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ORIGINAL ARTICLE

Clinical performance of volumetric finger-prick sampling for the monitoring of tacrolimus, creatinine and haemoglobin in kidney transplant recipients

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Aims: Finger-prick sampling has emerged as an attractive tool for therapeutic drug monitoring and associated diagnostics. We aimed to validate the clinical performance of using two volumetric devices (Capitainer® qDBS and Mitra®) for monitoring tacrolimus, creatinine and haemoglobin in kidney transplant (KTx) recipients. Secondarily, we evaluated potential differences between finger-prick sampling performed by healthcare professionals *vs*. self-sampling, and differences between the two devices.

Methods: We compared finger-prick and venous sampling in three settings: micro-sampling performed by healthcare personnel, self-sampling under supervision, unsupervised self-sampling. The finger-prick samples were analysed with adapted methods and results compared to routine method analysis of the venous blood samples.

Results: Twenty-five KTx recipients completed the main study and 12 KTx recipients completed a post hoc validation study. For tacrolimus measurements and predicted area under the curve, the proportions within ±20% difference were 79%–96% for Capitainer and 77%–95% for Mitra. For creatinine and haemoglobin, the proportions within ±15% were 92%–100% and 93%–100% for Capitainer and 79%–96% and 67%–92% for Mitra, respectively. Comparing sampling situations, the success rate was consistent for Capitainer (92%–96%), whereas Mitra showed 72%–88% and 52%–72% success rates with samples collected by healthcare personnel and the patients themselves.

Conclusions: Capitainer and Mitra are technically feasible for measuring tacrolimus, creatinine and haemoglobin. In the context of self-sampling, Capitainer maintained consistent sampling success and analytical quality. Implementing volumetric finger-prick self-sampling for the monitoring of tacrolimus, creatinine and haemoglobin may simplify and improve the follow-up of KTx recipients.

KEYWORDS
creatinine, dried blood spot, haemoglobin, tacrolimus, transplantation, VAMS

The authors confirm that the principal investigator for this paper is Karsten Midtvedt and that he had clinical responsibility for patients.

1 | INTRODUCTION

Volumetric microsampling devices allow finger-prick sampling of accurate capillary blood volumes and emerge as attractive tools for therapeutic drug monitoring (TDM) and associated diagnostics.[1,2] Implementation of self-sampling by finger pricks may enable a more flexible follow-up for the patient and lead to resource savings for the healthcare system. The methodology makes area under the curve (AUC)-targeted drug monitoring applicable when used for limited sampling strategies combined with Bayesian estimates.[3] Also, microsampling is interesting in rich sampling pharmacokinetic clinical studies, combining both a low total blood volume with more flexible study designs. Furthermore, utilization of finger-prick self-sampling at home could prevent the spread of infectious diseases in vulnerable patient populations, such as during the Covid-19 pandemic.

The collected blood dries on the device and the concept usually allows long storage time and transport at ambient temperature (e.g. standard mail service) to the laboratory for quantitative analysis.[4] Sample volumes in a low microliter range, the dried state of blood in specific absorbent materials, as well as the physical composition of the device, require carefully designed sample preparation methods in the laboratory to ensure adequate analytical quality. The panel of analytes that can be performed in a microsample should be appropriately extensive and relevant to the specific patient population. With respect to whole blood samples, nephrologists at our hospital initially pointed out **tacrolimus**, creatinine and haemoglobin as diagnostic tests of the highest priority in order to reduce the number of hospital follow-up visits by introducing self-sampling and telemedicine after kidney transplantation.

Clinical validation of the quality of results obtained by volumetric microsampling is mandatory before implementation in ordinary clinical follow-up, especially the total error, from the patient taking the sample to the final reported result. Unsupervised sampling is challenging and may be a source of variability, depending on the ease of use of the device, the training programme and acquired user skills. Non-volumetric and volumetric microsampling have previously been studied for combined monitoring of tacrolimus, cyclosporine and creatinine in kidney transplant recipients.[5–8] Nevertheless, there are knowledge gaps regarding how finger-prick self-sampling performs compared with assisted finger-prick sampling by healthcare personnel. So far, quantification of tacrolimus and creatinine has only been studied nonclinically with the use of the microsampling device Capitainer® qDBS (Capitainer AB, Solna, Sweden).[9] To the best of our knowledge, the clinical performance of Capitainer in connection with the monitoring of tacrolimus, creatinine and haemoglobin has not yet been studied, nor the direct comparison with another volumetric microsampling principle in the same population.

In the present study, we aimed to validate the clinical performance of two different volumetric microsampling devices in adult kidney transplant patients. The tacrolimus concentration pre-dose as well as 1 and 3 h post-dose, the predicted tacrolimus AUC, together with creatinine and haemoglobin concentrations were subjects for the validation. One microsampling device was based on volumetric capillary wicking of blood that is subsequently deposited on a filter paper (Capitainer® qDBS), and the other device was based on volumetric absorption of blood into a hydrophilic polymer (Mitra®). The agreement between measurements in finger-prick microsamples and liquid venous samples was the primary outcome of the validation. Secondarily, we evaluated potential differences between finger-prick sampling performed by healthcare professionals and by the patients themselves, as well as differences between the two devices.

What is already known about this subject

- Self-collection of volumetric finger-prick samples appears attractive for easier follow-up after kidney transplantation.
- Knowledge on the performance of volumetric self-sampling during the first weeks following kidney transplantation is sparse.
- The clinical performance of Capitainer® qDBS for monitoring tacrolimus, creatinine and haemoglobin has yet to be determined.

What this study adds

- Both Capitainer® qDBS and Mitra® demonstrated satisfactory quality for monitoring tacrolimus, creatinine and haemoglobin with finger-prick sampling performed by healthcare professionals.
- Capitainer showed consistent quality in the context of self-sampling.
- Monitoring of tacrolimus trough concentration and predicted AUC, combined with creatinine and haemoglobin, is feasible with volumetric finger-prick self-sampling after kidney transplantation.

2 | METHODS

2.1 | Study design and population

We asked kidney transplant recipients over the age of 18 to participate in the study conducted at Oslo University Hospital, Rikshospitalet, from January to June 2022. Patients invited to participate were followed at the out-patient clinic in the early phase (2–8 weeks) after kidney transplantation. During this early post-transplant phase, the participants either stayed at home, or in the patient hotel if they lived a long distance from the transplant centre. The study was approved by the Regional Committee for Medical Research Ethics (REK

South-East: reference 134 787) and by the data protection officer of the hospital prior to study start. All patients provided written informed consent for participation. The study was performed in accordance with the Helsinki Declaration.

The two microsampling devices studied were Capitainer® qDBS 10 µL from Capitainer AB, Solna, Sweden and Mitra® 10 µL from Trajan Scientific and Medical, Melbourne, Australia (Figure 1). All patients initially received written and oral information regarding how to use Capitainer® and Mitra®. In addition, they were shown and asked to download two YouTube videos on their smart phones (accessed via QR-codes). The videos have been made by the study personnel, are in Norwegian, easy to understand and show sampling procedure step-by-step for Capitainer® and Mitra®, separately. In the main study, three different sampling situations were carried out on separate study days: (i) microsampling performed by trained healthcare personnel, (ii) microsampling performed by the patient under supervision by trained healthcare personnel, and (iii) self-sampling alone (at home/hotel). Finger-prick samples were collected before medication, i.e. tacrolimus dosing in the morning (t_0), and 1 and 3 h after dosing (t_1 and t_3). Both Capitainer 2 × 10 µL and Mitra 2 × 10 µL were obtained at all sampling time points, in random order. We collected liquid venous blood samples close in time to all microsamples, except at t_1 and t_3 in relation to the unsupervised self-sampling situation. Due to the pharmacokinetic fluctuation of tacrolimus throughout the dose interval, the following predefined time limits between finger-prick and venous sampling were set as eligibility criteria in the data analysis: ±30 min at t_0, and ±10 min at t_1 and t_3.

In a post hoc study, we recruited a new group of kidney transplant recipients to provide an external data set for validation. The inclusion criteria were identical to the main study, but, due to the results obtained in the preceding study, we now only used Capitainer® as microsampling device. Each patient performed finger-prick sampling on four separate days (pre-dose sampling only) and healthcare personnel collected venous blood samples close in time. The participants received the same training as in the main study, and informed consent, ethics and regulatory aspects were also the same.

2.2 | Analytical methods

On each microsample device, one spot or tip was allocated for tacrolimus while the other was applied for creatinine and haemoglobin.

For tacrolimus assayed in liquid venous blood (routine assay), Capitainer and Mitra, we used separate sample preparation protocols. A common liquid chromatography tandem mass spectrometry (LC–MS/MS) method was applied for the quantification of tacrolimus.[4,10] The measurement range was 0.70–60 µg/L for all sample types. Analytical coefficients of variation (CV) were ≤7% for the liquid blood assay and ≤11% for the microsample assays.

In liquid venous blood plasma, creatinine was quantified with an enzymatic-colourimetric assay on the Cobas c702 instrument (Roche Diagnostics GmbH, Mannheim, Germany). The measurement range was 14–2700 µmol/L with CV ≤ 3%. Creatinine in extracts from the microsamples was measured with flow-injection MS/MS; measurement range 25–800 µmol/L. The CVs were ≤5% for Capitainer and ≤7% for Mitra.

Haemoglobin in liquid blood was quantified with a photometric assay using the Sysmex XN-9000 instrument (Sysmex Europe, Norderstedt, Germany). The measurement range was 1.0–26.0 g/dL with CV ≤ 2.5%. Haemoglobin in extracts from the microsamples was analysed with a low volume photometric assay on a plate reader. For the latter, the measurement range was 2.5–40.0 g/dL and the CVs were ≤8% for Capitainer and ≤7% for Mitra. Haemoglobin in g/dL can be converted to mmol/L by the conversion factor 0.6206.

The microsample assays are detailed in Data S1.

2.3 | Preliminary in vitro study

For the creatinine and haemoglobin microsample assays, we used liquid calibrator material directly in the analytical processes. Another important aspect was that the microsamples consisted of whole blood, while the routine creatinine samples consisted of plasma. Thus, we expected a systematic bias and need for correction factors. Surplus ethylenediaminetetraacetic acid (EDTA) blood samples and

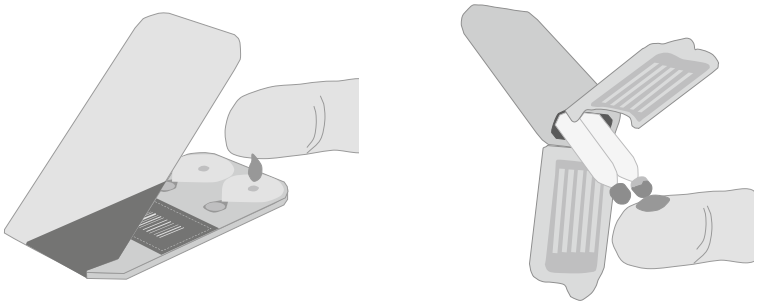

FIGURE 1 Illustrations of the devices used to collect volumetric finger-prick blood samples. On the left, Capitainer® qDBS 2 × 10 µL: a drop of blood is dripped into each of the two inlets, an accurate volume is collected by capillary wicking and the volumetric blood samples are deposited on shielded precut filter papers. The sampling card is closed and placed in a cardboard envelope. On the right, Mitra® 2 × 10 µL: blood is absorbed into a hydrophilic polymer that captures an accurate volume. The polymer is constructed at the end of two tips which are seated in a plastic cartridge. After sampling, the cartridge is closed and placed in a sealed bag with desiccant.

time-corresponding creatinine and haemoglobin results from the routine diagnostics were anonymized and used for this purpose. The blood was applied to the Capitainer and Mitra devices in vitro, and after drying, the microsamples underwent the respective assays and the results were compared with the routine measurements. We did not perform similar in vitro investigations for tacrolimus since its calibrators were prepared as dried microsamples based on whole blood material.

2.4 | Outcome assessments

The relative difference between the finger-prick microsample result and the corresponding liquid venous (routine method) result was the primary outcome variable. For tacrolimus, at least two-thirds of the results should be within ±20% according to the European Medicines Agency (EMA) guideline for bioanalytical method validation.[11] For this purpose, we assessed tacrolimus at t_0, t_1 and t_3 as well as predicted AUC results. We also assessed how the self-sampled microsamples at t_0 covaried with the venous samples regarding hitting within/outside the therapeutic ranges as defined at our hospital (standard 4–7 μg/L; immunological intermediate and high risk initially 10–12 μg/L, then 6–10 μg/L and eventually 5–8 μg/L in the maintenance phase). In order to control for differences in sampling times, we evaluated time-matched results obtained by pharmacokinetic model simulations. In an exploratory data analysis, tacrolimus was analysed in some microsamples not passing the visual inspection and compared with the parallel liquid venous sample. For haemoglobin and creatinine, the acceptance criteria, 80% within ±15%, was preset according to internal discretionary assessments of the clinical need related to self-sampling during routine follow-up. Surplus microsamples obtained at t_1 or t_3 were used for haemoglobin and creatinine measurements in cases of unsuccessful t_0 microsamples. We evaluated the sampling quality by visual inspection of the received microsamples, and the proportion of failed samples according to sampling situation was calculated. Additionally, we compared the rate of inadequate sampling between self-sampling and microsampling by healthcare personnel as well as between the two types of volumetric microsampling devices.

2.5 | Calculations and statistics

Tacrolimus $AUC_{0\text{-}12h}$ was predicted with a Bayesian forecasting model including the concentration measurements at t_0, t_1 and t_3.[3] The same model was used to simulate tacrolimus concentrations in liquid blood at the same time points as the corresponding microsamples were obtained, i.e. adjustment for the time difference between the sampling methods. Difference plots were constructed to assess the agreement between results obtained with finger-prick microsamples and liquid venous samples, using actual sampling times as the main analysis and estimated time-matched concentrations as sensitivity analyses. Proportions of relative differences within the acceptance limits are presented. We compared proportions with the McNemar's test for paired data, considering statistical significance to be $P < .05$. Potential covariations between analyte relative differences (finger-prick *vs.* venous) were explored in a post hoc Spearman correlation analysis stratified by Capitainer® and Mitra®. The results are presented as mean ± standard deviation (SD) if not otherwise specified.

2.6 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in https://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22.

3 | RESULTS

3.1 | Preliminary in vitro study

Linear regression between creatinine measured by the microsample assay (EDTA blood) and by the routine assay (plasma) gave the following results: $y = 0.75x + 5.0$ with $R^2 = 0.994$ for Capitainer ($n = 26$), and $y = 0.91x + 4.4$ with $R^2 = 0.991$ for Mitra ($n = 25$, one outlier excluded), where y is the microsample measurement and x is the routine method measurement. Overall, the difference was −12.9% ± 13.2% for Capitainer and 2.1% ± 13.5% for Mitra. For both devices, there was an increasing positive bias as the creatinine concentration decreased. The equations $x_{estimated} = (y - 5.0)/0.75$ and $x_{estimated} = (y - 4.4)/0.91$ were implemented as creatinine correction algorithms for Capitainer and Mitra, respectively.

Linear regression between haemoglobin measured by the microsample assay (EDTA blood) and by the routine assay (EDTA blood) gave the following results: $y = 0.84x + 0.90$ with $R^2 = 0.958$ for Capitainer ($n = 30$), and $y = 0.90x + 1.10$ with $R^2 = 0.953$ for Mitra ($n = 30$). The difference was −7.4 ± 5.0% for Capitainer and 0.7 ± 5.4% for Mitra. There was no apparent concentration-dependent bias. We implemented the corrections factors 1.080 and 0.993 for Capitainer and Mitra, respectively.

3.2 | Patient characteristics

In the clinical study, we included 25 kidney transplant recipients: median age 51 (range 21–74) years, male/female 17/8, living donor/deceased donor 9/16. The first three patients included were stable and transplanted 3, 20 and 21 years earlier. These patients were included to allow the study programme to be 'run in'. Twenty-two newly transplanted patients were then consecutively asked for participation when they reached steady-state creatinine post-engraftment. No patient declined to participate. The time since transplantation was median 3 (range 2–7) weeks ($n = 22$). All patients were on triple immunosuppression, i.e. original tacrolimus twice daily (except three patients on original tacrolimus prolonged-release once daily), original

mycophenolate mofetil twice daily, and prednisolone once daily. Four patients were familiar with self-monitoring of glucose concentrations, 17 were on acetylsalicylic acid, and one patient was on low molecular weight heparin. We collected data from January to August 2022.

3.3 | Study outcome

The time differences achieved, between finger-prick and venous sampling, were considered equivalent for the two microsampling tools; 88%–98% of the sample pairs were within the predefined time limits for tacrolimus assessments (Table 1).

For Capitainer, the rate of successful samplings assessed in the laboratory was 92%–96% regardless of whether the samples collected by healthcare personnel or by self-sampling. There was a trend towards a higher sampling success rate when Mitra microsamples were collected by healthcare personnel compared to self-sampling (72%–88% *vs.* 52%–72%; not statistically significant difference). The frequency of inadequate sampling was significantly lower with Capitainer than Mitra in the self-sampling situation (Table 2).

When analysing tacrolimus concentrations in technically valid samples, the agreement between finger-prick and venous samples was acceptable for both Capitainer and Mitra, regardless of whether the samples had been taken by healthcare personnel or by the patients themselves. The proportions within ±20% difference ranged from 77% to 96% (Table 3 and Figure 2). We did not observe significant differences between sampling situations nor device types. The agreement between capillary and venous results was comparable for observed finger-prick results *vs.* simulated time-matched venous blood concentrations, although the relative differences tended to be lower when

TABLE 1 Time difference between finger-prick and venous blood sampling.

	Capitainer qDBS			Mitra VAMS		
	t_0	t_1	t_3	t_0	t_1	t_3
Median (10th and 90th percentiles), minutes	8 (3–29)	3 (1–7)	4 (2–7)	8 (3–48)	4 (2–7)	4 (2–8)
Within ±10 min, %	–	98	90	–	94	90
Within ±30 min, %	91	–	–	88	–	–

Note: Capitainer and Mitra finger-prick microsamples were collected simultaneously from kidney transplant recipients ($n = 25$) during three different sampling situations on separate days: sampling by healthcare personnel, self-sampling under supervision and self-sampling alone. Venous blood samples were taken in parallel, but not post-dose in connection with 'self-sampling alone'. Time differences from the three situations are pooled. Pre-dose (t_0), 1 and 3 h after tacrolimus dose (t_1 and t_3).

TABLE 2 Technical quality of microsampling per two-sampler device.

	Capitainer qDBS card (2 × 10-µL filters)			Mitra VAMS cartridge (2 × 10-µL tips)		
	Healthcare personnel	Self-sampling (supervision)	Self-sampling (alone)	Healthcare personnel	Self-sampling (supervision)	Self-sampling (alone)
Pre-dose						
1 inadequate, %	4.0	12	4.0	12	16	24
2 inadequate, %	4.0	0.0	0.0	8.0	4.0	16
1 or 2 inadequate, %	8.0	12	4.0*	20	20	40*
1-h post-dose						
1 inadequate, %	8.0	8.0	4.0	28	16	32
2 inadequate, %	0.0	0.0	4.0	0.0	4.0	16
1 or 2 inadequate, %	8.0	8.0	8.0*	28	20	48*
3-h post-dose						
1 inadequate, %	4.0	16	0.0	8.0	12	20
2 inadequate, %	0.0	0.0	4.0	4.0	0.0	8.0
1 or 2 inadequate, %	4.0	16	4.0[a]	12	12	28[a]

Note: Capitainer and Mitra finger-prick microsamples were collected simultaneously from kidney transplant recipients ($n = 25$) during three different sampling situations on separate days. Technical quality per two-sampler device was registered in the laboratory. Failure categories of Capitainer qDBS microsamples: underfilled (user-related) and incomplete blood transfer to filter spot (device-related). Failure categories of Mitra microsamples: underfilled, overfilled, and transfer of blood from tip to inside of cartridge (all user-related). Two-tailed McNemar test; Mitra vs. Capitainer self-sampling alone.
*$P < .05$, and [a]$P = .07$.

normalized for sample collection time (Table S1). We retrospectively assessed whether results based on the self-collected microsamples at t_0 would not have led to other clinical actions. In this context, the agreement was 100% for Capitainer ($n = 19$) and 100% for Mitra ($n = 15$) compared with venous samples hitting within/outside the therapeutic range. Dose increase of tacrolimus would have been considered in one of the patients based on the venous sample (3.8 μg/L) as well as the finger-prick microsamples (Capitainer 3.2 μg/L, Mitra 3.3 μg/L). Predictions of AUC based on three finger-prick samples (t_0, t_1 and t_3) showed good agreement with corresponding predictions based on liquid venous samples; mean difference range −2.4%–0.5%, and 85%–96% of sample sets within ±20% difference (Table 3). We verified the predictive performance of the Bayesian forecasting model by comparing predicted *vs.* observed tacrolimus concentrations in the

TABLE 3 Tacrolimus in finger-prick microsamples versus venous blood samples.

	Capitainer qDBS			Mitra VAMS		
	Healthcare personnel	Self-sampling (supervision)	Self-sampling (alone)	Healthcare personnel	Self-sampling (supervision)	Self-sampling (alone)
Pre-dose						
Valid samples, *n*	24	24	19	23	23	15
Tacrolimus, mean ± SD, (range), μg/L	6.9 ± 2.2 (4.2–12.1)	6.6 ± 1.5 (4.2–9.6)	6.7 ± 1.7 (3.2–9.7)	6.9 ± 2.3 (3.7–12.8)	6.9 ± 1.9 (4.0–10.2)	6.9 ± 1.5 (3.3–9.0)
Difference *vs.* venous, mean ± SD, (95% CI), %	−2.6 ± 10.1 (−6.9–1.6)	−4.3 ± 12.2 (−9.5–0.9)	−5.9 ± 10.3 (−10.8–−0.9)	−3.7 ± 14.1 (−9.8–2.4)	−0.4 ± 15.2 (−6.9–6.2)	−1.5 ± 12.8 (−8.6–5.6)
Proportion within ± 20% difference, %	96	88	95	78	87	87
1-h post-dose						
Valid samples, *n*	23	24	–	22	23	–
Tacrolimus, mean ± SD, (range), μg/L	18.9 ± 10.1 (5.5–50.8)	19.7 ± 9.6 (7.5–44.7)	–	19.4 ± 8.6 (8.2–40.6)	19.0 ± 8.3 (7.8–36.5)	v
Difference *vs.* venous, mean ± SD, (95% CI), %	2.2 ± 13.0 (−3.5–7.8)	7.4 ± 17.7 (−0.1–14.8)	–	8.5 ± 16.8 (1.0–15.9)	3.5 ± 11.5 (−1.4–8.5)	–
Proportion within ±20% difference, %	87	79	–	77	91	–
3-h post-dose						
Valid samples, *n*	21	22	–	21	22	–
Tacrolimus, mean ± SD, (range), μg/L	16.3 ± 5.6 (7.8–27.0)	16.1 ± 5.7 (8.1–29.4)	–	16.4 ± 5.0 (6.9–26.6)	16.5 ± 5.0 (8.5–25.2)	–
Difference *vs.* venous, mean ± SD, (95% CI), %	−5.4 ± 10.3 (−10.1–−0.7)	−4.2 ± 10.1 (−8.7–0.2)	–	−3.2 ± 14.5 (−9.9–3.4)	0.1 ± 10.2 (−4.4–4.6)	–
Proportion within ±20% difference, %	95	91	–	81	91	–
Estimated AUC						
Tacrolimus $AUC_{0\text{-}12h}$, mean ± SD, (range), μg × h/L	131 ± 37 (78–231) *n* = 19	138 ± 43 (75–233) *n* = 21	–	138 ± 34 (71–224) *n* = 18	140 ± 37 (75–201) *n* = 20	–
Tacrolimus $AUC_{0\text{-}24h}$, mean ± SD, (range), μg × h/L	331 ± 8 (325–337) *n* = 2	255 ± 24 (232–280) *n* = 3		334 ± 6 (329–338) *n* = 2	282 ± 33 (259–305) *n* = 2	
Difference *vs.* venous, mean ± SD, (95% CI), %	−2.4 ± 10.5 (−7.2–2.4) *n* = 21	−2.0 ± 9.9 (−6.2–2.2) *n* = 24	–	−1.4 ± 12.3 (−7.1–4.4) *n* = 20	0.5 ± 8.7 (−3.4–4.3) *n* = 22	–
Proportion within ±20% difference, %	90	96	–	85	95	–

Note: Capitainer and Mitra finger-prick microsamples were collected from kidney transplant recipients ($n = 25$) during three different sampling situations on separate days. Venous blood samples were taken in parallel, but not in connection with post-dose 'self-sampling alone'. Samples of insufficient technical quality, and samples outside the predefined time differences (±30 min pre-dose and ±10 min post-dose), were excluded. The area under the blood concentration *vs.* time curve (AUC) was estimated with a Bayesian forecasting model using the concentration measurements at pre-dose, and 1 and 3 h post-dose (also including samples outside the predefined time differences). $AUC_{0\text{-}24h}$ was estimated for three patients on once-daily tacrolimus and $AUC_{0\text{-}12h}$ was estimated for the rest on twice-daily dosing.

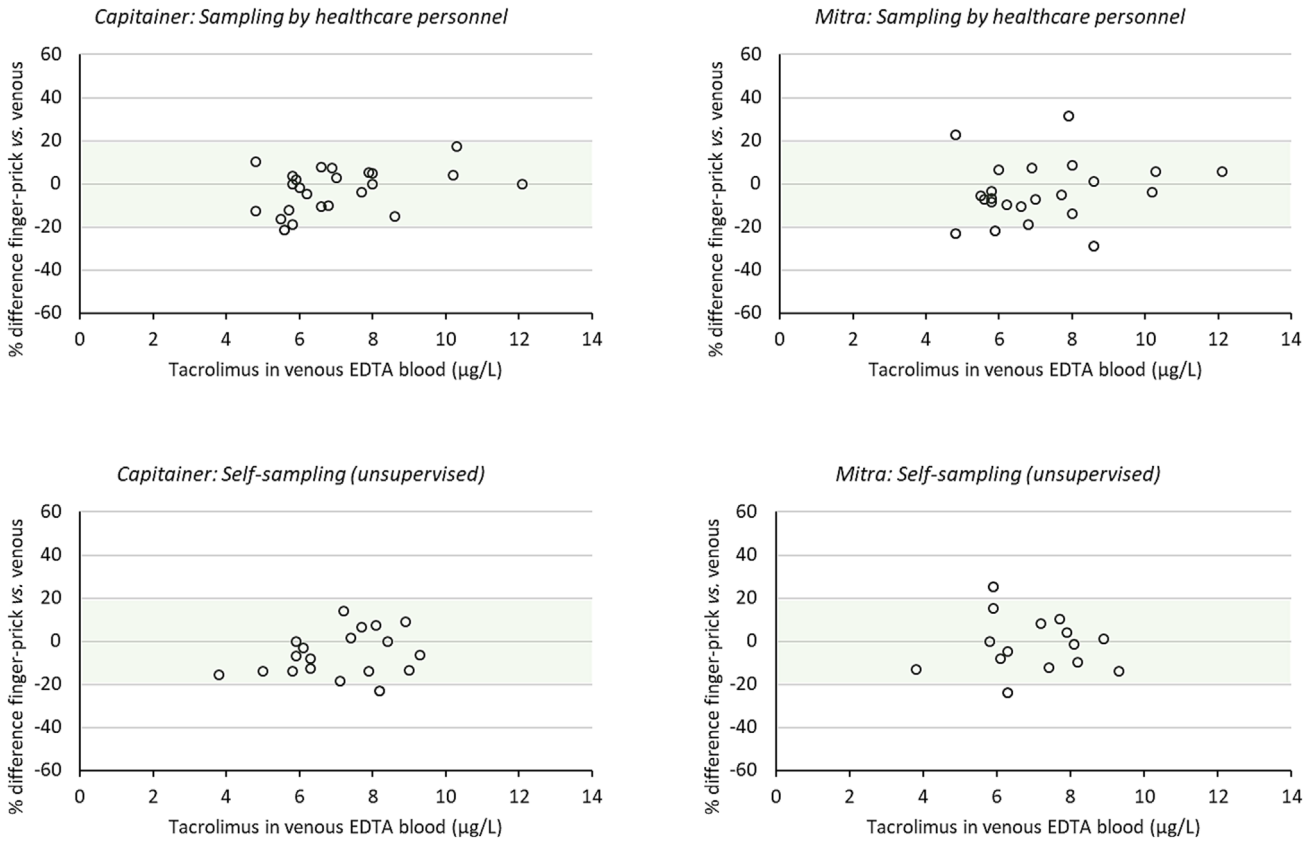

FIGURE 2 Difference plots for tacrolimus measured pre-dose in finger-prick *vs.* venous samples. Capitainer qDBS and Mitra VAMS microsamples were collected from kidney transplant recipients ($n = 25$); first by healthcare personnel and then by the patient themselves after training (separate sampling days). Venous blood samples were collected in parallel. Samples with inadequate quality, and samples outside the predefined time differences (±30 min), were excluded.

venous samples (mean difference range −0.8%–1.0%, Table S2). An ad hoc analysis of eight Mitra samples that were visually assessed to be overfilled resulted in a significant positive deviation from measurements in venous samples; 19.4% ± 13.2% (95% CI 8.4%–30.4%).

The mean difference between creatinine levels estimated from Capitainer measurements and routine method measurements in plasma was 11%–13%. The corresponding difference was 2.8%–4.6% for Mitra. Furthermore, the proportions within ±15% difference were 68%–76% for Capitainer and 79%–92% for Mitra (Table 4). Due to the suboptimal agreement when using the in vitro-derived correction algorithms, we adjusted the algorithms to achieve a median bias of zero in the clinical data set. Then $x_{estimated} = (y - 5.0)/0.83$ was used for Capitainer and $x_{estimated} = (y - 4.4)/0.93$ was used for Mitra. With these adjusted algorithms for creatinine, the proportions within ±15% were 92%–100% for Capitainer and 79%–96% for Mitra (Table 4 and Figure 3).

The mean difference between haemoglobin levels estimated from Capitainer measurements and routine method measurements was −5.7% to −4.6%. The corresponding difference was −11% to −7.3% for Mitra. Proportions within ±15% difference were 92%–100% for Capitainer and 58%–88% for Mitra (Table 5). Due to the suboptimal agreement when using the in vitro-derived correction factors, we adjusted the factors to achieve a median bias of zero in the clinical data set. Then 1.134 was used for Capitainer and 1.102 was used for Mitra. With adjusted correction factors for haemoglobin, the proportions within ±15% were 100% for Capitainer and 67%–92% for Mitra (Table 5 and Figure 4).

The relative differences between finger-prick and venous samples, stratified by sampler device, tended towards a positive correlation when pairs of analytes were explored (Spearman rho 0.16–0.61; statistically significant only for creatinine *vs.* haemoglobin in Mitra samples, $P = .0014$).

3.4 | Validation study in a separate group

We consecutively included 12 kidney transplant recipients: median age 51 (range 25–74) years, male/female 7/5, living donor/deceased donor 7/5. The time since transplantation was median 3 (range 2–5) weeks. All participants were on tacrolimus, mycophenolate mofetil and prednisolone. None had previous experience with dried blood samples or measurement of blood glucose. We collected data from October to December 2022.

For tacrolimus, six sample-pairs were excluded due to exceeding the time limit ±30 min and one sample was excluded because the dose had been taken before sampling, i.e. 41 samples from the four

TABLE 4 Creatinine in finger-prick microsamples *vs.* venous blood plasma.

	Capitainer qDBS			Mitra VAMS		
Valid samples, *n*	**Healthcare personnel 25**	**Self-sampling (supervision) 25**	**Self-sampling (alone) 25**	**Healthcare personnel 24[a]**	**Self-sampling (supervision) 25**	**Self-sampling (alone) 24[a]**
Correction A	$x_{estimated} = (y - 5.0)/0.75$			$x_{estimated} = (y - 4.4)/0.91$		
Creatinine, mean ± SD, (range), μmol/L	136 ± 31 (89–222)	136 ± 31 (93–238)	133 ± 26 (84–200)	126 ± 28 (83–197)	126 ± 31 (80–218)	124 ± 33 (73–222)
Difference *vs.* venous, mean ± SD, (95% CI), %	11.6 ± 5.5 (9.4–13.9)	12.7 ± 8.1 (9.4–16.0)	11.0 ± 6.8 (8.2–13.8)	2.9 ± 7.4 (−0.2–6.0)	4.6 ± 11.0 (0.1–9.1)	2.8 ± 12.5 (−2.5–8.1)
Proportion within ±15% difference, %	68	68	76	92	80	79
Correction B	$x_{estimated} = (y - 5.0)/0.83$			$x_{estimated} = (y - 4.4)/0.93$		
Creatinine, mean ± SD, (range), μmol/L	122 ± 28 (80–200)	122 ± 28 (84–214)	120 ± 23 (76–180)	123 ± 28 (81–193)	124 ± 30 (78–214)	122 ± 32 (72–218)
Difference *vs.* venous, mean ± SD, (95% CI), %	0.6 ± 4.9 (−1.5–2.6)	1.4 ± 7.2 (−1.6–4.4)	0.0 ± 6.0 (−2.4–2.5)	0.8 ± 7.3 (−2.2–3.9)	2.5 ± 10.8 (−2.0–7.0)	0.8 ± 12.2 (−4.4–6.0)
Proportion within ±15% difference, %	100	92	100	96	84	79

Note: Capitainer and Mitra finger-prick microsamples were collected from kidney transplant recipients ($n = 25$) during three different sampling situations on separate days. Venous blood samples were taken in parallel and analysed by the routine method. The correction A algorithms were derived from an in vitro study with EDTA blood. Correction B was derived by adjustment of A to achieve median bias equal to zero.

[a]One microsample result excluded due to lack of acceptable sampling quality.

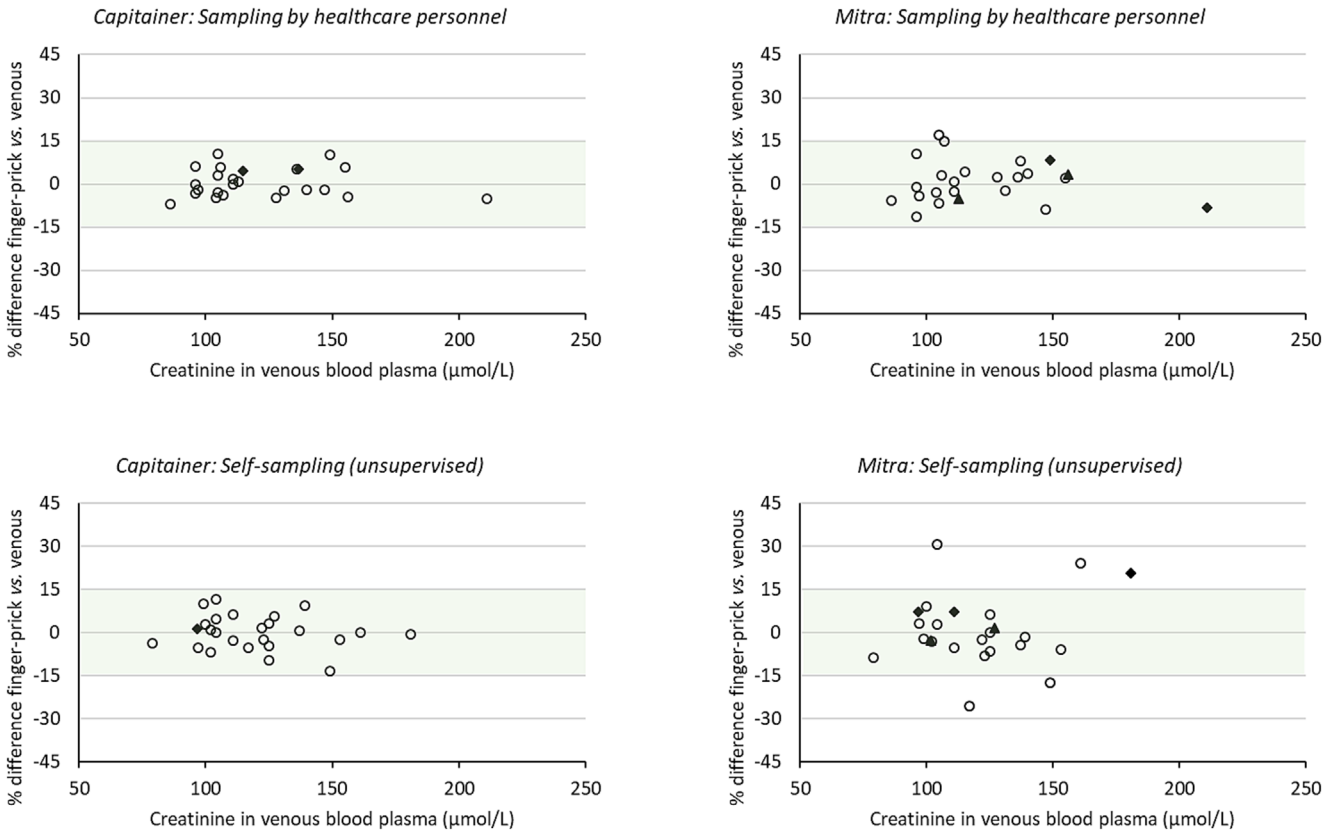

FIGURE 3 Difference plots for creatinine plasma levels estimated from finger-prick measurements *vs.* routine method measurements in plasma. Correction algorithms were applied to achieve median bias equal to zero. Capitainer qDBS and Mitra VAMS microsamples were collected from kidney transplant recipients ($n = 25$); first by healthcare personnel and then by the patient themselves after training (separate sampling days). Microsamples and parallel venous samples were generally collected immediately before the tacrolimus dose in the morning. In cases with no available microsamples of acceptable quality, we used samples taken 1 h after (filled diamonds) or 3 h after (filled triangles) the tacrolimus dose. Excluded due to inadequate sample quality: one Mitra sampled by healthcare personnel and one self-sampled Mitra.

TABLE 5 Hemoglobin in finger-prick microsamples *vs.* venous blood samples.

	Capitainer qDBS			Mitra VAMS		
Valid samples, *n*	**Healthcare personnel 25**	**Self-sampling (supervision) 25**	**Self-sampling (alone) 25**	**Healthcare personnel 24**	**Self-sampling (supervision) 25**	**Self-sampling (alone) 24**
Correction A	Factor 1.080			Factor 0.993		
Hemoglobin, mean ± SD, (range), g/dL	11.2 ± 1.2 (8.4–13.1)	11.2 ± 1.4 (9.3–14.7)	11.4 ± 1.2 (9.4–14.2)	11.0 ± 1.5 (7.7–14.6)	10.9 ± 1.6 (7.3–13.5)	10.7 ± 2.0 (7.5–14.8)
Difference *vs.* venous, mean ± SD, (95% CI), %	−5.2 ± 4.6 (−7.0–−3.3)	−5.7 ± 5.7 (−8.0–−3.3)	−4.6 ± 5.0 (−6.7–−2.6)	−7.3 ± 7.8 (−10.6–−4.0)	−8.0 to 12.0 (−13.0–−3.0)	−10.8 ± 12.4 (−16.1–−5.6)
Proportion within ±15% difference, %	92	92	100	88	64	58
Correction B	Factor 1.134			Factor 1.102		
Hemoglobin, mean ± SD, (range), g/dL	11.7 ± 1.3 (8.8–13.8)	11.8 ± 1.4 (9.8–15.4)	12.0 ± 1.3 (9.9–14.9)	12.2 ± 1.7 (8.5–16.2)	12.1 ± 1.8 (8.1–15.0)	11.9 ± 2.2 (8.3–16.4)
Difference *vs.* venous, mean ± SD, (95% CI), %	−0.4 ± 4.8 (−2.4–1.6)	−0.9 ± 6.0 (−3.4–1.6)	0.1 ± 5.2 (−2.0–2.2)	2.9 ± 8.8 (−0.8–6.6)	2.2 ± 13.4 (−3.3–7.7)	−1.0 ± 13.8 (−6.8–4.8)
Proportion within ±15% difference, %	100	100	100	92	72	67

Note: Capitainer and Mitra finger-prick microsamples were collected from kidney transplant recipients ($n = 25$) during three different sampling situations on separate days. Venous blood samples were taken in parallel and analysed by the routine method. The correction A factors were derived from an in vitro study with EDTA blood. Correction B factors were derived by adjustment of A to achieve median bias equal to zero.
[a]One microsample result excluded due to lack of acceptable sampling quality.

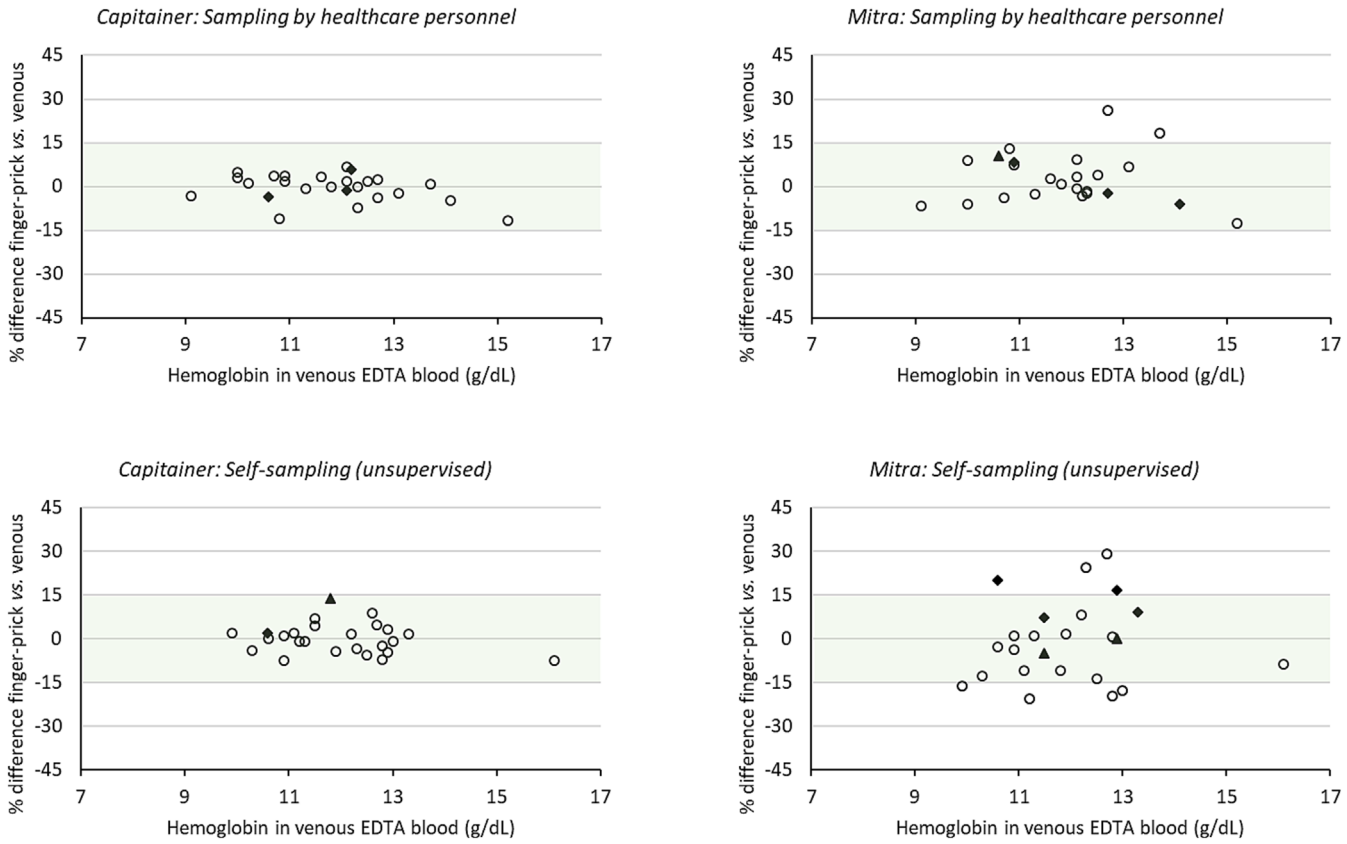

FIGURE 4 Difference plots for haemoglobin levels estimated from finger-prick measurements *vs.* routine method measurements. Correction factors were applied to achieve median bias equal to zero. Capitainer qDBS and Mitra VAMS microsamples were collected from kidney transplant recipients ($n = 25$); first by healthcare personnel and then by the patient themselves after training (separate sampling days). Microsamples and parallel venous samples were generally collected immediately before the tacrolimus dose in the morning. In cases with no available microsamples of acceptable quality, we used samples taken 1 h after (filled diamonds) or 3 h after (filled triangles) the tacrolimus dose. Excluded due to inadequate sample quality: one Mitra sampled by healthcare personnel and one self-sampled Mitra.

study days were eligible for the data analysis. We used the correction factor 1.051 which was derived from the preceding study. The time difference between valid finger-prick samples and corresponding venous samples was median 14 (8–27) min. Mean difference for Capitainer *vs*. venous samples was −5.4% ± 12% (minimum −32%, maximum +21%) with 90% of results within ±20%.

For creatinine and haemoglobin, we excluded four samples (two with incomplete transfer of blood to the filter paper, one with too little applied blood, and one due to technical error during sample preparation), i.e. 44 samples from the four study days were eligible for the data analysis. We used the correction $x_{estimated} = (y - 5.0)/0.83$ for creatinine and correction factor 1.134 for haemoglobin, derived from the preceding study. Mean difference for Capitainer *vs*. venous samples: creatinine −3.0% ± 7.8% (minimum −36%, maximum +12%) with 93% of results within ±15% and haemoglobin +2.6% ± 6.9% (minimum −13%, maximum +17%) with 93% of results within ±15%.

4 | DISCUSSION

In this study, we demonstrated the performance of Capitainer® qDBS and Mitra® VAMS® for the monitoring of tacrolimus, creatinine and haemoglobin in kidney transplant recipients. We found that tacrolimus trough concentration and predicted AUC can be reliably monitored with both sampling devices, provided that the microsamples are technically qualified upon arrival in the laboratory. Creatinine and haemoglobin could be reliably monitored when both types of microsamples were collected by trained healthcare professionals, but only Capitainer showed the same reliability with self-collected samples, verified during external validation in a separate group of kidney transplant recipients. Despite apparently adequate sample quality, a proportion of the creatinine and haemoglobin measurements had too large total error when analysed in self-collected Mitra samples. The quality of the dried blood samples was consistent for Capitainer in the situations where healthcare personnel performed the sampling and where the patients collected the samples themselves. Overall, we experienced a lower rate of sampling success in the Mitra self-sampling situation when compared to Capitainer.

With respect to self-sampling at home, adequate patient training and instruction is important. Our study results indicate that there should be a special focus on correct sampling technique and patient training with regard to Mitra. The challenge of incorrect Mitra samples has also been highlighted in a previous study, where trained healthcare personnel collected finger-prick samples from kidney transplant recipients and 32% of the samples were registered with inadequate quality.[12] Our ad hoc analysis of overfilled Mitra samples showed that it gave a significantly elevated tacrolimus result, which underlines the importance of visual inspection in the laboratory. Overfilled samples can be detected by a smoother film with a darker colour on the surface of the tip. We cannot rule out that the patient training was too sparse in the present study, although we did our best to succeed by engaging healthcare personnel with long-term experience in finger-prick sampling. Due to small sub-group numbers, we did not assess whether self-monitoring of glucose or the use of anticoagulants influenced the finger-prick performance. With Capitainer, the sample quality is usually considered to be acceptable if the filter spot is coloured red with blood. In a couple of cases, nevertheless, we observed that some of the blood remained in the capillary and that the filter spot was only partially filled. With Mitra, experienced laboratory personnel made a visual assessment of the technical sample quality. Although the results for creatinine and haemoglobin are based on what appeared to be valid samples, we observed somewhat large deviations in certain Mitra samples that were taken by the patients themselves. It could be that these samples did not have sufficient technical quality, but at the same time we were not able to detect this through visual inspection. Since we did not observe the same with Mitra samples taken by healthcare personnel, it may be that some of the patients obtained a deviant amount or composition of blood in the Mitra tip (e.g. by squeezing out blood or by applying blood multiple times to the same tip). If the total amount of blood was still correct, it may also be that our extraction method was unable to ensure reproducibility with varying microsampling techniques. A previous study also reported more variability with Mitra compared to another volumetric microsampling device (i.e. HemaXis, DBS System SA, Gland, Switzerland).[5] The positive correlation between the relative differences (finger-prick *vs*. venous) for creatinine and haemoglobin in Mitra samples indicated that either blood volume or recovery could be the cause of the variability rather than the subsequent quantifications performed with separate analytical principles.

The included cohort was smaller than what is recommended in guidelines for validation of dried blood spot-based methods for therapeutic drug monitoring when there is only a single sample per patient.[13] In the present study, multiple sampling time points per participant were utilized and we therefore consider that the overall study size was sufficient for the purpose. We chose to assess the comparative data using difference plots with fixed percentage acceptance limits rather than Bland–Altman plots with limits of agreement based on standard deviations of the differences. Thus, the drawn limits represent the predefined acceptance criteria in the study. Since we considered the routine method measurement in venous samples as the 'gold standard', we assessed the relative differences against those measurements and not against a method mean. The distribution widths of the analyte concentrations were limited, and we did not include Deming regression or Passing-Bablok regression to assess potential proportional differences. However, the difference plots did not indicate any concentration-dependent biases.

The exact timing of blood sampling is of crucial importance for pharmacokinetic monitoring, especially when samples are taken shortly after an oral dose where the concentration is changing rapidly. Our comparative results could potentially be confounded by the time differences between finger-prick and venous sampling. With regard to this, we performed a sensitivity analysis based on the simulation of time-matched tacrolimus concentrations. This analysis indicated that the actual time differences between the sample types had a modest effect on the results, and we can assume that the observed

differences in concentration between finger-prick and venous samples were mainly due to measurement conditions.

In the early post-transplant phase, kidney transplant recipients are closely monitored, often several times per week. If self-sampling could replace a proportion of these attendances, the overall implementation would be easier for the patient and the hospital could save resources. In a further perspective, self-sampling also has the potential to supplement and replace some of the follow-up consultations after discharge and it may pave the way for more sophisticated TDM by multiple sample collections during the dose interval and AUC-targeted dose individualization.[3] The present study demonstrates that Capitainer and Mitra finger-prick samples are feasible for this purpose as there was good agreement with AUC estimates based on the liquid venous blood samples.

During the Covid-19 pandemic, regular out-patient surveillance check-ups were complicated. Due to fear of being infected during the journey or in the hospital, many kidney transplant recipients either postponed their appointment or just decided they would not come. Some health care providers also told the patients not to come or did not have the capacity to conduct out-patient consultations. Introduction of self-sampling at home for tacrolimus, creatinine and haemoglobin creates a possibility to monitor some key elements in the transplant recipient without the fear of being infected. Implementation of this type of activity in clinical practice requires a carefully thought-out process to ensure feasibility.[14]

In conclusion, dried blood samples in the form of Capitainer® qDBS and Mitra® are technically feasible for measuring tacrolimus, creatinine and haemoglobin. In the real-life clinical setting, Capitainer showed consistent sampling success and analytical quality when the patients performed the sampling themselves. Monitoring of tacrolimus, creatinine and haemoglobin in volumetric finger-prick samples has the potential to simplify and improve the follow-up of kidney transplant recipients.

AUTHOR CONTRIBUTIONS

All authors contributed to the design of the study. Nils Tore Vethe, Anders Mikal Andersen, Ragnhild Heier Skauby and Stein Bergan contributed to the establishment of bioanalytical methods. Nils Tore Vethe, Karsten Midtvedt and Anders Åsberg contributed to the acquisition of data. Karsten Midtvedt was responsible for recruitment and follow-up of participants. Nils Tore Vethe and Anders Åsberg performed the data analyses. Nils Tore Vethe drafted the manuscript. All authors contributed to the interpretation of data, reviewing and revising the manuscript, and they approved the final version.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data may be available 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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