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Research Article

Use of volumetric absorptive microsampling and parallel reaction monitoring mass spectrometry for tacrolimus blood trough measurements at home in pediatric heart transplant patients

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ABSTRACT

Background: Measurement of trough levels for calcineurin inhibitors by venipuncture sampling is a mainstay of patient management in solid organ transplant recipients but challenging in pediatric patients. Volumetric Absorptive Microsampling (VAMS) is a patient-friendly, minimally invasive sampling technique to accurately collect blood. An assay for measurement of tacrolimus in blood using VAMS, coupled with parallel reaction monitoring (PRM) mass spectrometry, was validated in pediatric heart transplant patients.

Methods: Tacrolimus was measured by a newly developed high-resolution PRM assay and compared with low-resolution tandem mass spectrometry (MRM). Dried blood samples were collected from pediatric heart transplant patients (n = 35) using VAMS devices and a satisfaction survey was completed by patients/guardians. Tacrolimus concentrations were compared across whole liquid blood, dried blood spots, and capillary blood, and shipping stability determined.

Results: The PRM assay was linear over a range 1–50 ng/mL, similar to MRM but had greater specificity due to reduced background noise. No significant differences in tacrolimus concentrations were observed between VAMS and venous blood. Tacrolimus dried on VAM tips was stable for 14 days and concentrations were unaffected by postal shipping. The variability in two simultaneously collected at-home patient samples was minimal – average concentration difference was 0.12 ± 0.94 ng/mL ($p = 0.6$) between paired samples.

Conclusion: A high resolution PRM mass spectrometry assay was developed for home-based dried blood collections for therapeutic monitoring of tacrolimus. The advantage of PRM was enhanced specificity and the VAMS devices provided a simple and convenient approach to blood sampling at home in pediatric heart transplant patients.

Introduction

Measurement of trough levels for calcineurin inhibitors is a mainstay of patient management in many solid organ transplant recipients. Achieving and maintaining an appropriate level is key in preventing the sequelae of excessively elevated values (infection, renal injury, cancer risk, neurological symptoms) and the risk of low values (organ rejection). To achieve this level of control, frequent blood samples from venipunctures are the gold standard. Dried blood spots collected on paper allows for smaller sample size collection, and collection outside of a

Abbreviations: AGC, automatic gain control; CAP/CLIA, college of American pathologist/clinical laboratory improvement amendments; CI, confidence interval; CV, coefficient of variation; EDTA, ethylenediaminetetra-acetic acid; HCD, high-energy collision dissociation; HESI, heated electrospray ionization; HRMS, high-resolution, accurate mass; HT, hematocrit; IA, immunoassay; IS, internal standard; LC-MS, liquid chromatography-mass spectrometry; MRM, multiple reaction monitoring; MS^2, tandem mass spectrometry; NCE, normalized collision energy; PCC, Pearson's correlation coefficient; PRM, parallel reaction monitoring; QC, quality control; SD, standard deviation; TAC, tacrolimus; UHPLC, ultra-high-performance liquid chromatography; VAMS, volumetric absorptive microsampling.

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traditional clinical setting.

However, there is potential for sampling error, and accuracy is influenced by hematocrit (HT) [1–3]. More recently, improved methods such as volumetric absorptive microsampling (VAMS) have been introduced that improve the reliability of sample collection [4,5]. Use of VAMS is of particular value in pediatric heart transplant patients both for the ease of obtaining a finger or heel stick rather than venipuncture in smaller and often more anxious patients, while permitting home collections from patients living remote to a transplant center or clinic [6].

Parallel reaction monitoring (PRM) is a novel, targeted quantification approach performed in a high-resolution, accurate mass (HRMS) orbitrap mass spectrometer [7,8]. Applications of PRM in various biological studies have shown it to be powerful for quantitative proteomics [8,9]. The popularity of PRM can be attributed to the simple and straightforward data acquisition method, and high selectivity and specificity because full MS/MS spectra of each target ion is acquired using high resolution and mass accuracy. Continuous improvement in scan rate and resolving power in mass spectrometers has extended the capability of PRM to efficiently discriminate the targeted compounds from interfering background matrices, thereby yielding more reliable and accurate quantification without compromising the sensitivity and selectivity of the assay. It can be expected that this approach could supplant the low-resolution targeted methods most frequently used in clinical assays.

In this study we explored and validated the combined use of the VAMS sampling device with a highly specific and accurate assay using PRM mass spectrometry and compared this with low resolution tandem mass spectrometry for home-based measurement of tacrolimus in a pediatric heart transplant population. Furthermore, we surveyed patients as to their response to this approach to monitoring of their tacrolimus levels.

Materials & methods

Study design

Thirty-five pediatric (<18-years-old) post-heart transplant patients were enrolled in the study and asked to participate in a home-based sample collection. The protocol was approved by the Institutional Review Board at Cincinnati Children's Hospital Medical Center (protocol number: 2019–0943), and informed-consent and assent (if applicable) were obtained.

To evaluate the performance of the VAMS devices three separate studies were performed. In the first validation phase, following patient consent, whole blood was collected by venipuncture into tubes containing ethylenediaminetetra-acetic acid (EDTA) anticoagulant. Tacrolimus concentrations measured in these samples were compared with the concentrations from the same blood that was spotted onto VAMS devices and immediately analyzed. Furthermore, to determine the validity of using mailed-in samples, the same venous blood was spotted onto VAMS devices that were then mailed to the laboratory. These samples were obtained and mailed in over a period from August – March when there was a wide range in weather temperatures. A total of 25 pediatric post-heart transplant patients presented to the clinic for blood work-up and duplicate samples of whole blood were obtained on 20 μL VAMS devices (The Mitra® Cartridge by Neoteryx) by gently touching the VAMS tip to the surface of the blood. The blood samples were left to dry for 4 h, sealed in a specimen bag containing a desiccant, and stored at ambient temperature. One VAMS sample was immediately delivered to the lab for analysis, and the other was mailed to the lab approximately 24 h later to test for tacrolimus stability and to account for time that overnight shipping may take in a "real-world" setting.

In the second validation phase, whole blood concentrations of tacrolimus were measured in venous blood collected from 10 pediatric post-heart transplant patients who came to the clinic and compared with capillary blood collected by finger-prick at the same time. Finally, the assay was applied in a 'real world' setting to monitor blood tacrolimus levels by home sampling of 24 patients and to evaluate the patient/guardian response to the process from a written survey. A kit provided by Neoteryx (The Mitra® Collection Kit), a prepaid FedEx envelope, and patient/parent survey were given to the participants at the time of enrolment. A trained research professional spoke with the families, provided an informational video and gave basic instructions on the collection technique and shipping of samples. Trough capillary blood samples were collected by finger-prick 30 mins prior to the morning dose of tacrolimus and mailed to the lab on the same day. The survey was filled out after using the VAMS device for sample collections.

Assays for blood tacrolimus concentrations

1. Chemicals and reagents

Tacrolimus certified solution standard and the stable isotope-labeled internal standard (IS), [$^{13}C_1$,$^{2}H_2$] tacrolimus (isotopic purity, 97.7 %) were purchased from Cerilliant (Round Rock, TX). LC-MS grade water, methanol, acetonitrile, formic acid (optima grade), ammonium acetate (optima LC-MS grade) and zinc sulfate (ACS grade) were all obtained from Fisher Scientific. VAMS devices (Mitra™, 20 μL) were purchased from Neoteryx (Torrance, CA, USA). Deidentified human whole blood samples containing ethylenediaminetetra-acetic acid (EDTA) as the anticoagulant were obtained from in-house patients not undergoing tacrolimus therapy and pooled for assay validation and quality control (QC).

2. Preparation of calibrators and quality control samples

Stock solutions of tacrolimus and IS were prepared in methanol/water (1:1, v/v) at a concentration of 50 μg/mL, and stored frozen at −80 ± 5 °C. A working solution of IS was prepared in acetonitrile at a concentration of 0.5 ng/mL. Calibration standards were prepared in EDTA whole blood by spiking tacrolimus at concentrations of 0, 1, 2.5, 5, 10, 20, 40 and 50 ng/mL. QC samples were prepared in whole blood at concentrations of 2 ng/mL (low limit of quantification, LLOQ), 5 ng/mL (low QC), 15 ng/mL (medium QC), and 20 ng/ mL (high QC). Calibration standards and QC samples were freshly prepared prior to sampling with the VAMS device.

3. Sample preparation and extraction of tacrolimus

Blood samples were loaded onto VAMS tips by capillary action by gently touching the surface of the blood, ensuring that the tip was not fully submerged. The tip was gently removed from the blood when completely red in color, typically after 2–3 s. The tips were dried for a minimum of 3 h under airflow. The VAMS tip (20 μL) was broken-off from the plastic holder and transferred into 1.5 mL microcentrifuge tube. Solutions of 40 % MeOH/60 % H_2O (200 μL) and 20 μL 0.4 M $ZnSO_4$ were added to each tube, and the sample sonicated for 30 min. Acetonitrile (400 μL) containing 0.5 ng/mL IS was added. The tubes were vortexed for 1 min and centrifuged at 21,100*g*, for 10 min at 4 °C. Finally, 150 μL of supernatant was transferred to autosampler vials and 20 μL injected on column for MS analysis. The patient samples and calibrators were treated identically.

Whole blood tacrolimus concentration was determined using a routine validated lab developed tandem mass spectrometry clinical assay (Supplemental Materials).

4. Chromatographic conditions

Tacrolimus was isolated by chromatography on a UPLC BEH 1.7 μm C18 column (2.1 × 50 mm) maintained at 55 °C with gradient elution. Mobile phase A (MPA) consisted of 100 % water with 2 mM ammonium

acetate and 0.1 % formic acid. Mobile phase B (MPB) consisted of 100 % methanol with 2 mM ammonium acetate and 0.1 % formic acid. The gradient elution was from 50 % MPB to 100 % MPB after initial and final hold periods of 2 min and an equilibration of 1 min between injections. The flow rate was 0.45 mL/min, and the total analysis time was 5 min per sample.

5. Liquid chromatography mass spectrometry analysis of tacrolimus by parallel reaction monitoring (PRM) mass spectrometry

PRM analysis was performed on a Q Exactive Plus Orbitrap mass spectrometer equipped with heated electrospray ionization (HESI-II) probe (ThermoFisher Scientific). A Thermo Scientific Vanquish UHPLC was used for the chromatographic introduction of the sample and isolation of tacrolimus. The optimized ionization spray voltage was set to 3 kV, sheath gas flow rate was set to 40, and auxiliary gas flow rate to 12 (both in arbitrary units). The method functioned in PRM, using an inclusion list containing the exact mass of the parent ions and the retention time windows. Resolution was set to 70,000 for the high-energy collision dissociation (HCD) fragmentation performed using a normalized collision energy (NCE) at 15, with automatic gain control (AGC) target of $1e^5$ and a maximum ion injection time (IT) of 100 ms. Data were acquired and processed with Xcalibur™ 4.3 (Thermo Scientific). During data processing, the full MS^2 spectrum was used for the confident identification of tacrolimus. The transition m/z 821.5158 → 576.3165 was monitored for the quantification of tacrolimus, and the corresponding transition m/z 824.5288 → 579.3325 was used for IS. Peak areas of fragment ions were extracted using a 10 ppm mass window and integrated across the elution profile.

6. Routine chemiluminescence assay for tacrolimus

Peripheral venous EDTA blood collected for therapeutic drug monitoring (TDM) of trough levels of tacrolimus were measured by a chemiluminescent microparticle immunoassay (IA) (Architect Tacrolimus IL77 kit, Abbott Laboratories).

Assay validation

The method validation was conducted in accordance with guidelines for bioanalytical method validation by the Food and Drug Administration [10] (FDA, May 2018) and followed CAP/CLIA guidelines as indicated in Supplemental Table S1.

Statistical analysis

Passing-Bablok regression analysis [11] was used to investigate system bias between the sampling methods. Bland-Altman plots [12] were employed to further assess agreement among the developed methods. Pearson's correlation coefficient (PCC) was computed to determine the linear correlation between results from dried capillary blood collected by finger-prick on VAMS tips and those from whole blood venipuncture. In addition, we compared the tacrolimus concentrations in venous blood measured by mass spectrometry with an immunoassay.

For samples collected at home, all data analyses were performed in SAS 9.4 (SAS Institute, Carry, NC). Values were summarized as frequency and percentage for categorical variables and mean, standard deviation, median, and inter-quartile range for continuous variables. The distribution of the tacrolimus difference between the two fingerstick samples for each subject was visually inspected for approximate normality. Bland-Altman limit of agreement (difference ± 1.96SD) and its 95 % confidence limits were calculated.

Results

Method development and validation

Consistent with several previous reports [13–21], efficient extraction of tacrolimus from VAMS tips was achieved using a solvent mixture of methanol/water. Overall the optimum results in terms of extraction efficiency and reproducibility were obtained by first sonicating the VAMS tip in a solution of H_2O:MeOH (60:40, v/v) with $ZnSO_4$ (0.4 M) for 30 min, followed by protein precipitation with acetonitrile containing the stable isotope-labeled IS. As summarized in Supplemental Table S1, the assay met all pre-specified acceptance criteria in accordance with analytical guidelines. Recovery, matrix effect and process efficiency were calculated with analyte/IS response ratios at both QC low and QC high levels using 5 different blank matrices (hematocrit range from 20 to 50 %). With this approach, quantitative extraction of tacrolimus was achieved with the extraction recovery 89.1 ± 14.5 % (Supplemental Table S3). Furthermore, tacrolimus was stable on the VAMS tip stored in a sealed bag with desiccant for up to 14 days at room temperature (Supplemental Table S4).

The linearity, LLOQ and imprecision of the assay using low resolution MRM instrumentation were compared with the newly developed high resolution, accurate mass, PRM method (Table 1). Both instrument approaches gave similar linearity over the dynamic range of 1–50 ng/mL and likewise, the lower limit of quantification achieved was comparable (Supplemental Table S2). In this regard, the two instruments and approaches, i.e., PRM vs MRM, performed similarly for quantification of tacrolimus. However, the PRM method offered greater specificity and selectivity because monitoring of the product ions with high resolution eliminated matrix interferences. This is shown in Fig. 1, which includes a typical mass chromatogram of a sample of human blood collected on the VAMS device before and after spiking with tacrolimus at a concentration of 2 ng/mL. In this example, the S/N ratio for the quantifier ion transition m/z 821.3 → 576.1 on the Waters TQ XS was 135, while S/N ratio was essentially infinite when the same sample was run on the Thermo QE plus mass spectrometer. The imprecision and accuracy using the two approaches were determined by comparing the same extracted samples under PRM and MRM conditions and these data are summarized in Table 1. For both MRM and PRM methods, the % bias and % CV at each QC level were all within ±10 % indicating that PRM high-resolution mass spectrometry quantifies tacrolimus concentration with similar performance to low resolution MRM on a triple quadrupole instrument. Comparison of PRM with MRM was further evaluated by measuring tacrolimus concentrations from the patient samples. PRM showed an excellent correlation (R^2 = 0.986, n = 53) with the lower resolution MRM method (Fig. 2).

Clinical validation

A total of 35 pediatric heart transplant patients fulfilled the clinical protocol requirements for enrolment in the study. The demographics of the patients participating in each study are summarized in Table 2. Two patients dropped out, leaving 33 venipuncture samples available for validation. Overall, a total of 20 matched samples were used for the comparison study of tacrolimus concentrations collected on the VAMS tips delivered immediately to the laboratory with those collected, dried and mailed approximately 24 h after collection. For the comparison of capillary and venous blood levels, 10 patients were enrolled but in two patients the samples were unusable due to incorrect collection on the VAMS tips. Thus 8 matched patient samples qualified to compare fingerstick capillary blood collected on VAMS devices with venous whole blood. Finally, the tacrolimus concentrations in venous blood samples (n = 33) measured by mass spectrometry were compared to the values obtained by a routine clinical immunoassay (IA). All measured tacrolimus concentrations were within the analytically validated range as measured by high resolution PRM assay. The Passing-Bablok regression

Table 1
Comparison of inter- and intra-run imprecision of tacrolimus in whole dried blood collected on VAMS devices for QC samples measured by high-resolution PRM and low-resolution MRM mass spectrometry.

PRM on Thermo QE plus								
Nominal added Tac concentration (ng/mL)	Inter-run (n = 24)				Intra-run (n = 6)			
	Mean(ng/mL)	SD	Precision(%CV)	Accuracy (%bias)	Mean (ng/mL)	SD	Precision (%CV)	Accuracy (%bias)
LLOQ, 2.0	1.96	0.17	8.61	−1.85	1.83	0.09	4.95	−8.28
QCLOW, 5.0	4.87	0.48	9.82	−2.54	4.63	0.36	7.75	−7.48
QCMED, 15.0	14.90	1.10	7.39	−0.68	14.82	0.66	4.44	−1.17
QCHIGH, 20.0	19.93	1.83	9.16	−0.36	19.14	0.99	5.18	−4.29
MRM on Waters TQ-XS								
Nominal added Tac concentration (ng/mL)	Inter-run (n = 24)				Intra-run (n = 6)			
	Mean(ng/ml)	SD	Precision(%CV)	Accuracy (%bias)	Mean (ng/mL)	SD	Precision (%CV)	Accuracy (%bias)
LLOQ, 2.0	2.06	0.22	10.55	2.96	1.89	0.14	7.18	−5.75
QCLOW, 5.0	4.94	0.40	8.14	−1.21	4.64	0.34	7.40	−7.27
QCMED, 15.0	15.14	1.11	7.32	0.95	14.21	0.66	4.66	−5.24
QCHIGH, 20.0	19.75	1.92	9.73	−1.25	18.82	0.77	4.11	−5.91

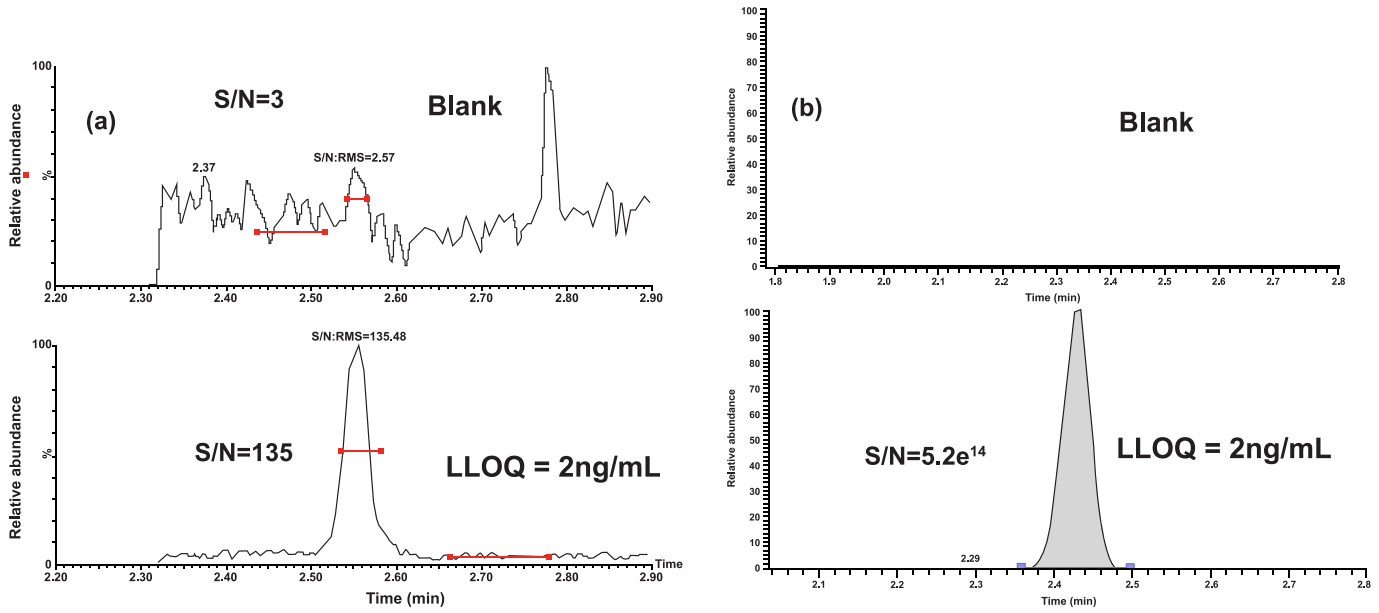


Fig. 1. Typical mass chromatograms of comparing human blood collected on VAMS devices and the same blood that was spiked with tacrolimus at the LOQ. The mass transitions monitored were *m/z* 821.3 → 576.1 on Waters TQ XS (a) and *m/z* 821.5158 → 576.3162 on Thermo QE plus (b). Peak areas of fragment ions were extracted using 10 ppm mass window on Thermo QE plus.

and a Bland-Altman plot for the methods comparison are summarized in Fig. 3. For the methods comparison, i.e. VAMS samples delivered immediately to the lab gave comparable tacrolimus values to samples that were mailed-in; and similarly, venous blood sample concentrations of tacrolimus collected on a VAMS device were not significantly different from those of whole liquid blood (Passing-Bablok fit was y = 1.1x − 0.41 (95 % CI slope 0.80–1.32; 95 % CI intercept −1.37 to 0.57), vs y = 1.05x − 0.06 (95 % CI slope 0.89–1.16; 95 % CI intercept −0.78 to 0.58, respectively). Pearson's correlation coefficients were 0.92 and 0.97, respectively, revealing a good agreement between the two methods. The Bland-Altman plot showed that the mean differences were 0.12 ng/mL (95 % CI −2.03 to 2.27) and −0.24 ng/mL (95 % CI −1.83 to 1.35). Our results also confirmed that the tacrolimus concentrations were stable and not influenced by the time taken in postal shipment. In comparing the measured tacrolimus concentrations in venous blood by PRM mass spectrometry with the immunoassay the Passing-Bablok regression showed differences between the two approaches, i.e., y = 0.89x – 0.14 (95 % CI slope 0.82–0.94; 95 % CI intercept −0.55 to 0.28) (Fig. 3). The Bland-Altman plot also showed a noticeable mean difference between the two methods was 1.15 ng/mL (95 % CI −0.54 to 2.84). LC-MS/MS method gave consistently lower concentrations than the immunoassay method (Fig. 3). This comparison with the routine IA method was done in response to requests from clinicians to understand whether there were differences between the two analytical assays. The IA method has historically been the routine assay that clinicians use to monitor trough levels of tacrolimus and the comparison was considered important to demonstrate that the clinical values generated by IA yield higher concentrations than the specific mass spectrometric assay. This is because IA assays measure not only tacrolimus but also metabolites of the drug and suffer from lack of specificity that can also be due to matrix effects. Our findings indeed confirmed this is the case and these data are helpful to the clinicians in providing an indication of how 'off' the IA values could be for the parent drug.

Pearson's correlation coefficient (PCC) was computed to determine the linear correlation between results from dried capillary blood collected by finger-prick on VAMS tips and whole blood simultaneously collected by venipuncture (Fig. 3). Pearson's correlation coefficient was 0.95 (95 % CI 0.73–0.99, P < 0.001) indicating a good correlation

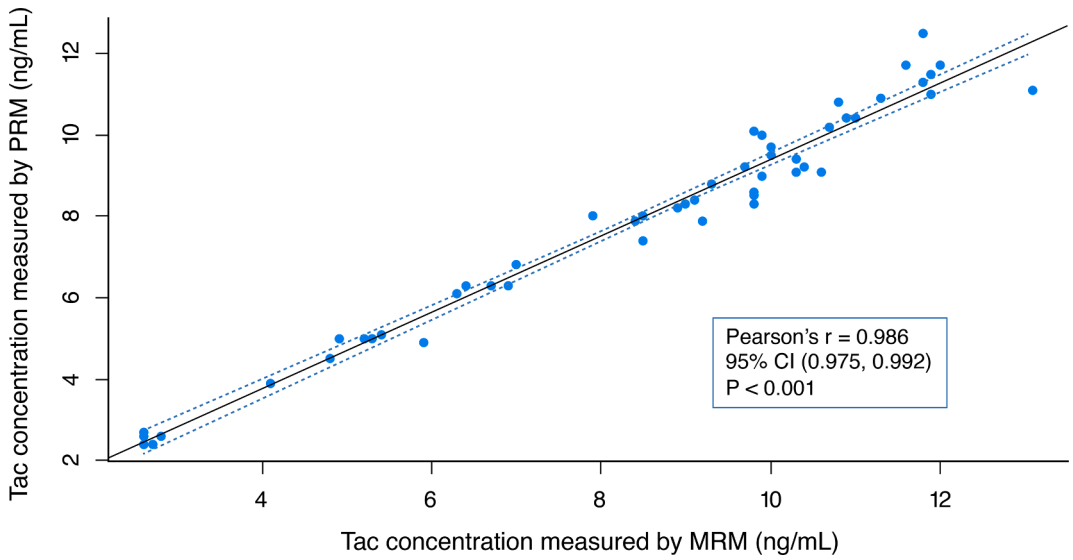


Fig. 2. Method comparison for blood tacrolimus concentrations in pediatric heart transplant patients measured using low resolution tandem mass spectrometry (MRM) and high resolution (PRM) instrumentation (n = 53).

Table 2
Patient demographics and lab values for the assay development and validation cohorts, and the subgroup used for testing the assay using home sampling.

Variable	Assay Development Cohort (N = 24)	Assay validation Cohort (N = 10)	Home Assay Subgroup (N = 18)
Age	9.66 (4.39, 13.8)	8.58 (1.00, 17.08)	11.6 (4.64, 15.9)
Sex			
Male	11 (45.8 %)	7 (70 %)	8 (44.4 %)
Female	13 (54.2 %)	3 (30 %)	10 (55.6 %)
Race			
White	23 (95.8 %)	8 (80 %)	17 (94.4 %)
Unknown/not checked	1 (4.2 %)	2 (20 %)	1 (5.6 %)
Ethnicity			
Not Hispanic or Latino	24 (100 %)	10 (100 %)	18 (100.0 %)
Hematocrit (%)	39.5 (36.4, 41.7)	37.74 (27, 45.1)	39.5 (36.9, 42.6)
Hemoglobin (g/dL)	12.9 (12.1, 13.9)	12.25 (8.7, 15.1)	12.7 (12.2, 13.9)
BUN (mg/dL)	12 (7, 16)*	15.1 (7, 23)	11 (6.5, 15.5)**
Creatinine (mg/dL)	0.4 (0.3, 0.5)*	0.44 (0.2, 1.0)	0.45 (0.3, 0.6)**

*Data available for 22 patients at time of VAMS sample collection.
**Data available for 16 patients at time of VAMS sample collection.

between the methods.

Our study revealed no significant differences in measured tacrolimus concentrations between VAMS and venous blood collection techniques, consistent with previous published findings [13–21]. Overall, we show that for pediatric heart transplant recipients undergoing TDM of their immunosuppressant levels, the use of a dried blood spot approach with a VAMS device is an ideal method for home blood sampling because tacrolimus was stable for up to 14 days (Supplemental Table S4) when dried on VAMS tips and not influenced postal shipment of the samples.

Home assay and parent/patient survey

Twenty-one patients of 24 initially enrolled in the home study returned samples using the home collection kit. Two patients failed to complete the accompanying paperwork to enable accurate identification of the samples, and one patient shipped back only one VAMS device and not duplicate samples as was indicated in the protocol. These patients' samples (n = 3) were not included in the data analysis. The final number of data points (Table 3 and Supplemental Fig. S1) was obtained from 18 patients' blood samples measured from duplicate VAMS devices.

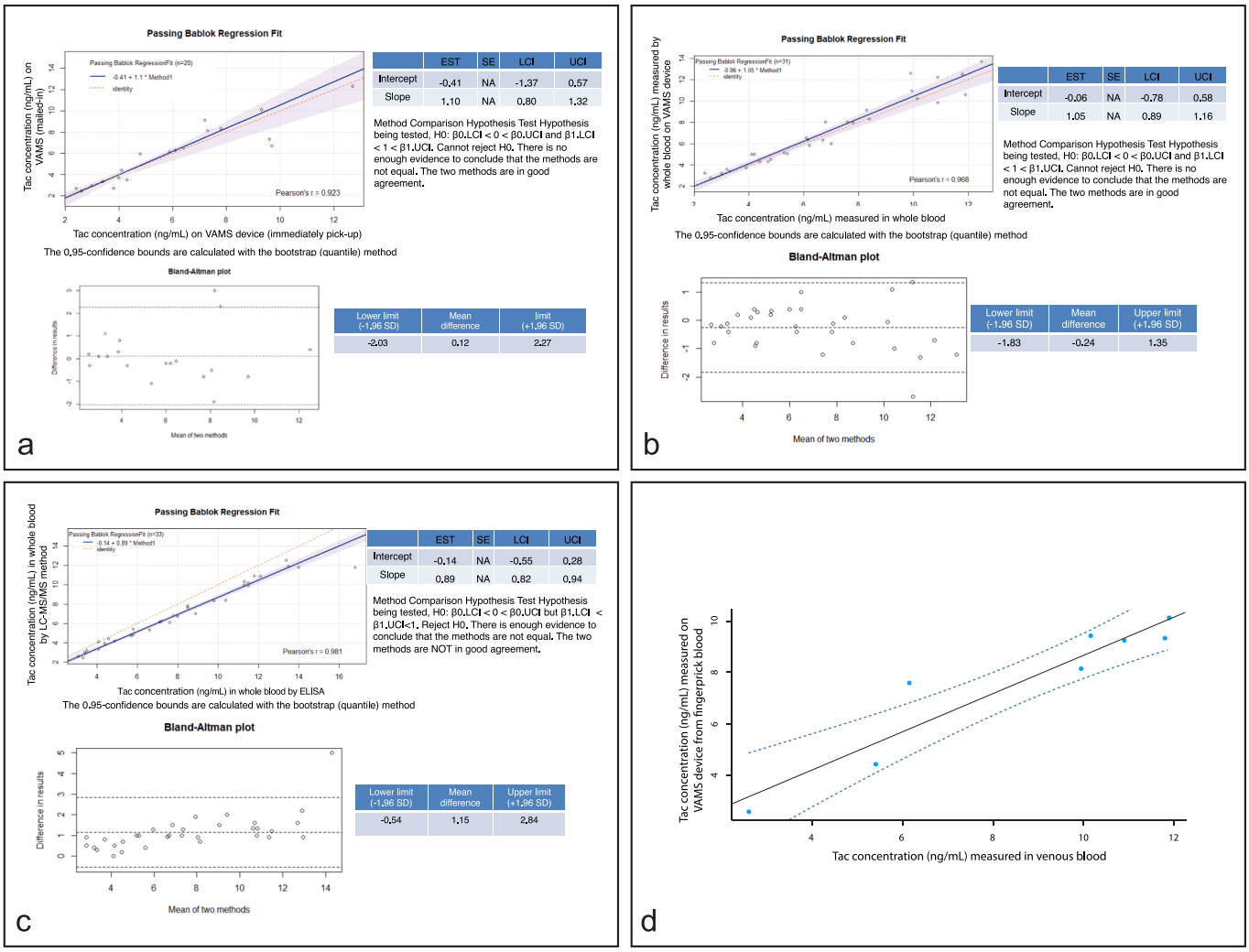
The mean time for a sample to reach the hospital laboratory after home collection was 50 h (range 24–175 h; Table 3). Each patient provided two samples from a single finger-stick and the average difference in tacrolimus concentrations was 0.12 ng/mL (95 %CI: −0.32–0.55) between the pairs. Bland-Alman limit of agreement (LOA) was (−1.7–2.0) (Supplemental Fig. S1). Results of the survey questionnaire completed at home by the patient/parent(s) indicated that the VAMS sample collection was "very easy" or "somewhat easy," and all would use this method for remote collection if necessary (Table 3).

Discussion

We describe a validated home-based assay for measurement of tacrolimus in pediatric heart transplant patients using VAMS devices for microsampling coupled with high resolution PRM mass spectrometry for analysis. A head-to-head comparison of low-resolution tandem mass spectrometry and high-resolution accurate mass spectrometry found blood tacrolimus concentrations to be similar, however the advantage of high-resolution approach was enhanced specificity and accuracy due to elimination of non-specific interferences and metabolites and from the microsampling devices, which can compromise immunoassay- based assays [22,23].

The patients and families involved in the study found the commercially available collection device easy to use, and the trough levels of tacrolimus in two simultaneously collected samples was similar and within the therapeutic range (6–15 ng/mL) for pediatric heart transplant patients. The main problem encountered with dry blood spot microsampling using paper collection is that variations in hematocrit can influence the concentration measured but this problem is eliminated using VAMS devices. Consequently, in this study we did not compare tacrolimus levels for dried blood spots collected on paper (DBS) with that on VAMS devices. Tacrolimus concentration for blood collected on VAMS devices has been previously reported to be independent of hematocrit levels for concentrations in the therapeutic range, or for samples within the hematocrit range of 20–60 % [13–21]. Most patient samples are within this range and for this reason the effect of hematocrit was not systemically examined.

The benefits of a reliable home collection in pediatric solid organ



	EST	SE	LCI	UCI
Intercept	-0.41	NA	-1.37	0.57
Slope	1.10	NA	0.80	1.32

Method Comparison Hypothesis Test Hypothesis being tested, H0: β0.LCI < 0 < β0.UCI and β1.LCI < 1 < β1.UCI. Cannot reject H0. There is no enough evidence to conclude that the methods are not equal. The two methods are in good agreement.

Lower limit (-1.96 SD)	Mean difference	limit (+1.96 SD)
-2.03	0.12	2.27

	EST	SE	LCI	UCI
Intercept	-0.06	NA	-0.78	0.58
Slope	1.05	NA	0.89	1.16

Method Comparison Hypothesis Test Hypothesis being tested, H0: β0.LCI < 0 < β0.UCI and β1.LCI < 1 < β1.UCI. Cannot reject H0. There is no enough evidence to conclude that the methods are not equal. The two methods are in good agreement.

Lower limit (-1.96 SD)	Mean difference	Upper limit (+1.96 SD)
-1.83	-0.24	1.35

	EST	SE	LCI	UCI
Intercept	-0.14	NA	-0.55	0.28
Slope	0.89	NA	0.82	0.94

Method Comparison Hypothesis Test Hypothesis being tested, H0: β0.LCI < 0 < β0.UCI but β1.LCI < β1.UCI<1. Reject H0. There is enough evidence to conclude that the methods are not equal. The two methods are NOT in good agreement.

Lower limit (-1.96 SD)	Mean difference	Upper limit (+1.96 SD)
-0.54	1.15	2.84

Fig. 3. Method comparison between: (a) VAMS samples immediately delivered to lab and a duplicate sample that was dried for 24 h and then mailed to the lab (n = 20); (b) tacrolimus concentrations measured in whole blood on VAMS device compared with whole liquid blood (n = 31); (c) LC-MS/MS measurement of tacrolimus in pediatric heart transplant patients compared with the routine clinical assay using ELISA (n = 33); and (d) capillary blood obtained by finger-prick and collected as a dried blood sample using the VAMS device with that of samples of whole blood collected by venipuncture from the same patients (n = 8).

transplantation are several. Firstly, venipuncture can be challenging in children who may be small, unable to cooperate, or have a history of multiple lab draws contributing to difficulties in obtaining access. A simple finger-stick done by a family member is easier to tolerate, and is a technique used frequently in other applications, like blood glucose monitoring. Secondly, the volume needed for VAMS collection can be as little as 10 μL, while that needed for venipuncture is usually 50–100 times greater. In a neonate or infant in need of daily monitoring of levels early after transplant, limiting blood volume waste is of utmost importance. Thirdly, pediatric transplant centers often serve a large geographical region, meaning patients may live several hours away from laboratories capable of providing immunosuppression blood levels. In our study, the average time to receive samples in lab was about two days and analysis was accomplished in a matter of hours. This is a vast improvement over the timeframe of a week that is often the case in clinical practice and it allows monitoring from home. Finally, a home-based assay allows for social distancing and limited exposure to a healthcare environment for patients that are immunosuppressed, decreasing the risk of acquiring transmissible infections.

The future use of VAMS for collection of blood samples in pediatric solid organ patients offers significant room for expansion. A single sample collection has the potential to allow analysis of several elements at once, including multiple immunosuppression medications. This technology has also been used to collect multiple samples within a single day without the need for indwelling lines or repeated venipuncture, thereby allowing for pharmacokinetic and pharmacodynamic studies to improve dosing [24]. Given the stability of the samples over several days or more after collection, these devices could also prove useful in carrying out biomarker studies collected remotely or over several days. The only drawback of the VAMS device, which is also similar for dried paper blood spot collection, is the small failure rate of sample collection. However, provided careful instructions are given to the patients/guardians on how to collect blood on VAMS this will minimize collection failures.

Conclusion

In summary, we have demonstrated that the use of a dried blood spot approach with a VAMS device is an ideal mode of blood sampling at home for pediatric heart transplant recipients undergoing therapeutic drug monitoring of their immunosuppressant levels. This is because the tacrolimus concentration is not influenced by delays in postal shipment of samples. This study is also the first to evaluate a high resolution PRM liquid chromatography-mass spectrometry approach, comparing it with low resolution tandem mass spectrometry and using VAMS sampling devices for quantitative application in a clinical laboratory for

Table 3
Shipping information and patient/parent survey results.

Variable	N = 18
Shipment time (hours)*	
Median (min–max)	50 (24–175)
Median (Q1, Q3)	50 (29.7, 75.5)
Patient/Parent Survey Questions	
1. How would you describe the process of collecting a drug level sample?	
Very easy	14 (77.8 %)
Somewhat easy	1 (5.6 %)
No opinion	0 (0 %)
Somewhat difficult	0 (0 %)
Very difficult	0 (0 %)
I could not collect a sample	0 (0 %)
Did not answer/survey not returned	3 (16.7 %)
2. If you could use this method to collect some of your child's required drug levels, would you?	
Yes	15 (83.3 %)
No	0 (0 %)
Did not answer/survey not returned	3 (16.7 %)

*Onesample was not appropriately marked to allow calculation of shipment time.

tacrolimus drug monitoring.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics statement

This work was approved by the Institutional Review Board at Cincinnati Children's Hospital Medical Center (protocol number: 2019-0943), and informed consent, and assent if applicable, were obtained.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmsacl.2023.11.004.

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