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Results From a Proficiency Testing Pilot for Immunosuppressant Microsampling Assays

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Background: Therapeutic drug monitoring (TDM) of immunosuppressive drugs is important for the prevention of allograft rejection in transplant patients. Several hospitals offer a microsampling service that provides patients the opportunity to sample a drop of blood from a fingerprick at home that can then be sent to the laboratory by mail. The aim of this study was to pilot an external quality control program.

Methods: Fourteen laboratories from 7 countries participated (fully or partly) in 3 rounds of proficiency testing for the immunosuppressants tacrolimus, ciclosporin, everolimus, sirolimus, and mycophenolic acid. The microsampling devices included the following: Whatman 903 and DMPK-C, HemaXis, Mitra, and Capitainer-B. All assays were based on liquid chromatography with tandem mass spectrometry. In round 2, microsamples as well as liquid whole blood samples were sent, and 1 of these samples was a patient sample.

Results: Imprecision CV% values for the tacrolimus microsamples reported by individual laboratories ranged from 13.2% to 18.2%, 11.7%–16.3%, and 12.2%–18.6% for rounds 1, 2, and 3, respectively. For liquid whole blood (round 2), the imprecision CV% values ranged from 3.9%–4.9%. For the other immunosuppressants, the results were similar. A great variety in analytical procedures was observed, especially the extraction method. For the patient sample, the microsample results led to different clinical decisions compared with that of the whole blood sample.

Conclusions: Immunosuppressant microsampling methods show great interlaboratory variation compared with whole blood methods. This variation can influence clinical decision-making. Thus, harmonization and standardization are needed. Proficiency testing should be performed regularly for laboratories that use immunosuppressant microsampling techniques in patient care.

Key Words: dried blood spots, external quality control, microsampling, immunosuppressants, therapeutic drug monitoring

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INTRODUCTION

Therapeutic drug monitoring (TDM) of immunosuppressant drugs, such as tacrolimus, is a cornerstone of transplant patient care.[1,2] Narrow therapeutic windows and great intraindividual and interindividual variation of blood concentrations of these drugs require frequent monitoring of blood drug levels to ensure the balance between therapeutic and toxic effects.[2,3] Over the past 15 years, dried blood spot (DBS) sampling has emerged, and it allows patients to sample at home by using a fingerprick and applying blood drops to a sampling card, which can be sent to a laboratory using regular mail.[4] The amount of blood on the sampling card varies (nonvolumetric); therefore, a subpunch of the DBS is analyzed. As a potential solution to the varying volume of free falling blood drops, several other microsampling devices were introduced, most of which can sample an exact amount of blood volume, which is also known as volumetric absorptive microsampling.[5] Examples of volumetric microsampling devices include the Neoteryx Mitra, Capitainer-B, and HemaXis samplers.[6] Multianalyte liquid chromatography with tandem mass spectrometry methods that can analyze up to 5 immunosuppressants in microsampling devices have been developed and validated.[4,7–16] Several of these microsampling methods were clinically validated by comparing paired fingerprick microsamples and venous whole blood samples obtained from transplant patients.[14,17–23] These methods have differences in the analytical procedures, such as the type of paper or

absorbent used, method of extraction, and type of internal standard (IS). Such differences could induce variations during the comparison of analytical results between laboratories. The International Organization for Standardization states that all medical laboratories are required to participate in interlaboratory comparison or proficiency testing to ensure the quality, comparability, and acceptability of analytical results.[24] Therefore, methods of analyzing immunosuppressants in venous whole blood are usually part of a quality control program. Studies assessing results from these programs show variations between laboratories, thus implying an urgent need for quality improvement.[25–29] A small proficiency testing pilot for tacrolimus in DBS was performed in 2014; however, it contained very little data.[30] Several hospitals currently use a form of home microsampling in routine transplant patient care for immunosuppressant TDM, and a microsampling proficiency testing program is urgently required.[31,32] In this study, we report the results from the first microsampling proficiency testing pilot for the immunosuppressants tacrolimus, ciclosporin, everolimus, sirolimus, and mycophenolic acid, and it consisted of 3 rounds.

MATERIALS AND METHODS

Selection of Participating Laboratories

The first round of the pilot started in 2017, and the second and third rounds were performed in 2018 and 2019, respectively. The only requirement to participate in this proficiency testing pilot was a validated microsampling liquid chromatography with tandem mass spectrometry assay for at least one of the 5 immunosuppressants (tacrolimus, ciclosporin, everolimus, sirolimus, and mycophenolic acid). The method did not have to be used in routine patient care for inclusion. Based on a PubMed search for immunosuppressant microsampling assays and the associated authors, laboratories were identified that could participate in the pilot. This search was repeated before each round. In round 1, 6 laboratories participated; in round 2, 7 laboratories participated; and in round 3, 14 laboratories participated. A total of 16 laboratories were contacted, and of these laboratories, 14 participated (Netherlands n = 7, United States n = 1, France n = 2, United Kingdom n = 1, Austria n = 1, Germany n = 1, and Norway n = 1).

Sample Preparation Round 1

Citrate whole blood from 1 donor was purchased from Sanquin (Amsterdam, the Netherlands) and used within 2 weeks after blood donation. The whole blood was stored at 4°C. Hematocrit (Ht) was measured using a XN-9000 hematology analyzer from Sysmex (Hyogo, Japan). An Ht value of 0.39 (vol/vol) represents the average for transplant patients.[7,18,32] In addition, most microsampling assays are analytically validated at Ht ranges of 0.23–0.50 for therapeutic concentrations.[9,10] Because the measured Ht value was 0.41 (vol/vol), no correction of the Ht value was needed.[33] Certified reference materials for tacrolimus and ciclosporin were purchased from Sigma-Aldrich (Cerilliant St. Louis, MO; lot numbers FN04231802 and FN01231702, respectively). Stock solutions were prepared in methanol. To prevent cell lysis, the volume of the spiked stock solution never exceeded 3% of the total blood volume. Target concentrations were chosen to reflect in vivo concentrations when measuring trough and peak concentrations in transplant patients (Table 1). In addition, concentrations should be within analytically validated ranges, which are usually 1.0–50 μg/L for tacrolimus, sirolimus, and everolimus; 100–12,000 μg/L for mycophenolic acid; and 20–1000 μg/L for ciclosporin.[9,29] Blood was spiked and homogenized for 30 minutes on a tumble mixer. All samples were prepared on both Whatman 903 and Whatman FTA DMPK-C cards (GE Healthcare, Chicago, IL) to match the

TABLE 1. Weighed-In Concentrations of Samples for the 3 Rounds of Proficiency Testing

Sample	Tacrolimus (μg/L)	Ciclosporin (μg/L)	Everolimus (μg/L)	Sirolimus (μg/L)	Mycophenolic Acid (μg/L)*
Round 1					
R1S1	3.0	50			
R1S2	18.0	200			
R1S3	40.0	700			
Round 2†					
R2S1	5.0	100			
R2S2	30.0	600			
R2P1	11.2‡				
Round 3					
R3S1	50.0	1200	48.0	46.0	16,000
R3S2	40.0	960	38.4	36.8	12,800
R3S3	20.0	480	19.2	18.4	6400
R3S4	8.0	192	7.7	7.4	2560
R3S5	3.0	72	2.9	2.8	960

*Spiked value in whole blood (not plasma/serum).
†Samples were prepared both as liquid whole blood samples and as dried microsamples.
‡All samples were spiked samples, with the exception of sample R2P1, which was from a patient undergoing tacrolimus therapy. Reference value for the patient sample was the laboratory result for the samples analyzed as part or routine care.
R1S1, round 1, sample 1, etc.

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paper type used by each participant. On each sampling card, a total of 4 spots of 50 μL blood were pipetted and allowed to dry for 24 hours at ambient temperature. To verify the sample preparation, a DBS sample (n = 1) of each spiked concentration and spiked whole blood were analyzed using the DBS method and the reference whole blood or plasma analysis method applied in the laboratory of the department of Clinical Pharmacy and Pharmacology of the University Medical Center Groningen, the Netherlands (UMCG), respectively, which is where the samples were prepared.[9,10,34] Results from all samples and spiked whole blood were within 15% of the weighed-in (theoretical) value. All DBS samples were labeled, placed in an airtight plastic sealed bag with a desiccant, and stored at −20°C until shipment. Shipment occurred under ambient conditions using regular mail because immunosuppressant stability was previously validated in dried microsamples for up to 28 days at room temperature.[10,15] Analysis by participants occurred within this timeframe. A questionnaire was sent to each participating laboratory that included questions on the specifications of the analytical method, including the extraction method, IS, quality control samples (QCs), analytical and clinically validated ranges,, and clinical application of the method. The complete questionnaire can be found in **Supplemental Digital Content** (see **Supplement 1**, http://links.lww.com/TDM/A594). In the following rounds, each newly participating laboratory received the same questionnaire.

Sample Preparation Round 2

Based on the preliminary results from round 1, microsamples and spiked liquid whole blood samples were sent in round 2. The spiked whole blood used to prepare the microsamples was sent as a liquid sample. Because tacrolimus whole blood assays mostly require EDTA anticoagulated blood and microsamples are usually prepared in citrated anticoagulated blood in the UMCG, a cross-validation was performed for all samples 8 times to determine the interchangeability between microsamples prepared from EDTA blood and microsamples prepared from citrate blood.[25,34] A volunteer provided both EDTA whole blood and citrate whole blood because such samples from the same donor cannot be purchased in the Netherlands. Results from the cross-validation using the method applied at the UMCG showed interchangeability between the EDTA and citrated microsamples (data not shown). The blood was spiked, and samples were prepared and sent as described in section "Sample Preparation Round 1," which included every quality control step described using the same certified reference materials. In addition, to accommodate laboratories that use the HemaXis sampling technique, samples were prepared on 10 μL HemaXis sampling cards.[35] The weighed-in values can be found in Table 1. The Ht value of the used blood was 0.36 (v/v). In addition to spiked whole blood, a leftover EDTA whole blood tacrolimus sample from a patient undergoing tacrolimus TDM was anonymized and used to prepare both microsamples and whole blood samples. Because of the absence of a weighed-in value for the patient sample, the results from the laboratory where the patient liquid whole blood sample was analyzed (UMCG) was chosen. The spiked whole blood samples and patient whole blood samples were prepared/sampled, sent under ambient conditions, and analyzed within 2 weeks to ensure that the results were obtained within the validated stability timeframe.[36]

Sample Preparation Round 3

Before sample preparation in round 3, all participating laboratories were asked to send the microsampling device or paper that was used in their laboratory. Sampling of these devices was performed per the manufacturer's instructions. Citrate blood was spiked, and samples were prepared and sent as described in section "Sample Preparation Round 1," including every quality control step described, with some alterations mentioned hereafter. After spiking the whole blood but before preparing the samples, the blood was homogenized for 24 hours on a tumble mixer. This step was introduced because discussions of the outcomes from round 1 and 2 with the participants indicated that tumble mixing was needed to obtain stable extraction recoveries of ciclosporin from microsampling devices. In vivo, ciclosporin is highly bound to erythrocytes, and a period of 24 hours was applied to provide a homogenous sample.[37] In addition to ciclosporin and tacrolimus, the blood samples were also spiked with sirolimus, everolimus, and mycophenolic acid. The weighed-in values can be found in Table 1. The Ht of the blood used was 0.38 (v/v). Sirolimus and everolimus–certified reference materials were purchased from Sigma-Aldrich (Cerilliant, Round Rock, TX. lot numbers FN06111802 and FN05101802, respectively). Mycophenolic acid with purity ≥98% was also purchased from Sigma-Aldrich.

Data Analysis

All results were recorded anonymously. Weighed-in values were used to calculate the imprecision (CV%) for tacrolimus per level using Microsoft Excel (2010, Microsoft, Redmond, WA).[25] To allow for comparisons between levels and rounds, the relative deviation from the weighed-in concentration was determined by subtraction of 100%, and inaccuracy was defined as the absolute deviation from the weighed-in concentration.[38] For the patient sample, the result from the whole blood analysis in the UMCG was set at 100% to calculate inaccuracy. The results were categorized by the sampling device (volumetric and nonvolumetric).

RESULTS

Participants

Details on the participating laboratories can be found in Table 2. All of the participating laboratories can analyze tacrolimus in microsamples. For ciclosporin, everolimus, sirolimus, and mycophenolic acid, analyses were only possible at 8, 7, 6, and 2 of the participating laboratories, respectively. For tacrolimus, 50% of the laboratories used an isotope-labeled IS while the other half used ascomycin as the IS. In 12 of 14 laboratories, the IS was added during the extraction of the samples. Six laboratories used a volumetric device, while 8 laboratories used nonvolumetric DBS devices. Nine out of 14 laboratories use microsampling in patient care. From these 9 laboratories, 7 laboratories performed a clinical validation study.

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TABLE 2. Details on Participating Laboratories and Their Immunosuppressant Microsampling Analysis Methods

Participating Laboratory	Microsampling device	Punch Size (mm)	Participated in Round	Immunosuppressants Analyzed*	Internal Standard (IS) for Tacrolimus	Moment of IS Addition	Calibrators and QCs	Microsampling method Used In Patient Care?	Clinical Validation Study Performed?
1	HemaXis 10 μL	N/A	2,3	T,E,M	Ascomycin	During extraction	Bought	Yes	Yes
2	Whatman 903	6	1,2,3	T,C,E,S	13CD2-tacrolimus	During extraction	Self-made	Yes	Yes
3	Whatman 903	7.94	1,2,3	T,C,E,S	13CD2-tacrolimus	During extraction	Self-made	Yes	No
4	Whatman 903	6	1,2,3	T,C,E,S	13CD2-tacrolimus	During extraction	Bought	No	N/A
5	Whatman 903	8	1,2,3	T	Ascomycin	During extraction	Bought	Yes	Yes
6	Whatman 903	6	1,2,3	T,C	Ascomycin	During extraction	Self-made	Yes	Yes
7	Whatman DMPK-C	8	1,2,3	T,C,E,S,M	13CD2-tacrolimus	During extraction	Self-made	Yes	Yes
8	Whatman 903	8	3	T,C	Ascomycin	During extraction	Self-made	Yes	No
9	Mitra 10 μL	N/A	3	T	Ascomycin	During extraction	Bought	Yes	Yes
10	Mitra 10 μL	N/A	3	T	Ascomycin	During extraction	Bought	Yes	Yes
11	Mitra 10 μL	N/A	3	T,C,E,S	13CD2-tacrolimus	During extraction	Bought	No	N/A
12	Capitainer-B	N/A	3	T,C,E,S	13CD2-tacrolimus	On the sample	Bought	No	N/A
13	Mitra 10 μL	N/A	3	T	13CD2-tacrolimus	After extraction	Bought	No	N/A
14	Whatman DMPK-C	3	3	T	Ascomycin	During extraction	Self-made	No	N/A

In a clinical validation study, paired patient whole blood venous samples (reference) are compared with fingerprick microsamples (candidate).
*C, ciclosporin; E, everolimus; S, sirolimus; M, mycophenolic acid; T, tacrolimus.

Extraction Procedures

As part of the questionnaire, details on the extraction procedures were obtained from participating laboratories, which can be found in Table 3. A great variety of extraction procedures was observed, particularly for the extraction solvent(s) and extraction techniques, such as sonication, vortexing, and centrifugation.

Between-Laboratory Results

We determined the between-laboratory differences for each immunosuppressant in each round by calculating the inaccuracy, as shown in Table 4. For round 3, a distinction was made between the volumetric and nonvolumetric assays. For ciclosporin microsamples, the inaccuracy in round 1 and 2 were comparable, although the value was improved in round 3, which was probably due to the 24-hour homogenization step that was introduced during sample preparation for round 3. In round 2, the inaccuracy differed greatly for the whole blood samples compared with the microsamples for tacrolimus (3.3% versus 12.0%). In round 3, the volumetric microsampling assays had a somewhat lower inaccuracy than the nonvolumetric microsampling assays for tacrolimus and sirolimus but not for ciclosporin and everolimus. However, the range of inaccuracy for both volumetric and nonvolumetric microsamples was quite large. It should be noted that fewer laboratories measured ciclosporin, everolimus, and sirolimus than tacrolimus, and only 2 laboratories measured mycophenolic acid (Table 2).

For tacrolimus, an overview is given for the imprecision of each unique sample in Table 5. The results show that imprecision is somewhat higher when tacrolimus levels are high (>30 μg/L) or low (<3 μg/L). The range of imprecision (CV%) values for the tacrolimus microsamples reported by individual laboratories was 13.2%–18.2%, 11.7%–16.3%, and 12.2%–18.6% for rounds 1, 2, and 3, respectively, showing no improvement over time.

The range of imprecision (CV%) values for ciclosporin, sirolimus, everolimus, and mycophenolic acid was similar to the results from tacrolimus (data not shown).

Significant differences were not observed in an analysis of the CV% values and inaccuracies for participants who used ascomycin versus deuterated IS, used a purchased QCs versus a self-made QC, and performed a clinical validation versus no clinical validation (data not shown).

Patient Sample

The tacrolimus reference value for the patient sample in round 2 was 11.2 μg/L, for both DBS and liquid whole blood. This value was close to the median (11.1 μg/L) and mean (11.3 μg/L) of all reported liquid whole blood values. The anonymized patient was a transplant recipient whose leftover blood sample was a tacrolimus trough sample. The target trough concentration for this patient was 10–12 μg/L. In Figure 1, the results from the 7 participating laboratories are shown in a boxplot for both the whole blood and the microsamples. For the whole blood, 6 of 7 participating laboratories reported a value within the therapeutic range in accordance with the reference value. For the microsamples, 2 of 7 laboratories report a value within the therapeutic range. One participant reported a value lower than the therapeutic range, and 4 of 7 participants reported a higher value than the therapeutic range.

DISCUSSION

We report the results from the first global immunosuppressant microsampling assay proficiency testing study, which consisted of 3 rounds. We observed a great variation in analytical procedures between laboratories, with the greatest variations observed in the applied extraction methods. A comparison of laboratories revealed consistent imprecision and inaccuracy for all 5 immunosuppressants. A patient sample containing tacrolimus that was sent both as a whole

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TABLE 3. Details on the Extraction Procedures Used in the Immunosuppressant Microsampling Analysis Methods

Participating Laboratory	Is $ZnSO_4$ Used during Extraction?	Extraction Solvent(s)	Is Acetonitrile Used During Extraction?	Is Sonication Used during Extraction?	Is Vortexing Used During Extraction?	Do You Use a Tumble Mixer During Extraction?	Is Centrifuging Used During Extraction?	Additional Relevant Details
1	Yes	80:20 MeOH:H_2O	No	No	15 min	No	20,784*g* for 5 min	
2	Yes	66:33 Acetontrile:H_2O	Yes	No	0.25 min	20 min	20,784*g* for 2 min	
3	No	66:33 MeOH:H_20	No	15 min	No	No	No	
4	Yes	60:40 MeOH:H_20	No	15 min	0.25 min	No	No	
5	No	82:18 MeOH:Acetonitrile	Yes	No	No	60 min	No	
6	No	50:50 MeOH:EtOH	No	10 min	0.33 min	No	No	
7	No	80:20 MeOH:H_2O	No	15 min	2 min	No	No	Samples are put in −20°C for 10 minutes postextraction
8	No	80:20 MeOH:H_2O	No	15 min	No	No	No	
9	Yes	H_2O	Yes	10 min	17 min	No	17,709*g* for 5 min	Ammonium sulfate 40% is added during extraction
10	Yes	H_2O	Yes	No	8 min	No	769*g* for 5 min	
11	Yes	Methanol, ZnSO4, and acetonitrile*	Yes	No	1 min	No	24,104*g* for 5 min	
12	Yes	Methanol, ZnSO4, and acetonitrile*	Yes	No	1 min	No	13,047*g* for 5 min	Bead rupture is used before adding extraction solvent
13	Yes	H_2O and then 66:33 MeOH:$ZnSO_4$ 0.1 M	No	No	21 min	No	2274*g* for 10 min	
14	No	80:20 MeOH:H_2O	No	15 min	0.75 min	No	12,493*g* for 10 min	

*External IVD kit containing unknown amounts of methanol, $ZnSO_4$, and acetonitrile.

blood sample and as a microsample, which was prepared from the whole blood sample, showed that analytical results from the microsample would have led to a different clinical decision compared with the whole blood sample result for several participants.

The number of participants in this study was relatively small. However, we are confident that almost all laboratories that were able to participate joined our study. Therefore, the results provide a good reflection of the state of immunosuppressant microsampling in the years in which this proficiency testing pilot was performed. Because this study was a pilot, the design of the rounds changed during the process based on new insights in each round, which influenced the interpretation of the results. For example, the results for ciclosporin in round 1 and 2 can be considered unreliable, which was probably due to the samples not being properly homogenized

TABLE 4. Microsampling Assay for Immunosuppressant Absolute Inaccuracy

Round	Median Absolute Inaccuracy, % (range)*				
	Tacrolimus	Ciclosporin	Everolimus	Sirolimus	Mycophenolic Acid
Round 1 microsamples	13.8 (5.0–26.1)	24.0 (8.0–42.6)			
Round 2 microsamples	12.0 (2.7–40.2)	16.9 (4.0–25.9)			
Round 2 whole blood	3.3 (0.9–15.0)	15.9 (8.7–21.9)			
Round 3 volumetric microsamples	8.5 (0.0–38.4)	10.8 (3.3–33.4)	15.6 (0.1–32.3)	10.4 (2.2–22.6)	11.7 (N/A)
Round 3 nonvolumetric microsamples	14.0 (1.3–35.6)	7.6 (0.0–35.3)	4.6 (0.2–34.5)	13.5 (3.2–35.9)	11.2 (N/A)
Round 3 all microsamples	11.2 (0.0–38.4)	8.7 (0.0–35.3)	5.5 (0.1–34.5)	12.1 (2.2–35.9)	11.5 (3.8–17.9)

Amount of samples per round can be found in Table 1. Absolute inaccuracy is defined as the relative deviation from the weighed-in concentration.
*Table 2 describes the participants per round and which participating laboratories measured which immunosuppressant.

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TABLE 5. Results for the Imprecision (CV%) of Unique Samples per Round for Tacrolimus

Sample	Tacrolimus (μg/L)*	Mean (μg/)†	Range of reported Values (μg/L)	CV%
Round 1				
R1S1 microsample	3.0	3.2	2.3–3.7	17.4
R1S2 microsample	18.0	18.2	14.0–20.6	13.2
R1S3 microsample	40.0	39.4	29.6–47.8	18.2
Round 2				
R2S1 microsample	5.0	5.3	4.4–6.5	16.3
R2S2 microsample	30.0	31.1	25.9–36.2	11.7
R2P1 microsample	11.2	12.5	9.7–15.7	15.8
R2S1 whole blood	5.0	5.2	4.6–5.6	3.9
R2S2 whole blood	30.0	32.2	30.7–34.5	4.2
R2P1 whole blood	11.2	11.1	10.8–12.5	4.9
Round 3				
R3S1 microsample	50.0	53.4	42.7–69.2	16.3
R3S2 microsample	40.0	38.8	28.8–53.1	18.6
R3S3 microsample	20.0	20.1	17.2–24.8	12.3
R3S4 microsample	8.0	7.8	6.4–9.7	12.2
R3S5 microsample	3.0	3.1	2.4–3.6	14.3

*Weighed-in concentration of tacrolimus with the exception of sample R2P1, for which the reference concentration was the result from a reference laboratory. Round 1, 2, and 3 had 7, 8, and 14 participating laboratories, respectively.

†The mean is calculated per sample per round because of differences in the amount of participating laboratories.

R1S1, round 1; sample 1, etc.

because of the short period of tumble mixing. In round 3, the inaccuracy for ciclosporin was lower (8.7%) than that in round 1 and 2 (24.0% and 16.9%, respectively), which may help explain these results. It is important to note that not all laboratories participated in each round and not all laboratories measured each immunosuppressant (Table 2). In addition, everolimus, sirolimus, and mycophenolic acid were only added in round 3. Therefore, the results for everolimus, sirolimus, ciclosporin, and mycophenolic acid should be interpreted with caution. The inaccuracy from round 3 (Table 4) was similar for all 5 immunosuppressants, suggesting no major differences in performance for these drugs. The CV% values for tacrolimus can be considered high when the suggested target imprecision of <10% for all immunosuppressants was considered.[29] Because most immunosuppressant microsampling assays were multianalyte assays containing up to 5 different immunosuppressants, the observed inaccuracy was probably not caused by the properties of the individual drugs but rather by the analytical method as a whole.[9] For mycophenolic acid, only 2 laboratories reported results. Therefore, these results were reported for whole blood so that they could be compared with the weighed value, which was performed for the other 4 immunosuppressants. In clinical practice, only the DBS results for mycophenolic acid are converted to plasma values using a correction factor before they are reported.[13] This conversion formula could be a source of variation, which can be investigated in future studies.

For this study, the weighed-in values were used as reference values for all samples that were spiked, in accordance with Lempers et al.[38] Because certified reference materials were used to prepare the samples for tacrolimus, ciclosporin, everolimus, and sirolimus, the samples were traceable to higher-order reference materials. In the absence of a true reference value for the patient sample in round 2, the result from the immunosuppressant whole blood analytical method of the UMCG (singular analysis) was selected. This method is validated and is currently used in routine patient care. The result from the reference method (11.2 μg/L) was close to the median (11.1 μg/L) and mean (11.3 μg/L) of all reported whole blood values during round 2. Therefore, this reference value can be considered reliable.

The tacrolimus microsample imprecision CV% values were considerably higher than the CV% values of the liquid samples in round 2 (3.9%–4.9% versus 11.7%–16.3%, respectively), which shows a great discrepancy between the performance of microsampling assays compared with the whole blood assays. The patient sample in round 2 illustrates this discrepancy (Fig. 1). Based on the whole blood results, 6 of 7 participating laboratories reported a value within the therapeutic range for tacrolimus. For the microsamples, only 2 of 7 participating laboratories reported results within the therapeutic range, which would lead to incorrect clinical decision-making for 5 of 7 participating laboratories. These data indicate that immunosuppressant microsampling assays do not produce the same quality of results as whole blood immunosuppressant assays. In addition, the results from microsampling are not interchangeable between laboratories, which can influence the immunosuppressive therapy of transplant patients because 9 of 14 participating laboratories use

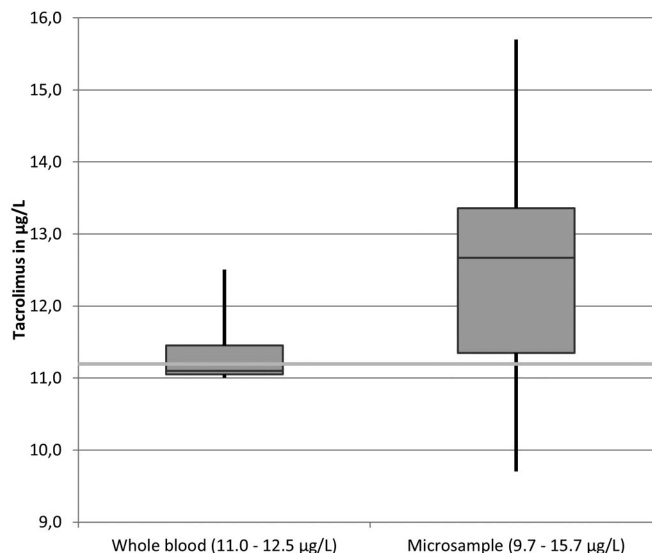

FIGURE 1. Boxplot showing the distribution of results from 7 participating laboratories for the patient tacrolimus samples. The whole blood sample was used to produce the microsample. The gray line shows the reference value of 11.2 μg/L. The boxes show the values between the 25th and 75th percentile of the median value. The whiskers represent the min–max values.

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the microsampling method for patient care. Incorrect adjustments of the tacrolimus dose can lead to either increased side effects or transplant rejection risks.[2] A likely explanation for the high CV% values observed between laboratories might be the extraction methods used by participants. Table 3 shows characteristics of the extraction methods used. A great variety in the use of extraction solvents, vortexing, centrifuging, and sonication was observed. Although microsampling methods are usually developed in-house and validated according to the FDA, EMA, and microsampling guidelines, these differences might have been related to differences between laboratories observed in this study.[32] Other possible explanations might be the differences in chromatography, mass spectrometry, microsampling devices, and drying time or storage conditions.[23] The proficiency testing samples could have also been a source of variation. Although traceable substances were used to prepare the samples, the samples might not be commutable. A true patient microsample is prepared from a fingerprick, which can lead to different results relative to venous whole blood.[32] Thus, standardization and harmonization of analytical methods are urgently needed. A possible solution might be to use 1 sampling device worldwide with a well-investigated and robust extraction method that is feasible for use in every laboratory. Although the sample size was too small to investigate differences for each individual sampling device, the differences between volumetric and nonvolumetric sampling devices were small and probably not clinically relevant. It should be noted that from the 7 participants for which both whole blood and a microsample were analyzed in round 2, only 1 used a volumetric sampling device. In future studies, samples should be prepared using both whole blood and volumetric microsamples and sent to participating laboratories. In addition, more samples can be included in the panel of patient samples. Ht likely did not influence these results because the Ht value was close to the reference value used during validation (0.39 vol/vol).[32] In addition, during analytical validation of the microsampling methods, the influence of Ht should be part of the validation.[32]

Although the use of isotope-labeled tacrolimus as an IS is considered state-of-the-art, 50% of the participants reported that they used ascomycin.[27] However, the observed CV% values and inaccuracy were comparable between participants who used ascomycin or an isotope-labeled IS. Commercially acquired QCs, especially freeze-dried QCs, might have shown different absorption on a microsample compared with QCs that were freshly prepared using human blood, thereby influencing the analytical results.[27] However, according to our results, major differences were not observed in the performance for laboratories that used commercially acquired QCs compared with laboratories that prepared their QC samples with fresh unfrozen whole blood. In round 3, creatinine levels measured in microsamples were reported for 4 participants and Ht was reported for one participant. Owing to the small sample size, these results were not reported here.

A limitation of this study is that it only shows between-laboratory comparisons and not within-laboratory comparisons, as shown by Levine et al.[25] In future studies, within-laboratory assay imprecision can be investigated. In several studies, common calibrators are used to potentially correct for interlaboratory variations.[25,27] However, for whole blood immunosuppressant analytical methods, these common calibrators are suggested as a source of interlaboratory variation without a noticeable positive effect.[27] For microsampling methods, all methods are developed in-house and the microsampling devices differ between laboratories, thus making a unified method of calibrator sample preparation very challenging.

CONCLUSION

Transplant patients undergoing immunosuppressive therapy should be able to use a microsampling method for immunosuppressant blood concentration monitoring at home that produces reliable results. This study shows that the current microsampling methods show great interlaboratory variation compared with whole blood methods. This variation is of such a magnitude that it will influence clinical decision-making. Harmonization and standardization are needed for many aspects of immunosuppressant microsampling methods, most notably the extraction methods used. Proficiency testing should be performed regularly for laboratories that use immunosuppressant microsampling techniques in transplant patient care. Currently, such a proficiency testing program is offered by the SKML (Nijmegen, the Netherlands).

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