Clinical validation of a liquid chromatography-tandem mass spectrometry method for the quantification of calcineurin and mTOR inhibitors in dried matrix on paper discs

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ABSTRACT

Introduction: Advances in liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) have enabled the quantification of immunosuppressants using microsampling techniques. In this context, dried matrix on paper discs (DMPD) could be a useful alternative to conventional venipuncture. Although analytical validation is necessary to establish the suitability of method performance, it is not sufficient to proceed with its implementation into routine clinical practice. Also necessary is that equivalence between sampling methods be demonstrated in a clinical validation study.

Objectives: To clinically validate a LC-MS/MS method for the quantification of tacrolimus, sirolimus, everolimus and cyclosporin A using DMPD.

Methods: According to the recommendations of international guidelines, at least 40 whole blood (WB) and DMPD paired samples for each analyte were collected by skilled technicians and analyzed using LC-MS/MS. Results were evaluated in terms of statistical agreement and bias values at medical decision points.

Results: For all analytes, Passing-Bablok regression analysis revealed that confidence intervals (CIs) for slopes and intercepts included 1 and 0, respectively. It also showed that biases at medical decision points were not clinically relevant. No statistically significant differences between DMPD and WB were found using difference plots and agreement analysis. In this regard, CIs for bias estimators included 0, and more than 95% of the results fell within the limits of agreement.

Conclusion: The feasibility of the clinical application of simultaneous quantification of tacrolimus, sirolimus, everolimus and cyclosporin A in DMPD was demonstrated. Results showed that this microsampling technique is interchangeable with conventional WB sampling when specimens are collected by trained personnel.

Keywords: Dried matrix on paper discs (DMPD) Immunosuppressants Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) Method comparison Clinical validation

Introduction

In the field of solid organ transplantation, calcineurin (tacrolimus and cyclosporin A) and mTOR (sirolimus and everolimus) inhibitors are two of the most widely prescribed groups of immunosuppressive agents. These drugs are long-life administrated to attenuate the recipient central role in the prevention and treatment of acute and chronic allograft rejection [1].

Due to their high between-subject pharmacokinetic variability and narrow therapeutic windows, therapeutic monitoring of immunosuppressants is highly recommended. The measurement of these drugs in biological samples is an essential tool to individualize dose regimens, obtain the best clinical outcome and avoid adverse effects [2,3].

One of the most distinctive characteristics of immunosuppressive drugs is their extensive binding to erythrocytes. Tacrolimus, sirolimus, everolimus and cyclosporin A are mainly distributed via red blood cells (75 %, 94.5 %, 75 % and 58 %, respectively) [4]. For this reason, whole blood (WB) is the gold standard matrix for patient monitoring. In this regard, pre-dose trough concentration (C0) is most generally used to adjust dosage in transplant patients. In addition, therapeutic ranges for these drugs are generally based on C0 values [5–10]. Nevertheless, either

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2-hour post-dose concentration (C₂) for cyclosporin A or abbreviated area under the concentration time curve (AUC) obtained by limited sampling strategy (LSS) is better in predicting response compared to C₀ [2,11–13]. However, these approaches still face financial and logistical issues that make them difficult to implement in clinical practice [14].

Regardless of the number of samples and their time of collection, venipuncture is the standard procedure to obtain WB specimens. This is an invasive technique that must be performed by skilled technicians in a clinical setting. In addition, analytes are usually poorly stable in this matrix at room temperature. Therefore, samples should be rapidly delivered to the clinical laboratory to prevent degradation, which is generally a time-consuming and costly process [15].

Advances in liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) have enabled the quantification of immunosuppressive drugs in small sample volumes with adequate selectivity and sensitivity [16]. In this context, microsampling techniques have increasingly emerged as useful alternatives to venipuncture. These strategies refer to procedures for collecting small volumes of blood (usually <100 µL) in a non-invasive manner. Microsampling procedures allow home sampling by minimally trained persons (patients themselves or caregivers). Besides, most analytes exhibit adequate stability in microsampling devices versus frozen samples, which enables their shipment to the laboratory via standard post [15,17]. It should be noted that while this action may be feasible in temperate climates with adequate mail services, in regions where the daily temperature rises above 40 °C, the temperature in the mailbox may reach values over 60 °C [18]; for that reason, temperature conditions need to be properly validated.

In the field of therapeutic monitoring of immunosuppressants, dried blood spots (DBS) is the most used microsampling technique [19]. This method of sample collection consists of applying a drop of capillary blood onto a sampling paper. After drying and transportation, a sample disc is punched and analytes are extracted and analyzed. Nevertheless, DBS shows limitations that strongly affect the applicability of the analytical results. These include the volume of blood spotted, the chromatographic effect (homogeneity), and the hematocrit (Ht). The latter is undoubtedly the most widely discussed DBS-related problem [19–23].

The viscosity of blood is directly proportional to the Ht, which affects the flux and diffusion properties of the matrix on the paper. Moreover, a linear inverse relationship between DBS area and Ht has been well-established. Hence, partial punches taken from DBS prepared from blood with different Ht values will contain different amounts of blood and analytes [20,24]. Considering that calcineurin and mTOR inhibitors are extensively bound to erythrocytes, this phenomenon is highly significant.

One approach to avoid the Ht effect is the volumetric absorptive microsampling method (VAMS). This is a fixed volume sampling strategy used to obtain dried blood specimens via a porous hydrophilic tip attached to a plastic handle. Compared with DBS, VAMS allows accurate volume collection without being influenced by the Ht [25]. Although this procedure has shown promising results, only a few studies have been performed to assess its feasibility as sampling strategy in therapeutic monitoring of immunosuppressive drugs [26–30].

The analysis of whole-cut DBS discs is a simple and practical way to overcome the Ht problem, because it guarantees the same physical blood spot size at each Ht level [31]. In this approach an accurate and precise volumetric application of the blood onto the filter paper is mandatory. This can be accomplished by using capillary tubes or micro-collection pipettes. Thus, the influence the spot volume and the chromatographic effects can be assumed as negligible [20].

When pre-cut paper discs are placed in a special support prior to the volumetric application of the matrix, the method is called “dried matrix on paper discs” (DMPD). This microsampling technique has been successfully applied for the quantification of nevirapine and acyclovir in human samples and seems to be promising for therapeutic monitoring of calcineurin and mTOR inhibitors [31,32]. However, an analytical method validation step is not sufficient to assure the quality of the results and implement the procedure as part of routine clinical practice. Therefore, a clinical validation study is necessary to demonstrate the interchangeability between the results obtained from DMPD and WB [33].

In a clinical validation study, paired DMPD and venous blood samples are obtained and analyzed. Then, the analytical results are compared and statistically evaluated to demonstrate the interchangeability between methodologies [33].

In a previous work, we fully validated a simple LC-MS/MS method for the simultaneous quantification of tacrolimus, sirolimus, everolimus and cyclosporin A in DMPD. We also showed that the Ht had no clinical impact on the analytical results [34]. The aim of this study is to clinically validate our method and assess its potential use for routine analysis.

**Materials and methods**

**Clinical validation study**

The study was designed, planned and performed according to the recommendations of the Clinical Laboratory Standards Institute (CLSI) Evaluation Protocol (EP) guidelines EP-09-A3, “Method Comparison and Bias Estimation Using Patient Samples” [35]. Briefly, at least 40 paired samples (WB and DMPD) for each analyte were collected and analyzed. Results were evaluated in terms of statistical agreement and bias values at medical decision points.

**Patients and paired sample collection**

Paired samples were collected from pediatric and adult transplant patients on single or multiple drug maintenance therapy during their
routine clinical follow-ups at the Hospital Italiano de Buenos Aires. Specimens were collected from September through December of 2018. Both WB and DMPD samples were taken by trained phlebotomists. To obtain trough concentrations, collection was coordinated prior to dose administration (30–60 min). Fingerp lick blood samples were collected within 10 min of the venous sample. The study was approved by the Institutional Review Board (IRB00010193). All participating patients or legal guardians gave their written consent after receiving information about the study.

Whole blood samples
K$_2$EDTA was used as anticoagulant. Specimens were analyzed within a day as they were part of routine care.

DMPD samples
After warming of the hands, patients’ ring fingertip was disinfected using alcohol 70 % v/v and dried. Capillary samples were collected using Microtainer Contact-Rosa Lancets (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The first drop was discarded and 10 µL of the second drop were collected using a fixed-volume capillary micropipette (Roc town Technologies & Services, Libertyville, IL, USA) and spotted in a 5-mm pre-punched Whatman 903™ paper disc (Sigma Aldrich, St. Louis, MO, USA) that was previously placed in a DMPD cartridge (Rock Town Technologies & Services, Libertyville, IL, USA). DMPD samples were left to dry for at least for 6 h at room temperature (24 °C), and then packed in zip lock plastic mini bags with a desiccant prior to analysis, as previously described [34]. All DMPD specimens were analyzed on the day of sample collection.

Analytical procedures
The reference procedure was the measurement of tacrolimus, sirolimus, everolimus and cyclosporin A in WB obtained by conventional venous sampling. The analysis of these samples was performed using the MassTrak Immunosuppressants XE Kit (Waters Corporation, Milford, MA, USA) on an Acquity UPLC Classic System, equipped with Acquity UPLC Binary Sample Manager, an Acquity UPLC Sample Manager and a column heater (Waters Corporation, Milford, MA, USA). The chromatographic system was coupled to a Xevo TQ MS triple quadrupole mass spectrometer (Waters Corporation, Milford, MA, USA) as previously described [34]. The quantification of calcineurin and mTOR inhibitors in DMPD was performed according to our previously validated method, using the previously referenced instrument [34]. Linear ranges were (1.1–30.8) ng/mL, (1.0–27.5) ng/mL, (1.0–33.4) ng/mL and (27.7–1483) ng/mL for tacrolimus, sirolimus, everolimus and cyclosporin A, respectively. Ascomycin was the internal standard for tacrolimus and sirolimus, [13C2H4]-everolimus for everolimus and [13H12]-cyclosporin for cyclosporin A. The ionization was operated in positive electrospray (ESI+) mode. The mass spectrometer parameters for the quantification of immunosuppressants in WB and DMPD were as follows: capillary voltage, 2.80 kV; cone voltage, 20 V; desolvation temperature, 550 °C; desolvation gas (N₂), 800 L/h; cone gas (N₂), 50 L/h; and collision gas (Ar), 0.15 mL/min. Multiple reaction monitoring (MRM) transitions for ammoniated adducts of each analyte and internal standard were the same as previously described [34]. The MassLynx™ software (Version 4.1, Waters Corporation, Milford, MA, USA) was used for instrument control, data acquisition and processing. A summary of the chromatographic conditions used in both WB and DMPD measurements is described in Table 1.

| Time (min) | Flow (mL/min) | Mobile Phase A (%) | Mobile Phase B (%) |
|-----------|---------------|-------------------|-------------------|
| 0.0       | 0.4           | 50                | 50                |
| 0.6       | 0.4           | 0                 | 50                |
| 1.2       | 0.4           | 50                | 100               |
| 2.0       | 0.4           | 50                | 50                |

Mobile phase A: 2 mmol/L ammonium acetate in water, 0.1 % formic acid.
Mobile phase B: 2 mmol/L ammonium acetate in methanol, 0.1 % formic acid.
Column: MassTrak™ TDM C18 column (2.1 x 10 mm 3.5 µm) (Waters Corporation, Milford, MA, USA).

Statistical analysis
Statistical analysis was performed using the Analyse-it® software version 5.01 (Leeds, United Kingdom). First, the assessment of constant or proportional errors between WB and DMPD concentrations was performed by non-parametric Passing-Bablok regression analysis. Intercepts and slopes were estimated with their 95 % confidence intervals (CIs). Significant constant or proportional bias was considered when the CIs of intercept and slope did not include 0 and 1, respectively. In addition, the clinical significance between sampling methods was determined by evaluating the overall bias and its CI calculated from the regression equation at medical decision points (lower and higher values of each analytes therapeutic range established at pre-dose trough concentrations) [10,34,36].

Secondly, the global bias between methods for each analyte was assembled by a graphical concordance analysis using difference plots. The selection of the best bias estimator (median or average) was made assessing the normality of the differences between methodologies by a Shapiro-Wilk test. Then, the limits of agreement (LoA) were calculated. The equivalence between the methods was concluded when CIs for the bias estimator contained the value 0 and the LoA were included within a previously defined tolerable range of ±15.0 % [36]. This range was established by a multidisciplinary team from our hospital, according to the recommendations of the International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT) [33].

Results

Demographic characteristics
A total of 160 paired samples collected from 125 transplant patients (58 (46.4 %) females; 67 (53.6 %) males) were analyzed. Median age was 50.5 years (range: 15 to 85 years; median of samples per patient: 1, range: 1 to 5). In 43 samples (26.9 %), more than one drug was quantified.

Regarding the types of patients, 14 (11.2 %) were inpatients and 111 (88.8 %) were outpatients. In this study 63 kidney (50.4 %), 32 liver (25.6 %), 14 reno-pancreas (11.2 %), 10 heart (8.0 %), 2 bone marrow (1.6 %), 2 liver-kidney (1.6 %), 1 heart-lung (0.8 %), 1 lung (0.8 %) transplant patients were included. The median Ht value for analyzed samples was 35.4 % (range: 16.6–58.1 %). Demographic characteristics for each immunosuppressive drug are summarized in Table 2.

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Clonal validation is concerned with patients receiving the right medical treatment based on laboratory test results. This procedure is intended to take a method that has undergone the bioanalytical validation step and evaluate whether it can be used for a specific clinical purpose before its implementation in the laboratory routine [36,37]. For this reason, the equivalence between DMPD-based results and those obtained in WB should be demonstrated by a method comparison procedure [38]. In this work, we assessed the feasibility of DMPD for therapeutic monitoring of four immunosuppressive drugs.

One of the most important points that must be considered when planning and performing a comparison between two methods is the number of paired samples to be included. In this regard, both the IATDMCT and the CLSI guidelines recommend that at least 40 specimens should be analyzed. In addition, the range of values should ideally span the entire analytical measurement range [33,36,38]. The first requirement was well accomplished in this work (89, 43, 42 and 43 paired samples were analyzed for tacrolimus, sirolimus, everolimus and cyclosporin A, respectively). Nevertheless, any analyte completely covered the measurement range, especially at high concentration values. These results were comparable to those obtained in other published clinical validation studies on DBS [26,39,45] and VAMS [26–29]. In our opinion, this could be attributed to the fact that all patients were under maintenance therapies, most of them were outpatients and all samples were taken prior to dose administration (C0 (Table 2).

Passing-Bablok regression analysis revealed the absence of statistically significant constant or proportional errors between DMPD and WB concentration for each analyte. In all cases, confidence intervals for intercepts and slopes contained the values 0 and 1, respectively. In addition, a strong linear relationship between DMPD and WB concentrations was obtained for all immunosuppressive drugs (Table 3). These results were similar to other previously published works on DBS [26,41,43,45] and VAMS [26–29].

The regression line for each analyte was used to estimate the bias value at medical decision points, which was a distinctive feature of our study. Medical decision points are concentrations commonly used as thresholds for making clinical statements [36]. In the field of therapeutic drug monitoring, lower and the higher values of therapeutic ranges are used as clinical decision limits [46,47]. In this work, the CIs for bias at each medical decision point included 0. Hence, it was stated with 95 % certainty that the measurement error was not clinically relevant (Table 3). The assessment of clinical significance at medical decision points is generally not informed in most clinical validation studies [26–28,39,40,42–45]. At present, only one work performed on WB and DBS samples from allogeneic stem cell transplant patients has published bias and CIs values for cyclosporin A [41]. At five medical decision points (60, 140, 225, 300 and 60 ng/mL), CIs included 0 and, consequently, no clinically relevant error was reported.

Difference plots and concordance analysis were used to evaluate whether the global bias between DMPD and WB met the acceptance criteria for interchangeability. In this regard, the 95 % confidence interval for the bias estimator of each analyte should contain the value 0 and the limits of agreement should be included within a previously defined allowable bias range [38]. According to IATDMCT, it should be defined by a multidisciplinary team of experts in each health center on the basis of clinical and analytical characteristics [36]. In this work, the allowable bias range was established at ±15.0 %, which was similar to other previously published studies [26,44]. Values outside this range would lead to different dosing advice.

For all analytes, lower concentrations in DMPD compared with WB were measured (−1.39, −1.01, −1.48 and −0.63 % for tacrolimus, sirolimus, everolimus and cyclosporin A, respectively) (Fig. 2). These results were reasonable considering the bias assessment on medical decision points (Table 3), and comparable to other previously published works on DBS [40–42,44] and VAMS [28]. Blood from skin puncture is a

| Table 2 | Demographic characteristics for tacrolimus, sirolimus, everolimus and cyclosporin A. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Variable        | Immunosuppressant | Tacrolimus | Sirolimus | Everolimus | Cyclosporin A |
| Number of samples | 89 | 43 | 42 | 43 |
| Number of patients | 71 | 30 | 29 | 35 |
| Sex n (%)       | Female | 36 (50.7) | 14 (46.7) | 10 (34.5) | 18 (51.4) |
|                 | Male     | 35 (49.3) | 16 (53.3) | 19 (65.5) | 17 (48.6) |
| Age (years) median (range) | 46 (15, 57) | 48 (17, 85) | 60 (23, 79) | 48 (19, 77) |
| Type of patient n (%) | Outpatients | 64 (90.1) | 25 (83.3) | 25 (86.2) | 31 (88.6) |
| Hematocrit (%) | Inpatients | 7 (9.9) | 5 (16.7) | 4 (13.8) | 4 (11.4) |
| (median; range) | 35.2 (20.3, 39.5) | 35.9 (20.3, 39.5) | 35.4 (16.6, 35.0) | 35.0 (16.6, 35.0) |
| Whole blood concentration range (ng/mL) | 1 (1.4, 17.1) | 1 (1.3, 16.5) | 1 (1.0, 16.6) | 28 (0.2, 854) |
| Type of transplant n (%) | Heart | 5 (7.1) | 0 (0.0) | 4 (13.8) | 1 (2.9) |
| Heart-lung | 1 (1.4) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Liver | 14 (19.7) | 5 (16.1) | 11 (37.9) | 12 (34.3) |
| Liver-kidney | 1 (1.4) | 0 (0.0) | 1 (3.4) | 1 (2.9) |
| Bone marrow | 2 (2.8) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Lung | 1 (1.4) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Kidney | 38 (53.5) | 17 (56.7) | 12 (41.4) | 19 (54.3) |
| Reno-pancreas | 9 (12.7) | 8 (26.7) | 1 (3.4) | 2 (5.7) |

Median and ranges for Ht values (%) per transplant type: heart: 35.0 (32.2, 42.8); heart-lung: 44.0; liver: 36.1 (26.8, 45.2); liver-kidney: 31.9 (30.3, 33.5); bone marrow: 35.4 (31.3, 39.5); lung: 27.7 (25.5, 30.1); kidney: 35.4 (16.6, 58.1); reno-pancreas: 38.2 (31.0, 56.8).

between DMPD and WB changed with increasing concentrations (data not shown). For that reason, the percent difference between measurement procedures was plotted on the y-axis in the difference plots (Fig. 2).

Lack of normality for the differences was found via the Shapiro-Wilk test for tacrolimus and sirolimus (p values obtained were 0.048 and 0.011, respectively). As a consequence, the mean value was selected as the bias estimator. In addition, the limits of agreement were calculated using a non-parametric method, considering both 2.5 and 97.5 percentiles. Regarding everolimus and cyclosporin A, the differences were found to be normal using the same statistical test (p values obtained were 0.9261 and 0.0897). As a consequence, the mean value was selected as the bias estimator and the limits of agreements were calculated as the mean of the relative differences ±1.96 standard deviations.

In all the cases, more than 95.0 % of the results fell within the limits of agreement (96.6, 97.7, 96.4 and 100 % for tacrolimus, sirolimus, everolimus, and cyclosporin A, respectively). Results outside these limits were included in the previously defined tolerable range of ±15.0 %. As plotted in Fig. 2, neither of the studied drugs showed significant bias between methodologies because the 95 % confidence intervals for the means or medians contained the value 0. In addition, the limits of agreement were tighter than the previously selected tolerable range. Finally, confidence intervals for bias at medical decision points for each analyte included zero. Thus, it can be stated with 95 % certainty that the measurement error was not clinically relevant and dosage change was not advised.
mixture of blood from arterioles, veins and capillaries and contains some interstitial and intracellular fluids [48]. Although during the sample collection procedure the first drop of capillary blood was discarded, it is possible that interstitial and intracellular fluids residuals resulted in a slight sample dilution that could be detected because of the high sensitivity of the instrument. In our previous work, we proved that the recovery of the analytical method was near 100% for all the analytes [34]. For that reason, lower concentrations in DMPD were not attributed to recovery issues. Nevertheless, as the CIs for the bias estimator of each analyte contained the value 0 and the limits of agreement were tighter than ±15.0%, it was concluded that both sampling methods were interchangeable.

Therapeutic ranges for immunosuppressants depend on several factors, including therapeutic approaches, the moment of sample collection and the transplant type. These aspects should be carefully considered before the formulation of analytical goals and the implementation of the method in the clinical laboratory [49,50]. The Hospital Italiano de Buenos Aires is one of the largest transplant centers for pediatric and adult patients in Latin America. Hence, quantification of calcineurin and mTOR inhibitors in blood samples collected from different transplant patient types is performed as part of the laboratory routine. C0 levels are regularly requested by physicians, because C2 and abbreviated AUC monitoring are not feasible for clinical practice. For this reason, in and out transplant patients from different age ranges on single or multiple drug maintenance therapy were selected in this study. In addition, a wide range of Ht values were included (Table 2). These values were comparable to others previously reported in clinical validation studies on VAMS [28] and DBS [28,42,45] in which various types of transplant

**Table 3**

Results of the Passing-Bablok regression analysis.

| Analyte        | r²     | Slope (CI)         | Intercept (CI) | Medical decision points (ng/mL), Calculated BIAS (ng/mL, %) | ICs (ng/mL) |
|----------------|--------|--------------------|----------------|-------------------------------------------------------------|-------------|
| Tacrolimus     | 0.995  | 0.980 (0.957, 1.006) | 0.022 (−0.100, 0.121) | Lower: 5.0 | −0.08 (−1.56) | (−0.15, 0.01) |
|                |        |                    |                | Upper: 20.0 | 0.38 (−1.89) | (−0.79, 0.00) |
| Sirolimus      | 0.996  | 0.970 (0.9437, 1.002) | 0.112 (−0.146, 0.285) | Lower: 5.0 | −0.04 (−0.73) | (−0.18, 0.10) |
|                |        |                    |                | Upper: 15.0 | 0.33 (−2.22) | (−0.66, 0.11) |
| Everolimus     | 0.995  | 1.000 (0.956, 1.045) | −0.100 (−0.289, 0.141) | Lower: 3.0 | −0.10 (−3.33) | (−0.20, 0.10) |
|                |        |                    |                | Upper: 8.0  | 0.10 (−1.25) | (−0.30, 0.14) |
|                |        |                    |                | Lower: 100 | 0.88 (−0.88) | (−4.01, 2.00) |
|                |        |                    |                | Upper: 400 | −12.88 (−3.22) | (−22.25, 4.09) |

![Fig. 1. Passing-Bablok regression lines for tacrolimus (A), sirolimus (B), everolimus (C) and cyclosporin A (D). Dotted lines are the identity lines and continuous lines are the regression lines.](image-url)
DMPD specimens collected by skilled technicians are suitable for more autonomous. At the present time, we have demonstrated the feasibility of the clinical application of DMPD immunosuppressant monitoring in clinical routine could decrease cost for patients and medical providers. For this reason, implementation of DMPD self- and trained personnel-sampling. In addition, patient satisfaction will be evaluated. This second study will be essential to assess the possibility of remote self-sampling and help patients to be more autonomous. At the present time, we have demonstrated that DMPD specimens collected by skilled technicians are suitable for therapeutic drug monitoring of calcineurin and mTOR inhibitors.

**Conclusion**

We have demonstrated the feasibility of the clinical application of simultaneous quantification of tacrolimus, sirolimus, everolimus and cyclosporin A in DMPD. Our results show that this microsampling technique is interchangeable with traditional WB sampling when specimens are collected by trained personnel. For this reason, implementation of DMPD immunosuppressant monitoring in clinical routine could be a helpful tool to attain target trough levels, reduce patient burden and decrease cost for patients and medical providers.

**Declaration of Competing Interests**

The authors declare that they have no known competing financial interests or personal relationships that could affect the work described in this article.

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