 Patient characteristics ($n = 25$)

Characteristic	*n* (%)	Mean	Range
Sex			
Male	18 (72%)		
Female	7 (28%)		
Age (y)		56.7	21.6–81.8
Total bodyweight (kg)		80.0	58.6–117
Transplant type			
Kidney transplant	24 (96%)		
Simultaneous kidney-pancreas transplant	1 (4%)		
Time after transplantation (y)		8.48	3.24–41.0
Haematocrit (fraction)		0.406	0.348–0.479
Immunosuppressive drug regimen			
Tacrolimus + MPA + prednisolone	15 (60%)		
Tacrolimus + prednisolone	4 (16%)		
Tacrolimus + everolimus + prednisolone	3 (12%)		
Tacrolimus + MPA	2 (8%)		
Tacrolimus + azathioprine + prednisolone	1 (4%)		

MPA, mycophenolic acid.

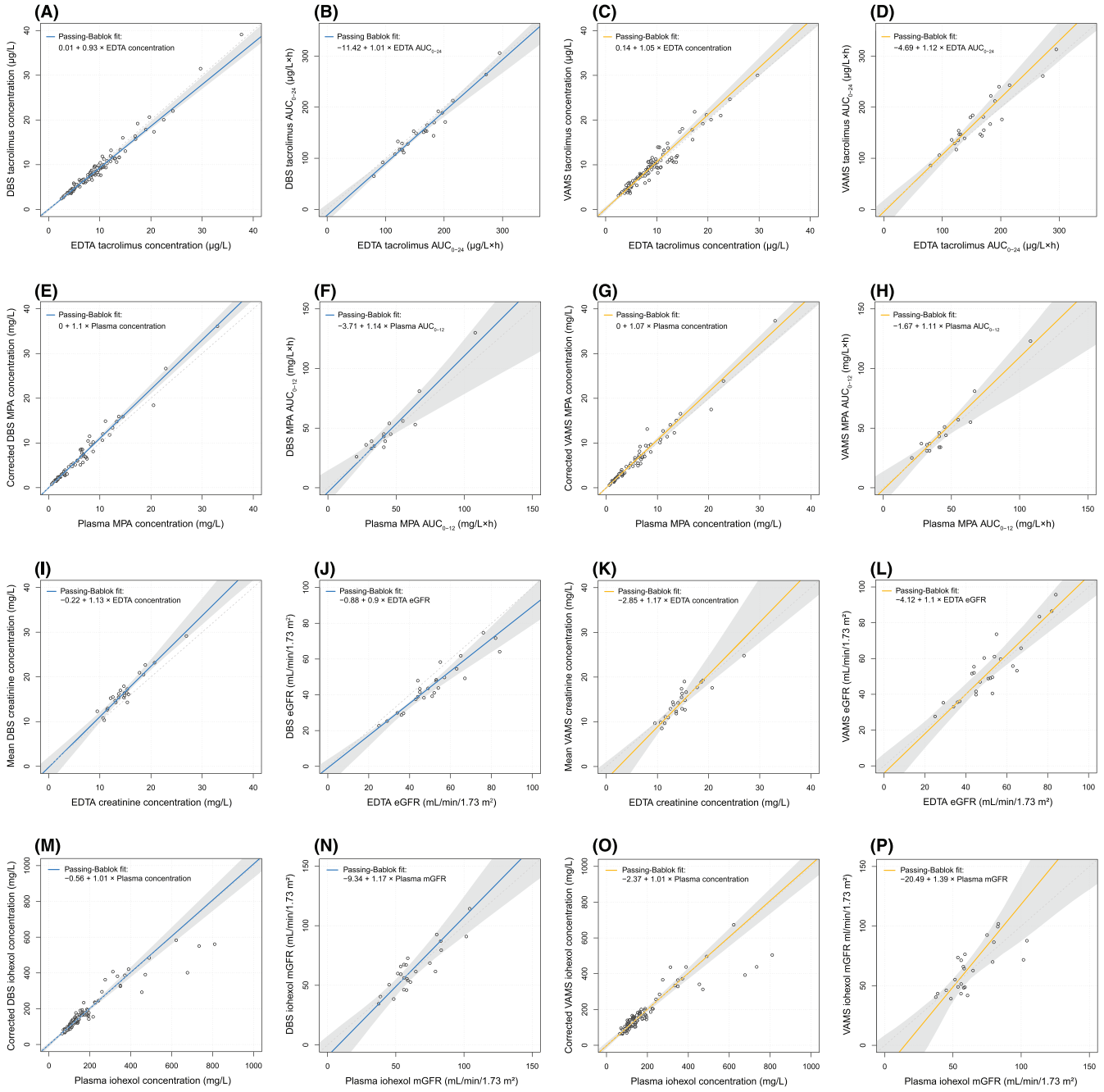

FIGURE 1 Passing–Bablok regression plots for the tacrolimus concentrations (A, C) and area under the concentration–time curves (AUCs; B, D), corrected mycophenolic acid (MPA) concentrations (E, G) and AUCs (F; H), creatinine concentrations (I, K) and estimated glomerular filtration rates (eGFRs; J, L), and corrected iohexol concentrations (M, O) and measured GFRs (mGFRs; N, P). Solid blue and gold lines represent the Passing–Bablok regression fits for the dried blood spot (DBS) and volumetric absorptive microsampling (VAMS) techniques, respectively, with their 95% confidence intervals shaded in grey

Therefore, the tacrolimus C_0 and AUC_{0-24} results quantified in DBS samples were interchangeable with those quantified in venous EDTA samples. The VAMS technique can be considered for tacrolimus AUC_{0-24} monitoring in clinically stable patients experiencing difficulties with the DBS technique, but should not be used for C_0 monitoring based on a single sample.

3.2.3 | MPA

As expected, the MPA, DBS and VAMS samples displayed a pronounced bias as compared to the reference EDTA plasma concentrations (Figures S3, S4), showing mean concentration ratios of 0.75 ± 0.10 (range: 0.56–0.98) and 0.73 ± 0.10 (range: 0.54–1.12),

TABLE 2 Agreement of the dried blood spot (DBS) and volumetric absorptive microsampling (VAMS) methods with the EDTA reference method

				Passing–Bablok regression		Bland–Altman ratio differences		
Analyte	**Marker**	**Method**	***n***	***Slope [95% CI]***	***Intercept [95% CI]***	***Mean ratio [95% LoA]***	***P_{20} (%)***	***P_{30} (%)***
Tacrolimus	Concentration	DBS[a]	97	0.93 [0.89; 0.99]	0.01 [−0.36; 0.29]	0.94 [0.78; 1.11]	95.9	100
		VAMS[a]	97	1.05 [0.98; 1.14]	0.14 [−0.37; 0.65]	1.07 [0.78; 1.35]	76.3	93.8
	C_0	DBS[a]	25	1.00 [0.82; 1.27]	−0.27 [−1.44; 0.47]	0.95 [0.80; 1.10]	100	100
		VAMS[a]	25	1.09 [0.88; 1.55]	−0.06 [−2.31; 0.85]	1.07 [0.84; 1.31]	88.0	96.0
	AUC_{0-24}	DBS[a]	24	1.01 [0.87; 1.10]	−11.4 [−24.6; 9.81]	0.94 [0.80; 1.08]	95.8	100
		VAMS[a]	24	1.12 [0.92; 1.32]	−4.69 [−34.3; 16.7]	1.06 [0.83; 1.30]	79.2	100
MPA	Concentration	DBS[a]	64	0.75 [0.70; 0.79]	0.01 [−0.14; 0.13]	0.75 [0.56; 0.95]	28.1	70.3
		DBS[b]	64	1.10 [1.04; 1.15]	0.00 [−0.22; 0.17]	1.11 [0.82; 1.40]	78.1	85.9
		VAMS[a]	64	0.72 [0.66; 0.77]	0.00 [−0.13; 0.14]	0.73 [0.52; 0.93]	17.2	57.8
		VAMS[b]	64	1.07 [0.97; 1.13]	0.00 [−0.21; 0.18]	1.07 [0.77; 1.37]	84.4	93.8
	AUC_{0-12}	DBS[b]	16	1.14 [0.64; 1.38]	−3.71 [−13.2; 13.9]	1.07 [0.79; 1.35]	68.8	100
		VAMS[b]	16	1.11 [0.69; 1.35]	−1.67 [−13.9; 13.8]	1.05 [0.76; 1.33]	87.5	93.8
Creatinine	Mean concentration	DBS[a]	25	1.13 [0.98; 1.37]	−0.22 [−3.71; 2.02]	1.12 [0.94; 1.30]	76.0	100
		VAMS[a]	25	1.17 [0.92; 1.71]	−2.85 [−9.74; 0.93]	0.98 [0.76; 1.20]	92.0	100
	eGFR	DBS[a]	25	0.90 [0.75; 1.09]	−0.88 [−10.0; 4.25]	0.88 [0.71; 1.05]	80.0	100
		VAMS[a]	25	1.10 [0.88; 1.33]	−4.12 [−15.9; 5.77]	1.04 [0.76; 1.33]	80.0	96.0
Iohexol	Concentration	DBS[a]	88	0.61 [0.55; 0.67]	1.24 [−7.05; 7.53]	0.61 [0.44; 0.79]	3.41	13.6
		DBS[b]	88	1.01 [0.92; 1.11]	−0.56 [−12.1; 10.2]	1.00 [0.72; 1.29]	85.2	92.0
		VAMS[a]	88	0.61 [0.54; 0.67]	1.00 [−8.78; 11.2]	0.61 [0.39; 0.83]	5.68	25.0
		VAMS[b]	88	1.01 [0.91; 1.11]	−2.37 [−15.8; 14.8]	1.00 [0.64; 1.36]	76.1	88.6
	mGFR	DBS[b]	24	1.17 [0.88; 1.51]	−9.34 [−30.2; 6.27]	1.01 [0.72; 1.29]	75.0	100
		VAMS[b]	24	1.39 [0.82; 2.08]	−20.5 [−66.1; 8.02]	1.02 [0.63; 1.40]	62.5	91.7

AUC_{0-12}, area under the concentration–time curve from time zero to 12 h after administration; AUC_{0-24}, area under the concentration–time curve from time zero to 24 h after administration; C_0, trough concentration; CI, confidence interval; DBS, dried blood spot; eGFR, estimated glomerular filtration rate; LoA, limits of agreement; mGFR, measured glomerular filtration rate; MPA, mycophenolic acid; P_{20}, percentage of observations within ±20% of those of the reference method; P_{30}, percentage of observations within ±30% of those of the reference method; VAMS, volumetric absorptive microsample.
[a]Based on the uncorrected concentration in capillary whole blood;
[b]Based on the corrected concentration in capillary whole blood.

respectively. These suggest DBS-to-plasma and VAMS-to-plasma conversion factors of 1/0.75 and 1/0.73, which are slightly higher but comparable to the 1/0.68 observed in our previous study.[7]

After correction with the 1/0.68 factor, the corrected DBS and VAMS MPA concentrations showed adequate linearity with the EDTA reference samples for the individual concentrations (Figure 1E, G) and AUCs (Figure 1F, H), displaying Passing–Bablok regression slopes of 1.10 and 1.07, and 1.14 and 1.11, respectively (Table 2). The DBS method showed biases of +11% (range: −18 to +44%) for the individual concentrations and +7% (range: −17 to +29%) for the AUCs, with P_{20} of 78.1 and 68.8%, respectively (Figure 2E, F; Table 2). Whereas the AUC P_{20} did not meet the prespecified acceptance criterium, its P_{25} and P_{30} of 93.8 and 100% did provide some reassurance of the potential of this approach.

The VAMS method showed superior performance, with mean biases of +7% (range: −21 to +64%) and +5% (range: −19 to +32%), and P_{20} of 84.4 and 87.5% for the individual concentrations and AUCs, respectively (Figure 2G, H; Table 2). The DBS and VAMS dosing recommendations were identical. For both methods, differences with the reference dosing recommendations occurred on 4/16 (25.0%) of occasions (Figure S2). On average, the DBS and VAMS dosing recommendations were 58.1 ± 190 mg (range: −500; +250) lower than for the reference (relative difference: −4.17% ± 14.6%, range: −33.3 to +25.0%).

Thus, the MPA AUC_{0-12} results quantified in VAMS samples were interchangeable with those quantified in venous plasma samples. The DBS technique yielded slightly different MPA AUC_{0-12} results, but can still be considered for routine clinical care as these differences were within 25% for most of the samples and did not exceed 30%.

3.2.4 | Creatinine

For creatinine, the DBS and VAMS methods showed adequate linearity with the EDTA reference method across the eGFR range, with Passing–Bablok regression slopes of 1.13 and 1.17 for the individual

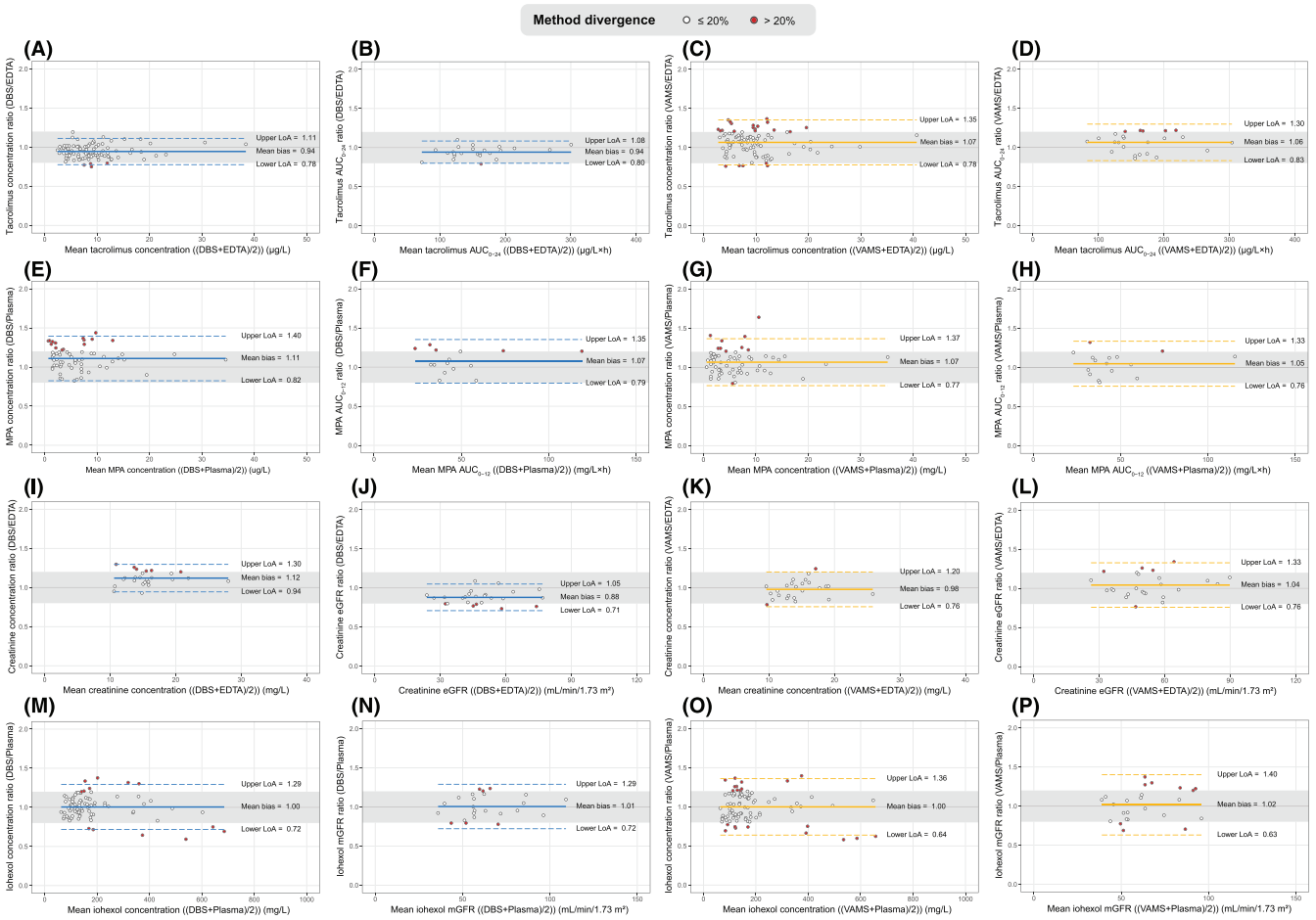

FIGURE 2 Bland–Altman ratio difference plots for the tacrolimus concentrations (A, C) and area under the concentration–time curves (AUCs; B, D), corrected mycophenolic acid (MPA) concentrations (E, G) and AUCs (F, H), creatinine concentrations (I, K) and estimated glomerular filtration rates (eGFRs; J, L), and corrected iohexol concentrations (M, O) and measured GFRs (mGFRs; N, P). Solid and dotted blue and gold lines represent the mean bias and the lower and upper limits of agreement (LoA) for the dried blood spot (DBS) and volumetric absorptive microsampling (VAMS) techniques, respectively. The grey-shaded area depicts the ±20% ratio difference limit around the line of identity, with any observations exceeding the ±20% ratio difference limit highlighted in red

concentrations (Figure 1I, K; Table 2) and 0.90 and 1.10 for the eGFRs (Figure 1J, L; Table 2), respectively. The DBS method yielded mean biases of +12% (range: −7 to +30%) for the individual concentrations and −12% (range: −27 to +9%) for the eGFRs, with P_{20} of 76.0 and 80.0%, respectively (Figure 2I, J; Table 2). The VAMS method showed superior performance, with mean biases of −2% (range: −22 to +24%) and +4% (range: −24 to +34%), and P_{20} of 92.0 and 80.0% of the individual concentrations and eGFRs, respectively (Figure 2K, L; Table 2).

Consistent with the superior overall precision for DBS samples observed during the analytical validation (ESM), the between-sample creatinine concentration variability was lower within DBS kits (mean: 4.58 ± 2.76% CV, range: 0.93–13.7) than within VAMS kits (mean: 6.18 ± 3.44% CV, range: 2.48–14.4). The variability was <5% CV for 18/25 (72%) and <10% CV for 23/25 (92%) of patients for the DBS samples, whereas this was 13/25 (52%) and 21/25 (84%) for the VAMS samples.

Thus, when quantified in 4-fold in tandem with immunosuppressant AUC assessment, the DBS or VAMS samples yielded eGFR results that are very similar to those quantified in venous EDTA samples and can be used for kidney function trend monitoring. Creatinine or eGFR assessment based on 1 sample is possible with the DBS technique, but not with the VAMS technique.

3.2.5 | Iohexol

For iohexol, 8 samples (8.3%) drawn at 5–15 minutes after administration showed plasma concentrations >1000 mg/L, demonstrating divergent discrepancies with the DBS and VAMS samples. As iohexol concentrations >1000 mg/L were suspected to result from sampling errors, these samples were excluded from the analyses.

In the evaluation of the required DBS-to-plasma and VAMS-to-plasma conversion factors, the DBS and VAMS samples showed mean

concentration ratios of 0.61 ± 0.09 (range: 0.36–0.84) and 0.61 ± 0.11 (range: 0.35–0.85) as compared to the EDTA plasma reference values (Figures S3, S4). This suggested a conversion factor of 1/0.61 for both matrices. Concentration conversion based on the mean population or individual haematocrit yielded comparable results, justifying the use of mean ratio-based conversion for this study (Figure S5).

The corrected iohexol concentrations showed adequate linearity of the DBS and VAMS samples with the reference values for the individual concentrations (Figure 1M, O) and mGFRs (Figure 1N, P), displaying Passing–Bablok regression slopes of 1.01 and 1.01, and 1.17 and 1.39, respectively (Table 2). The DBS method showed biases of 0% (range: −41 to +38%) for the individual concentrations and +1% (range: −22 to +24%) for the mGFRs, with a P_{20} of 85.2 and 75.0%, respectively (Figure 2M, N; Table 2). The VAMS method showed inferior performance, with mean biases of +0% (range: −42 to +40%) and +2% (range: −31 to +37%), and a P_{20} of 76.1 and 62.5%, respectively (Figure 2O, P; Table 2).

Thus, the iohexol mGFR results quantified in DBS and, particularly, VAMS samples differed from those quantified in venous EDTA plasma. This renders our assay inapplicable for iohexol mGFR determination, because the clinical application of this approach calls for particularly high reliability.

3.2.6 | Everolimus

For everolimus, albeit strictly exploratory owing to the limited sample size ($n = 12$), the DBS and VAMS methods showed adequate linearity with the EDTA reference method, with Passing–Bablok regression slopes of 0.95 and 1.13 for the individual concentrations (Figures S3, S4), respectively. On average, the DBS method showed superior results as compared to the VAMS method, yielding biases of −8% (range: −15 to +3%) vs. +21% (range: +12 to +38%), with a P_{20} of 100 and 50%, respectively (Figures S3, S4). The limited number of everolimus C_0 ($n = 3$) and AUCs ($n = 3$) thwarted statistically sound method comparison. However, exploratory analyses did provide preliminary confirmation that the concentration results translate to similar C_0 (DBS $P_{15} = 100$; VAMS $P_{15} = 0\%$) and AUC (DBS $P_{20} = 100$; VAMS $P_{20} = 33.3\%$) results.

Thus, these exploratory results suggested that our assay can be used for everolimus C_0 and AUC_{0-12} TDM in DBS samples, but not in VAMS samples. However, additional validation is warranted to confirm this.

3.3 | Clinical feasibility study

Complete SUS questionnaires were obtained from 20/25 (80%) of the included RRFD patients. The main results are depicted in Figure 3A, indicating adequate overall system usability for both devices, with median SUS scores of 65.0 (IQR: 52.5–72.5; range: 27.5–97.5) and 76.3 (IQR: 65.0–90.6; range: 35.0–100) for the DBS and VAMS devices, respectively. The VAMS device showed statistically significantly higher system usability than the DBS device ($P = .013$), with the difference originating mainly from question items on patient confidence with and ease-of-use of the devices (Figure 3B).

Laboratory data of 420 DBS kits from 341 unique patients were available for evaluation of the remote TDM process. Of these kits, 252 (60.0%) contained tacrolimus and MPA, 87 (20.7%) only tacrolimus, and 81 (19.3%) only MPA. This yielded 1611 DBS spots, of which 1463 spots (90.8%) were of adequate quality for quantification of the agent(s) of interest (Figure 4A). Of the 148 disapproved samples, 72 (48.6%) spots were discarded because no blood had been transferred from the capillary onto the filter paper, 38 (25.7%) contained a blood spot that was too small, 13 (8.8%) contained a blood spot that was too large, 13 (8.8%) spots were missing, 6 (4.1%) displayed blood spillage, 5 (3.4%) were sampled directly onto the filter paper and, for 1 spot (0.7%), no sampling time was recorded (Figure 4B). In total, 643 AUCs were anticipated from the 420 DBS kits, of which 591 (91.9%) could be estimated reliably (Figure 4C). Of the 52 failed AUCs, 44 (85%) AUCs could not be estimated due to disapproval of ≥2 blood spots. Additionally, 2 (3.8%) AUCs failed due to medication intake prior to the C_0 sample collection, 2 (3.8%) because no clear peak concentration was reached, 1 (0.6%) because the C_0 sample collection had failed, and 1 (0.6%) because only a C_0 sample was collected. In 2 cases (3.8%), the device contained no blood spots at all (Figure 4D).

4 | DISCUSSION

In this study, a novel LC–MS/MS assay was developed and clinically validated for the quantification of tacrolimus, MPA and creatinine in DBS and VAMS samples. Herewith, we aimed to enable simultaneous remote immunosuppressant TDM and kidney function assessment in kidney transplant recipients.

For tacrolimus, the assay showed high similarity between the DBS and reference samples with a P_{20} > 95% across the entire concentration and exposure range. While the Passing–Bablok regression analysis indicated a minor divergence between both methods, the Bland–Altman analysis and P_{20} results provided adequate assurance that the methods can be used interchangeably. Also, the developed assay showed adequate performance in the tacrolimus C_0 range for DBS samples. Its P_{15} of 92.0% is comparable with a previously published multianalyte assay for tacrolimus C_0 monitoring in kidney transplant recipients using DBS sampling, which displayed a P_{15} of 96.6%.[46] The VAMS method generally showed higher imprecision and P_{20} values just below the acceptance limit of 80%, but can be considered for AUC assessment in patients experiencing difficulties with the DBS device as most samples did fall within 30% of the reference. However, the P_{15} of 68.0% does not allow for VAMS-based C_0 monitoring.

For MPA, the assay showed moderate similarity of the corrected DBS and VAMS concentrations and AUCs with the plasma reference values. Notably, the DBS and VAMS samples displayed mean

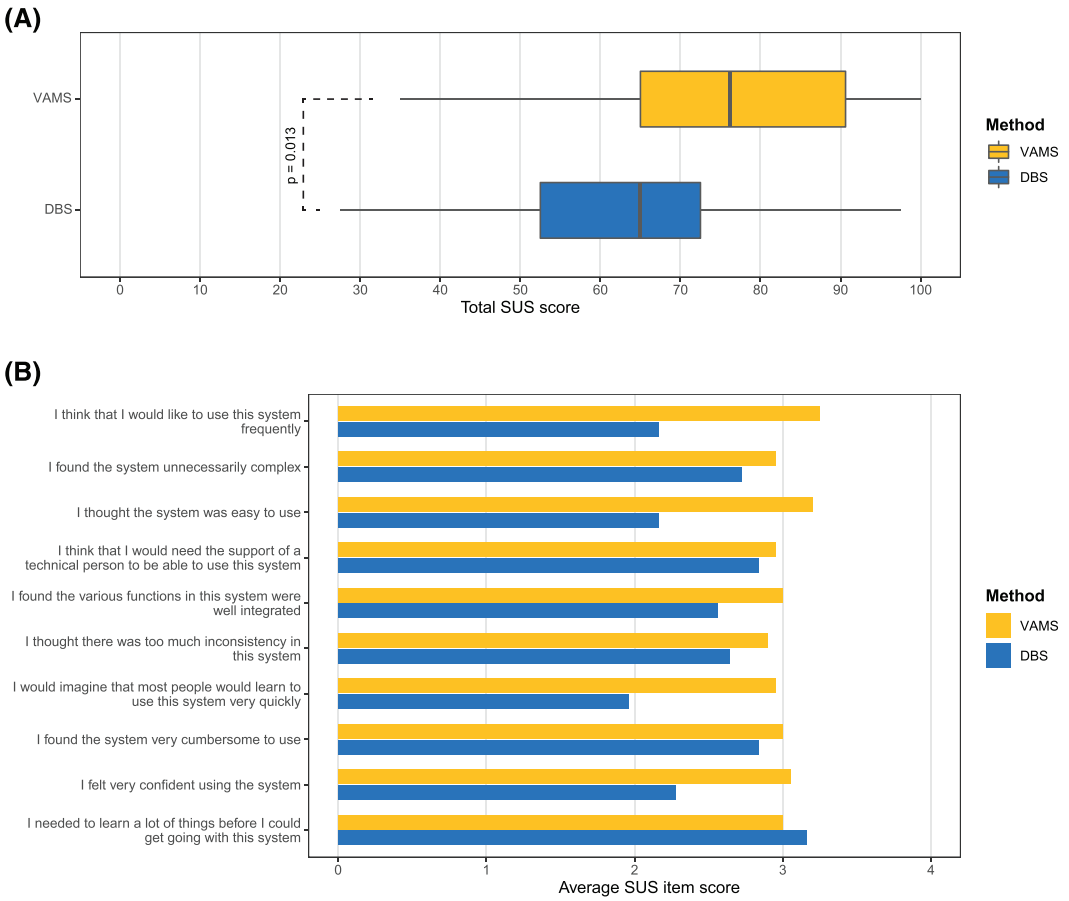

FIGURE 3 Feasibility of volumetric microsampling in kidney transplant recipients as evaluated with the System Usability Score (SUS) questionnaire. (A) Boxplots of the total SUS scores for the dried blood spot (DBS) and volumetric absorptive microsampling (VAMS) methods. (B) Bar graph of the average score for each SUS questionnaire item for the DBS and VAMS methods

concentration ratios of 0.75 and 0.73 as compared to the plasma samples, respectively. Hence, mean ratio-based conversion based on our previously derived factor of 1/0.68[7] resulted in slight overcorrection. Also, while the conversion factor corrects for the mean bias, it slightly inflates the overall imprecision, yielding broader limits of agreement around the mean bias. These findings may indicate a need for further optimization of our conversion factor. Previous studies have described various methods for DBS-to-plasma concentration conversion, including mean DBS-to-plasma ratio-,[2,7] individual haematocrit-[5,10,47] and mean population haematocrit-based[17] conversion. Disturbingly, a wide variability in the systematic DBS-to-plasma divergence is discernible between these studies, with Passing–Bablok slopes and mean concentration ratios ranging 0.51–0.78.[2,7,17,47] This translates to capillary DBS concentrations being generally 1.25- to 2-fold lower than venous plasma concentrations. Whereas individual haematocrit-based conversion seems the most sound approach from a theoretical perspective, the reported population haematocrits of 0.39–0.41 in these studies do to not fully explain the reported systematic biases. Clearly, this topic requires further study. Nevertheless, our VAMS method still showed a P_{20} of 87.5% in the AUC comparison, justifying clinical application of this method. Additionally, although the DBS method showed a P_{20} of merely 68.8%, its respective P_{25} and P_{30} of 93.8 and 100% in the AUC comparison provide reassurance that its performance is still acceptable for remote MPA AUC trend monitoring. This was confirmed by identical performance of the DBS and VAMS methods in the dosing recommendation comparison.

For creatinine, when quantified in 4-fold in tandem with immunosuppressant AUC assessment, the assay showed adequate similarity of the DBS and VAMS methods with the reference method, with eGFR P_{20} values of 80% and P_{30} values >96%. The DBS method displayed a mean bias of −12% but relatively low imprecision, whereas the VAMS method showed negligible mean bias but higher imprecision. Particularly, the low analytical within- and between-run imprecision (<4% CV; ESM) and low within-kit variability for DBS samples ensures that detection of kidney function changes exceeding approximately 15% at adequate certainty.[88] Whereas this provides slightly less certainty than conventional monitoring at the clinic (<2% CV), the limited patient discomfort associated with remote microsampling facilitates more frequent eGFR assessment and thus trend monitoring at a higher resolution. By contrast, the inferior between-run imprecision (ESM) and higher within-kit variability for VAMS samples yields less reliable detection of kidney function changes over time with this

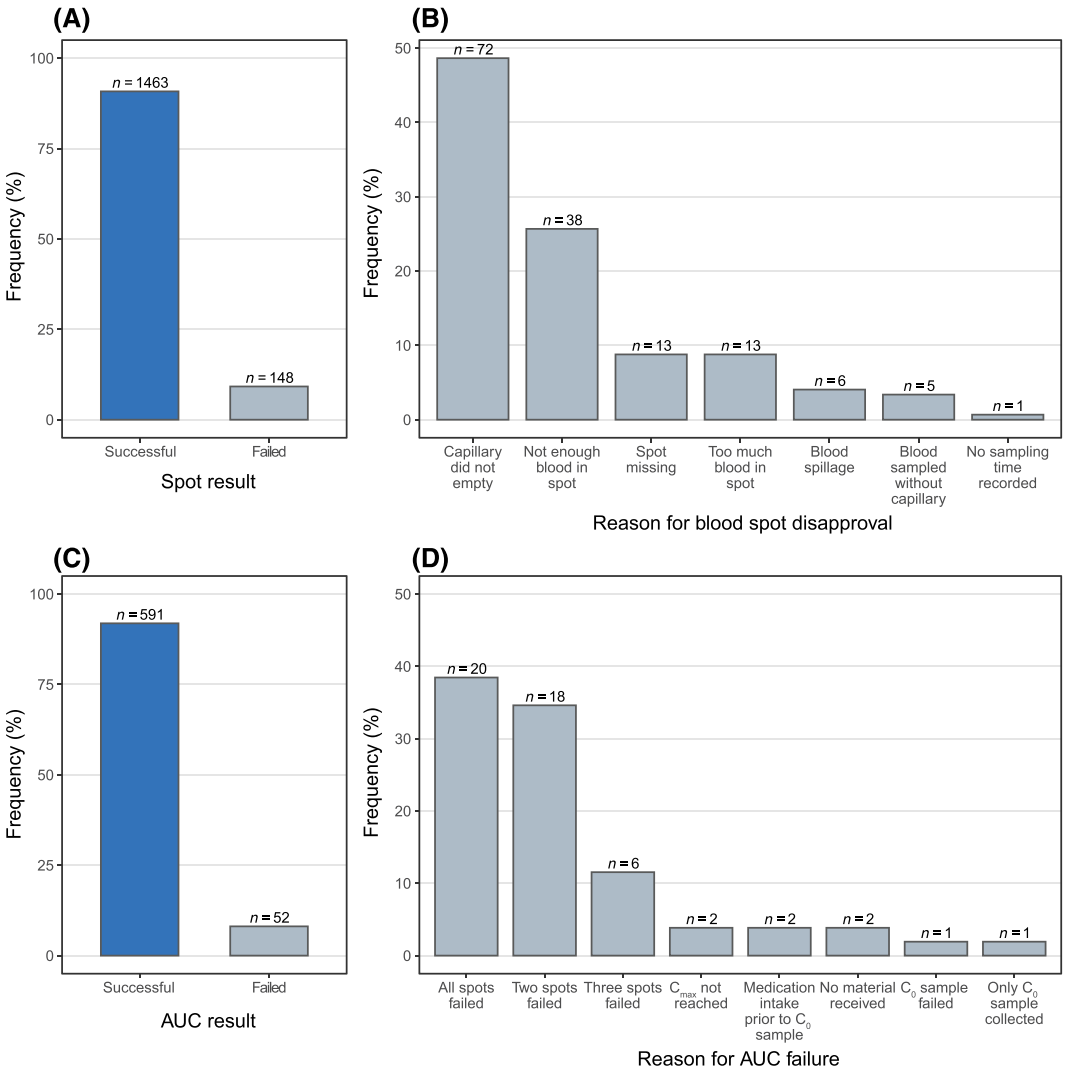

FIGURE 4 Feasibility of the remote dried blood spot (DBS)-based immunosuppressant therapeutic drug monitoring process. (A) Frequency of DBS spot disapproval. (B) Reasons for DBS spot disapproval. (C) Frequency of area under the concentration–time curve (AUC) failure. (D) Reasons for AUC failure

technique, especially when quantified from 1 sample per occasion. Kidney function trend monitoring based on eGFR values quantified with our assay is thus preferably performed using the DBS technique.

For iohexol, the assay showed P_{20} values below the acceptance threshold for the DBS ($P_{20} = 75.0\%$) and VAMS ($P_{20} = 62.5\%$) methods in the mGFR comparison. Our assay should thus not be applied to inform stand-alone clinical decisions based on iohexol mGFRs determined in microsamples. Also, while a clinically measured iohexol mGFR has added value over creatinine-based approaches in terms of kidney function determination,[50] it is questionable whether this still holds true at suboptimal bioanalytical method performance. Especially because patients still need to come in for the intravenous iohexol administration, our microsampling-based iohexol mGFR method has probably limited clinical potential at present. We suspected that the dissatisfactory results for iohexol were caused by variability in recovery, but this could not be confirmed based on our data. Aside from these aspects, the derived conversion factor of 1/0.61 requires further validation. Others have applied individual haematocrit-based corrections,[55,56,71,89,90] but this necessitates quantification of the haematocrit in the DBS or VAMS sample when applied remotely. Whereas nondestructive techniques for haematocrit quantification in DBS samples exist,[91,92] these are not yet widely available. Also, while our mean ratio-based conversion factor does not correct for the haematocrit dependency, the rather narrow haematocrit distribution in the stable kidney transplant recipient population probably does not result in clinically relevant bias. However, individual haematocrit correction does pose a more elegant approach and should be reconsidered when these techniques become more widely available. Finally, we encountered a number of iohexol concentrations >1000 mg/L for samples drawn within 5–15 minutes after iohexol administration, which were highly divergent from their paired DBS and VAMS concentrations. Although we suspected that these

were associated with sampling errors, another explanation could be that capillary iohexol distribution was not yet complete at these early time points. In that case, Bayesian estimation of the iohexol mGFR based on DBS or VAMS samples may require slightly later time points for the first concentration.

For everolimus, albeit exploratory, the assay showed excellent performance for the DBS samples with a C_0 and AUC P_{20} of 100%. By contrast, the VAMS samples showed C_0 and AUC P_{20} results of 0 and 33%, respectively. Hence, the assay can probably be applied for routine everolimus quantification in DBS samples after additional validation, but requires further optimisation before considering its application for VAMS samples.

Thus, the developed assay allows for C_0-based tacrolimus TDM combined with creatinine and/or eGFR trend monitoring from a single DBS sample, but not from a single VAMS sample. Similarly, AUC-based TDM for tacrolimus and/or MPA combined with creatinine and/or eGFR monitoring is preferably conducted with the DBS technique, based on blood samples drawn just before drug intake and at 1, 2 and 3 hours thereafter. The VAMS technique can be considered for tacrolimus and/or MPA AUC monitoring for patients experiencing difficulties with the DBS device, but yields less reliable detection of kidney function changes over time than the DBS technique. At present, our assay shows limited clinical potential for iohexol mGFR quantification in DBS or VAMS samples.

Overall, our assay generally showed higher imprecision for VAMS samples as compared to DBS samples. We suspect that this was related to higher variability in recovery for VAMS samples, as our initial DBS sample preparation method was only partly adjusted for VAMS samples. For instance, others have suggested to use an initial water:methanol ratio of 40:60 for the extraction solution to redissolve the erythrocytes,[43] whereas an initial ratio of 60:40 was applied here. This provides options to further optimise our assay for VAMS samples. Others have also suggested variability in sampling volume as an additional source of imprecision in VAMS samples, wherein particularly slightly undersaturated samples pose a problem as these cannot be identified by visual inspection.[46]

Additionally, both microsampling devices demonstrated adequate patient satisfaction. Interestingly, a preference for the VAMS device over the DBS device was observed. This confirms that this sampling device has potential as an alternative to the DBS device for a selection of patients. The current remote TDM process showed adequate clinical feasibility, with successful DBS sample collection and clinical DBS kit applicability in >90% of cases. Nevertheless, some shortcomings were identified. Most sampling issues originated from incomplete blood transfer from the capillary to the filter paper. This is likely to be associated with incomplete or untimely closure of the device, indicative of a need for additional patient training and guidance. Additionally, in some cases, the blood spot volume was either too small or too large. Although the DBS device allows for volumetric blood sample collection, it requires complete filling of the capillary to produce an adequately sized spot. This can be a problem when the generated blood drop is too small to completely fill the capillary at once. Patients are allowed to apply multiple blood drops to completely fill the capillary; however, this may lead to blood coagulation within the capillary. Most issues with the clinical DBS kit applicability concerned disapproval of ≥2 spots, thereby thwarting reliable AUC estimation. Also, a few issues were related to the shape of the pharmacokinetic curve, including premature oral immunosuppressant intake and the absence of a clear peak concentration. The former indicates a need for improved patient instruction, whereas the latter is associated with the applied Bayesian estimator. While the selected Bayesian estimator is generally considered optimal for tacrolimus AUC estimation,[59] it apparently does not capture all pharmacokinetic profiles within this population. However, the limited number of divergent pharmacokinetic profiles does not justify extended sampling in the entire population. Finally, it is important to acknowledge that remote monitoring with these alternative sampling devices will simply not be operable for all patients. Already in our limited study population ($n = 20$), SUS scores ranged as low as 35, suggesting dissatisfaction with the device for certain patients. To identify these patients in a timely manner, it is important to evaluate the remote monitoring process early after initiation.

Our study showed some limitations. First, the clinical validation study was performed at the clinic where the participants were partly assisted with the sample collection. While this approach ensures high sample quality and accurate sampling time recording, it does not capture the remote sampling process. However, the results of our clinical feasibility study provide adequate reassurance of the potential of this novel assay. Second, the SUS questionnaire results may have partly been influenced by the differences in user experience with both devices. All participants had elaborate experience with the independent and remote use of the DBS device, but not with the VAMS device. Our findings are thus based on only a first impression of the VAMS device, do not include remote sampling experience with the VAMS device, and may have been partly influenced by the user's prior experiences with the DBS device. Third, no separate stability studies were performed for the VAMS samples during the analytical validation. However, previous studies have shown that tacrolimus, MPA and everolimus are stable in VAMS samples for 50 days in the freezer (−20°C), 60 days at room temperature, 30 days at 37°C and 2 days at 50°C.[43] Additionally, iohexol is stable in VAMS samples up to 8 months in the freezer (−20°C) or refrigerator (2–8°C) and 3 months at room temperature,[58] and creatinine for at least 14 days at 20°C, 7 days at 37°C and 25 days in the freezer (−20°C) or refrigerator (4°C).[38] As all VAMS samples for the clinical validation were analysed within 1 month after sample collection and stored at −20°C before analysis, no pronounced interference of analyte stability with our results was anticipated. Fourth, whereas we evaluated whether any haematocrit-dependencies were discernible within the observed haematocrit range of the clinical validation population, linear regression-based extrapolation of these data to more extreme haematocrit values is associated with uncertainty. However, our results are comparable with those from previous studies, which showed that tacrolimus, MPA, everolimus and creatinine can be quantified reliably in DBS samples displaying haematocrit values between 0.23 and 0.53,[12,15] while no such data are available for iohexol.

Similarly, others showed that tacrolimus, MPA, everolimus, creatinine and iohexol can be quantified reliably in VAMS samples with haematocrit values between approximately 0.20 and 0.60.[43,48,58] As haematocrit values outside these ranges are seldomly encountered in adult kidney transplant recipients,[93] also in the clinically unstable phase, this probably allows for DBS- and VAMS-based monitoring without pronounced haematocrit interference for most patients. Nevertheless, DBS- and VAMS-based monitoring should still be considered carefully in the first weeks after transplantation and in otherwise clinically unstable patients, as these studies set their maximum tolerable haematocrit effect thresholds at ±15%, which still allows for substantial haematocrit-dependent assay variability.

5 | CONCLUSIONS

We developed and validated a novel LC–MS/MS assay for the simultaneous quantification of tacrolimus, MPA and creatinine in DBS and VAMS samples. Our DBS method allows for remote kidney function assessment combined with either tacrolimus and/or MPA AUC monitoring based on 4 sequential samples or tacrolimus C_0 monitoring from a single sample. Furthermore, we demonstrated that remote DBS-based immunosuppressant TDM is feasible within routine clinical care, offering a patient-friendly alternative to conventional venous EDTA-based TDM at the clinic. Our VAMS method has potential as an alternative to DBS sampling for a selection of patients, but generally yields less reliable results, demands more elaborate sample preparation and requires additional evaluation of the remote sampling process.

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COMPETING INTERESTS

Tom Zwart, Erik Metscher, Paul van der Boog, Jesse Swen, Johan de Fijter, Henk-Jan Guchelaar, Aiko de Vries and Dirk Jan Moes declare that they have no conflicts of interest that are directly relevant to the content of this article.

AUTHOR CONTRIBUTIONS

T.C.Z., P.J.M.v.d.B., A.P.J.d.V., H.J.G, .J.W.d.F and D.J.A.R.M. contributed to the study conception and design. T.C.Z., E.M. and D.J.A.R.M. performed the research and analysed the data. The initial draft of the manuscript was written by T.C.Z. and D.J.A.R.M., and all authors commented on previous versions of the manuscript. All authors read and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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