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Measurement of sirolimus concentrations in human blood using an automated electrochemiluminescence immunoassay (ECLIA): a multicenter evaluation

https://doi.org/10.1515/cclm-2017-0583
Received July 4, 2017; accepted October 28, 2017; previously published online December 5, 2017

Abstract

Background: Therapeutic drug monitoring (TDM) of sirolimus is essential in transplant recipients. We evaluated the performance of a new electrochemiluminescence immunoassay (ECLIA) for measuring sirolimus concentrations in whole blood at five European laboratories.
Methods: Study assessments included repeatability, intermediate precision and functional sensitivity (concentration at coefficient of variation [CV] of 20%) experiments. Method comparisons with liquid chromatography-tandem mass spectrometry (LC-MS/MS; reference method) and two immunoassays (chemiluminescent microparticle immunoassay [CMIA] and antibody-conjugated magnetic immunoassay [ACMIA]) were performed using native samples from patients with kidney transplants.
Results: Imprecision testing CVs were ≤6.4% and ≤10.7% across the sirolimus concentration range for both repeatability and intermediate precision, respectively. The ECLIA showed excellent functional sensitivity: the CV did not reach 20%; the CV at the assay's limit of quantitation (1.5 μg/L) was 7.0%. Agreement between the ECLIA and LC-MS/MS using native kidney samples was close, with weighted Deming regression analysis yielding a slope of 1.05, an intercept of 0.154 μg/L and a Pearson's correlation coefficient (r) of 0.94, while Bland-Altman analysis showed a combined mean bias of 0.41 μg/L (±2 standard deviation [SD], –1.96 to 2.68). The ECLIA also showed good correlation with the two other immunoassays: the CMIA (slope = 0.91, intercept = 0.112 μg/L and r = 0.89) and the ACMIA (slope = 0.99, intercept = 0.319 μg/L and r = 0.97).
Conclusions: The ECLIA showed good precision, functional sensitivity and agreement with other methods of sirolimus measurement used in clinical practice, suggesting that the assay is suitable for TDM in transplant recipients and provides an alternative to LC-MS/MS.

Keywords: ECLIA; immunoassay; sirolimus; therapeutic drug monitoring.

Introduction

Sirolimus, a mammalian target of rapamycin (mTOR) inhibitor, and its synthetic derivative, everolimus, are widely used immunosuppressant agents for the prevention of graft rejection in solid organ transplantation [1, 2]; sirolimus appears especially beneficial in the setting of renal transplantation [1]. In common with many other immunosuppressant drugs, sirolimus displays significant interpatient, and intrapatient, pharmacokinetic variability that can potentially lead to under or overdosing and possible severe side effects [3]. Moreover, because sirolimus has to be taken continuously by transplant recipients, and treatment compliance is essential to prevent organ rejection, precise therapeutic drug monitoring (TDM) of concentrations in a patient's blood is important [3, 4].

Concentrations of sirolimus in whole blood can be determined, either by liquid chromatography-tandem mass spectrometry (LC-MS/MS) or by immunoassay. Immunoassays, of which several are commercially available, offer the benefit of less complexity, automation and round-the-clock results [5]. However, some immunoassays suffer from relatively reduced analytical performances with respect to calibration bias, analytical sensitivity and specificity, as well as from cross-reactivity with other drugs (e.g. everolimus) or metabolites and interference from heterophilic antibodies and endogenous compounds [5–8].

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The purpose of the present multicenter study was to evaluate, under field conditions and in clinically relevant cohorts, the analytical performance of an electrochemiluminescence immunoassay (ECLIA; Elecsys® Sirolimus, Roche Diagnostics) for determining concentrations of sirolimus in human whole blood.

Materials and methods

Participating centers and instruments

Five European centers experienced in TDM of immunosuppressive drugs participated in this study. The investigational sites included two in Germany (Klinikum-Stuttgart, Stuttgart and Hospital of the University of Munich, Munich), two in Belgium (Ghent University Hospital, Ghent and Cliniques Universitaires St. Luc, Brussels) and one in Spain (Bellvitge University Hospital, Barcelona). The sirolimus ECLIA assays were assessed on the cobas e platforms (Roche Diagnostics): four of the five centers used the cobas e 411 platform, and the Barcelona site used a cobas e 601 platform. LC-MS/MS was used at all centers (Table 1). The sirolimus chemiluminescent microparticle immunoassay (CMIA) was used on the Architect system in Ghent, and the sirolimus antibody-conjugated magnetic immunoassay (ACMIA) was used on the Siemens Dimension platform at Stuttgart.

The study received independent ethics committee approval and was conducted according to the Declaration of Helsinki and ICH Good Clinical Practice guidelines with respect to subject confidentiality and the use of data in subject management.

Samples sources and handling

A range of specimens was used for the ECLIA performance evaluation that included inaccuracy tests, imprecision, functional sensitivity and method comparisons experiments.

To test inaccuracy, international proficiency testing (IPT) samples were obtained from Analytical Services International (London, UK). All of the supplied inaccuracy samples were initially sirolimus-free blood samples to which sirolimus was added to produce the final target concentration. Target values for the IPT samples were assigned based on the mean of the LC-MS/MS laboratories in the UK Quality Assurance Program.

The imprecision and inaccuracy experiments used quality control (QC) material, PreciControl ISD (PC ISD), at three different sirolimus concentrations (PC ISD L1–3, Roche R&D, Penzberg): 2.66 µg/L (L1), 9.6 µg/L (L2) and 16.9 µg/L (L3). Target values for the PC ISD samples were assigned in house by Roche. For the imprecision experiments involving the three PC ISD samples, the concentration of sirolimus was measured as part of the in-house value assignment carried out by Roche Diagnostics using Elecsys® Sirolimus assay reagents and cobas e analyzers. In addition, five human sample pools (HSP) of whole blood samples containing native and spiked samples (concentration range of 0.5–30 µg/L) were used to test imprecision (HSP2–6).

Functional sensitivity experiments were performed for which 11 spiked HSP samples that covered the concentration range, 0.2–2.3 µg/L, of sirolimus were used.

Anonymized residual ethylenediaminetetraacetic acid (EDTA) whole blood samples were collected by a single venipuncture into plasma tubes with EDTA-K_2/-K_3, from patients who had received a kidney transplant and were undergoing sirolimus therapy. These native samples were used for the method comparison experiments; different anonymized samples were assayed at each center. In addition, leftover anonymized samples from the Medizinische Hochschule Hannover were used to supplement the targeted transplant sample type. Samples, if tested within 5 days of collection, could be stored at room temperature (18 °C–25 °C) or at 2 °C–8 °C for up to 1 week. Where longer storage was necessary, samples were frozen between –15 °C and –25 °C, or ideally at –80 °C, if available. Liquid whole blood aliquots for the method comparisons were used within 24 h on all systems, and for in-between measurements, the aliquot was stored at 2 °C–8 °C.

Immunoassays

The ECLIA for detection and measurement of sirolimus in whole blood was used at each center according to the manufacturer's instructions [12]. Before testing, blood samples, calibrators and controls were pretreated with Elecsys® ISD sample pretreatment, following which an aliquot of the drug-containing supernatant was measured using the ECLIA. The ECLIA was calibrated using the Elecsys® Sirolimus CalSet, which consists of two calibrators at concentrations of 1 and 25 µg/L [13]. Both calibrators were reconstituted according to manufacturer's instructions and stored in 0.3 mL aliquots for 7 days at 2 °C–8 °C or below –20 °C for 28 days. Reconstituted aliquots were used for calibration within 30 min of pretreatment. Calibration was performed once per reagent lot and as required for maintaining QC values within specified limits.

The measuring range of the sirolimus ECLIA is 0.5–30 µg/L. The lower value is based on the limit of detection (LoD), as is recommended to differentiate between the presence or the absence of an analyte [14]. The limit of blank (LoB) is 0.4 µg/L the LoD is 0.5 µg/L, and the limit of quantitation (LoQ) is 1.5 µg/L. The LoB, LoD and LoQ were determined in accordance with the CLSI EP17-A2 [15]. As reported in the CLSI guidelines, results between the LoD and LoQ should be reported as <LoQ [15].

The CMIA and ACMIA immunoassays were used according to manufacturers' instructions [16, 17]. Performance criteria for these assays have been described in previously published comparative studies [18, 19].

LC-MS/MS procedures

Each investigational site performed LC-MS/MS according to the protocols developed and used routinely at each center (Table 1).

Analytical performance measures

Inaccuracy: Inaccuracy was assessed according to CLSI EP05-A3 guidelines [20] by measuring the PC ISD (n = 3) and IPT (n = 6) samples using the ECLIA and LC-MS/MS at all sites. In addition, the same samples were measured using the CMIA at site 1 and the ACMIA at

Table 1: LC-MS/MS measurement methods used at the five investigational sites.

Center	Extraction[a]	Calibrators	LC/MS manufacturer/ model	Analytical column	Internal control	Method working range	Within-lab imprecision (CV)
Site 1[b]	PPT with $ZnSO_4$ and acetone followed by LLE with 1-chlorobutane; no online sample clean-up	Chromsystems 6PLUS1® Multilevel Calibrator Set Immunosuppressants	Waters Acquity TQD	Waters MassTrak TDM C18 2.1 × 10 mm	32-Demethoxy-sirolimus	0.5–30 µg/L	<8%
Site 2[b]	PPT with $ZnSO_4$ and acetonitrile; no online sample clean-up	Chromsystems 6PLUS1® Multilevel Calibrator Set Immunosuppressants	Waters H-class Acquity/Xevo-TQD	MZ-Analysentechnik MZ Aqua Perfect C18 150 × 3.0 mm, 5 µm	13Cd3-Sirolimus	0.6–50 µg/L	<6% [9]
Site 3 [10]	PPT with MeOH and $ZnSO_4$ (4:1), followed by on-line solid phase extraction (Oasis HLB trapping column) [10]	Chromsystems 6PLUS1® Multilevel Calibrator Set Immunosuppressants	Waters Quattro Ultima Pt	Waters HLB column, 90% MeOH/10% 0.1% formic acid, isocratic elution	d4-Sirolimus	0.6–50 µg/L	≤10.8%
Site 4[b]	PPT with MeOH and $ZnSO_4$, followed by online solid phase extraction	RECIPE ClinCal® (Whole Blood Calibrator Set level 0–6)	Agilent 6460 and HPLC Agilent 1290	Agilent Zorbax C18, 4.6 × 50 mm	d4-Everolimus	1.2–45.0 µg/L	≤4.26%
Site 5 [11]	PPT with $ZnSO_4$ and acetonitrile	RECIPE ClinCal® (Whole Blood Calibrator Set level 0–6)	Waters Acquity® UPLC/ Waters TQD®	Waters Acquity® UPLC® BEHTM C18 reverse-phase column 2.1 × 30 mm	13C2d4-Everolimus	1.52–49.5 µg/L	≤8.5[c]

[a]Precipitation with organic solvent mixture and centrifugation. [b]In-house protocols used. [c]Interday imprecision (CV). HPLC, high-performance liquid chromatography; LLE, liquid-liquid extraction; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MeOH, methanol; PPT, precipitation; UPLC, ultra-performance liquid chromatography; $ZnSO_4$, zinc sulfate.

site 2. All instruments were calibrated before the experimental run, as described previously. Samples were divided into three aliquots and measured in a single run with three replicates for each sample. Mean recovery (%) relative to target value was calculated for each sample in each center.

Assay imprecision: Repeatability (within-run precision) and intermediate precision (within-lab) of the ECLIA were determined according to CLSI EP05-A3 guidelines [20]. The experiments were carried out at four centers, three of which used the cobas e 411 platform and one the cobas e 601 platform. A total of 84 aliquots from each of the three PC ISD controls and the five HSP samples were tested over a 21-day period. A model with two runs per day and two replicates per run was used, with samples randomized with every run.

Site-to-site, lot-to-lot, day-to-day variance components were estimated as well as their sum (reproducibility) according to CLSI EP05-A3 guidelines [20]. Measurements were carried out at three centers, all of which used the cobas e 411 platform. Each center tested 25 aliquots in total, in which two lots were used over 5 days with one run conducted per day with five replicates for the three PC ISD samples and the five HSP samples.

Functional sensitivity: The functional sensitivity of the ECLIA was assessed at a single center on the cobas e 411 platform using the 11 spiked HSP samples, covering the sirolimus concentration range 0.2–2.3 µg/L. Samples were measured in two separate runs each day for 5 days in total, with a single measurement per aliquot. The functional sensitivity was initially defined as the sirolimus concentration at which the coefficient of variation (CV) was 20%. LoQ experiments were performed according to CLSI EP 17-A2 guidelines [15].

Method comparison experiments: Method comparisons between the ECLIA and LC-MS/MS were carried out at all five centers. In addition, the ECLIA and the CMIA assays were compared at site 1, and ECLIA and ACMIA assays were compared at site 2. Anonymized residual samples from kidney transplant recipients were used at each study center (site 1, n = 74; site 2, n = 120; site 3, n = 50; site 4, n = 38; site 5, n = 120).

Data analysis

ECLIA test results were captured directly (from a laptop computer attached to the cobas e 411/c 6000 analyzer) using the Windows-based Computer Aided Evaluation (WinCAEv) program [21] and, more specifically, version 2.2.2, CFR 21 Part 11 compliant electronic data capture software that had been developed and validated for Roche-sponsored studies. Reference assay output was entered offline into WinCAEv at each center, and source data were verified from analyzer printouts.

For the inaccuracy tests, the % recovery was calculated, and linear regression analyses were performed using Microsoft Excel. For imprecision, the mean, standard deviation (SD) and CV were estimated using statistical software R with the variance component analysis (VCA) package version 1.2.1. [22]. Imprecision results, including outlier handling, were calculated in compliance with the CLSI EP05-A3 guideline [20]. For repeatability and intermediate precision assessments, it was permitted to reject at most two results due to an outlier. For reproducibility, it was permitted to reject one result at most due to an outlier, and this applied for each precision sample and each 5-day experiment.

Method comparison analyses were performed with WinMC 2.0 using exported data from WinCAEv. These analyses were compliant with CLSI EP09-A3 guidelines [23]. Analysis of the method comparisons with each center's LC-MS/MS and the two immunoassays was performed using Weighted Deming regression, and Pearson's Correlation coefficients (r) were also calculated. To gain further information on method comparability, Bland-Altman difference plots were used [24]. For the ECLIA vs. LC-MS/MS method comparisons that were performed at multiple sites, the relative bias at clinically relevant medical decision points was calculated. These medically relevant decision points were defined as the therapeutic range for sirolimus: between 4 and 12 µg/L for patients receiving calcineurin inhibitors (CNIs) and between 12 and 20 µg/L for those not receiving CNIs [3, 7, 25–28]. This was not performed for the ECLIA vs. CMIA and ECLIA vs. ACMIA method comparisons, because these experiments were performed at single sites and may be subjected to a site-specific bias.

Results

Inaccuracy

With respect to the three PC ISD samples, at pre-determined concentrations of 2.66 µg/L, 9.6 µg/L and 16.9 µg/L, all of the ECLIA test results were within a recovery range of 88.6%–101.4%, the LC-MS/MS results were from 83.8% to 130.8%, CMIA was 56.1%–94.4% and ACMIA was 68.9%–110.3% (Table 2). Median percentage recovery ranged from 97.9% to 100.4% for the ECLIA and from 88.5% to 96.6% for LC-MS/MS.

For the spiked IPT samples, recovery relative to target value for the ECLIA ranged from 82.5% to 119.8%, compared with 101.7%–142.0% for LC-MS/MS, 80.3%–111.8% for the CMIA and 89.5%–113.7% for the ACMIA. Median percentage recovery ranged from 94.0% to 105.1% for the ECLIA and from 108.7% to 119.5% for LC-MS/MS.

Linear regression analyses of all the target values vs. the test values showed excellent agreement between the ECLIA-measured values vs. the target values at all sites (Figure 1). At site 1, linear regression of the inaccuracy results revealed slopes of 1.0044 for the ECLIA (Figure 1A), 1.1462 for LC-MS/MS (Figure 1B) and 1.0949 for the CMIA (Figure 1C). At site 2, the slope was 0.9562 for the ECLIA (Figure 1D), 1.0138 for LC-MS/MS (Figure 1E) and 1.176 for the ACMIA (Figure 1F). For the ECLIA inaccuracy tests, the ECLIA and LC-MS/MS, respectively, demonstrated slopes of 0.9422 and 1.1109 at site 3 (Figure 1I and J), and 0.8213 and 1.2802 at site 5 (Figure 1K and L) across all samples tested.

Table 2: Mean measured value (μg/L) and mean recovery rate relative to target value (%) in PC ISD and IPT samples.

Samples	Investigational center													
	Mean concentration measured (mean % recovery relative to target value)												Median measured conc. (median % recovery) across all sites	
	Site 1			Site 2			Site 3		Site 4		Site 5		All sites	
	ECLIA	LC/MS-MS	CMIA	ECLIA	LC/MS-MS	ACMIA	ECLIA	LC/MS-MS	ECLIA	LC/MS-MS	ECLIA	LC/MS-MS	ECLIA	LC/MS-MS
PC ISD L1	2.61	2.47	1.49	2.50	2.73	1.83	2.36	2.23	2.60	2.41	2.64	3.13	2.60	2.47
(2.66 μg/L)	(98.1)	(92.7)	(56.1)	(93.9)	(102.5)	(68.9)	(88.6)	(83.9)	(97.9)	(90.4)	(99.1)	(117.8)	(97.9)	(92.7)
PC ISD L2	9.65	8.20	7.78	9.64	8.75	8.10	8.98	8.50	9.74	8.05	9.16	11.68	9.64	8.50
(9.6 μg/L)	(100.5)	(85.4)	(81.0)	(100.4)	(91.1)	(84.4)	(93.5)	(88.5)	(101.4)	(83.8)	(95.4)	(121.5)	(100.4)	(88.5)
PC ISD L3	17.07	14.50	15.96	15.68	16.34	18.63	16.41	16.37	17.07	15.39	15.47	22.10	16.41	16.34
(16.9 μg/L)	(101.0)	(85.8)	(94.4)	(92.8)	(96.6)	(110.3)	(97.1)	(96.8)	(101.0)	(91.1)	(91.5)	(130.8)	(97.1)	(96.6)
IPT – 180B	9.56	9.90	9.23	10.07	9.35	8.23	8.99	10.0	9.51	10.18	8.82	11.53	9.51	10.0
(9.2 μg/L)	(103.9)	(107.6)	(100.4)	(109.5)	(101.7)	(89.5)	(97.8)	(108.7)	(103.4)	(110.6)	(95.9)	(125.4)	(103.4)	(108.7)
IPT – 181A	26.29	31.30	28.03[a]	26.17	27.16	29.80	24.62	29.87	24.44	37.22	21.61	32.77	24.62	31.30
(26.2 μg/L)	(100.4)	(119.5)	(107.0)	(99.9)	(103.7)	(113.7)	(94.0)	(114.0)	(93.3)	(142.0)	(82.5)	(125.1)	(94.0)	(119.5)
IPT – 182A	2.68	3.20	2.41	3.57	3.03	2.93	2.73	3.50	3.30	3.49	3.07	3.70	3.07	3.49
(3.0 μg/L)	(89.2)	(106.7)	(80.3)	(119.1)	(110.1)	(97.8)	(91.1)	(116.7)	(110.0)	(116.3)	(102.3)	(123.3)	(102.3)	(116.3)
IPT – 185A	6.59	6.8	6.26	7.48	7.13	5.80	6.62	7.40	6.95	7.18	5.67	8.50	6.62	7.18
(6.3 μg/L)	(104.6)	(107.4)	(99.4)	(118.7)	(113.2)	(92.1)	(105.1)	(117.5)	(110.3)	(114.0)	(90.0)	(134.9)	(105.1)	(114.0)
IPT – 188A	13.13	16.0	15.43	14.23	14.03	13.80	13.24	15.40	13.51	16.33	12.56	18.57	13.24	16.0
(13.8 μg/L)	(95.2)	(115.9)	(111.8)	(103.1)	(101.7)	(100.0)	(95.9)	(111.6)	(97.9)	(118.3)	(91.0)	(134.5)	(95.9)	(115.9)
IPT – 188C	4.37	4.93	4.23	5.39	4.90	3.93	4.56	5.13	4.98	4.85	4.48	5.07	4.56	4.93
(4.5 μg/L)	(97.1)	(109.6)	(94.1)	(119.8)	(108.8)	(87.4)	(101.3)	(114.1)	(110.7)	(107.8)	(99.5)	(112.6)	(101.3)	(109.6)

[a]2/3 results > measuring range (30 μg/L) therefore not used. IPT, international proficiency testing; PC ISD, PreciControl ISD.

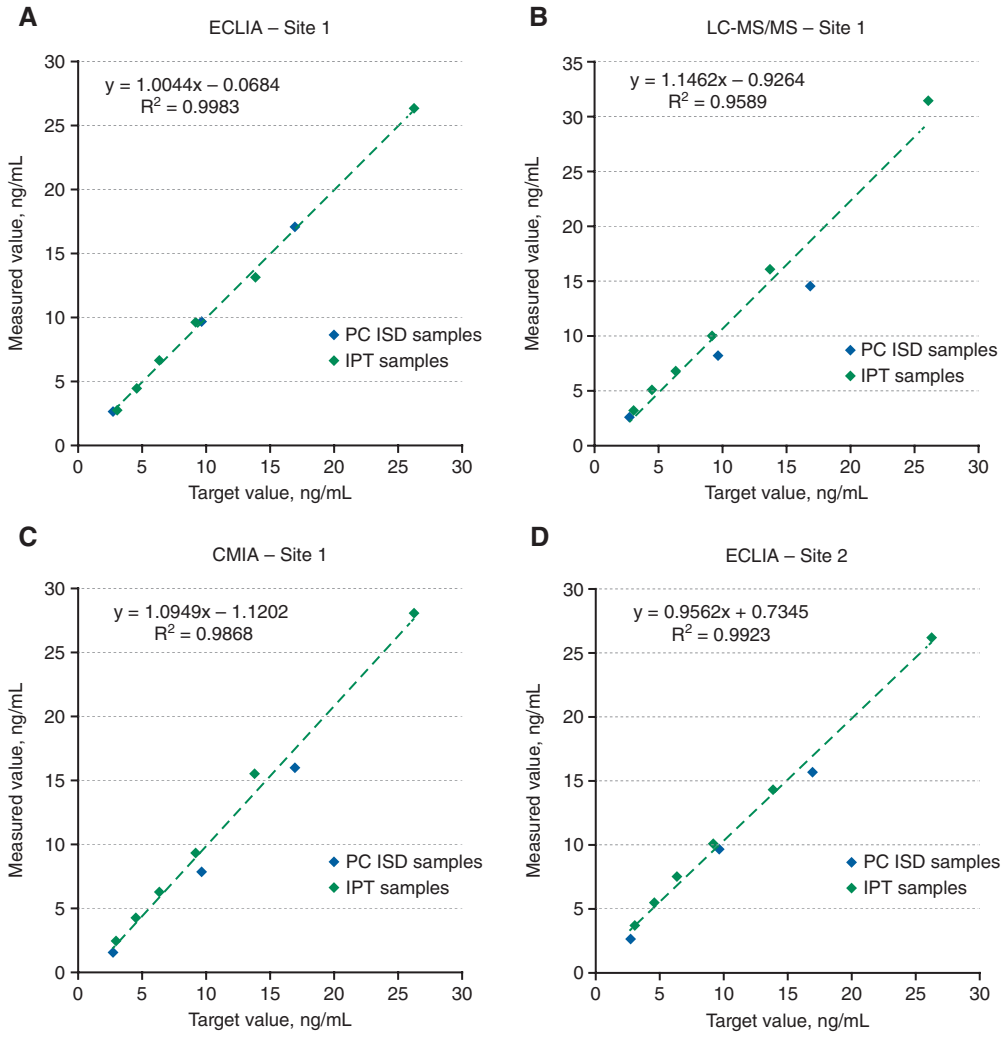

Figure 1: Linear regression analysis of inaccuracy test results for sirolimus concentration in whole blood against predetermined target values.
ECLIA, electrochemiluminescence assay; IPT, international proficiency testing; LC-MS/MS, liquid chromatography-tandem mass spectrometry; PC ISD, PreciControl ISD.

Assay imprecision

With respect to repeatability, estimated from pooling data from all four sites, the SDs of the lower concentration control samples (PC ISD L1 and HSP2; 0.5–3.0 µg/L) were ≤0.173 µg/L (Table 3). Repeatability CVs for the samples containing the higher concentrations of sirolimus (PC ISD L2-3 and HSP3-6; 3–30 µg/L) were ≤6.4%. Corresponding results for intermediate precision were SDs of ≤0.288 µg/L for the control samples containing low concentrations of sirolimus, whereas for the control samples in the higher concentration ranges, the intermediate precision CVs were ≤10.7%.

Reproducibility results are displayed in Table 4. For samples of low sirolimus concentration (≤3.0 µg/L), the SDs were ≤0.311 µg/L, and for the higher concentration samples (3.0–30.0 µg/L), the CVs were all ≤12.7%.

Functional sensitivity

Figure 2 shows a plot of CVs determined from single measurements of nine evaluable pools spiked with sirolimus over ten runs in 5 days (two runs per day) at each concentration. Two of the 11 samples fell below 0.3 µg/L, the LoB for sirolimus, and therefore were not included. Across the concentration range of 0.5–2.5 µg/L sirolimus, CVs were low and did not go above 20.0%. Therefore, the CV at the LoD (<0.5 µg/L) of the assay was determined as the functional sensitivity. This was calculated as 10.9% at 0.5 µg/L.

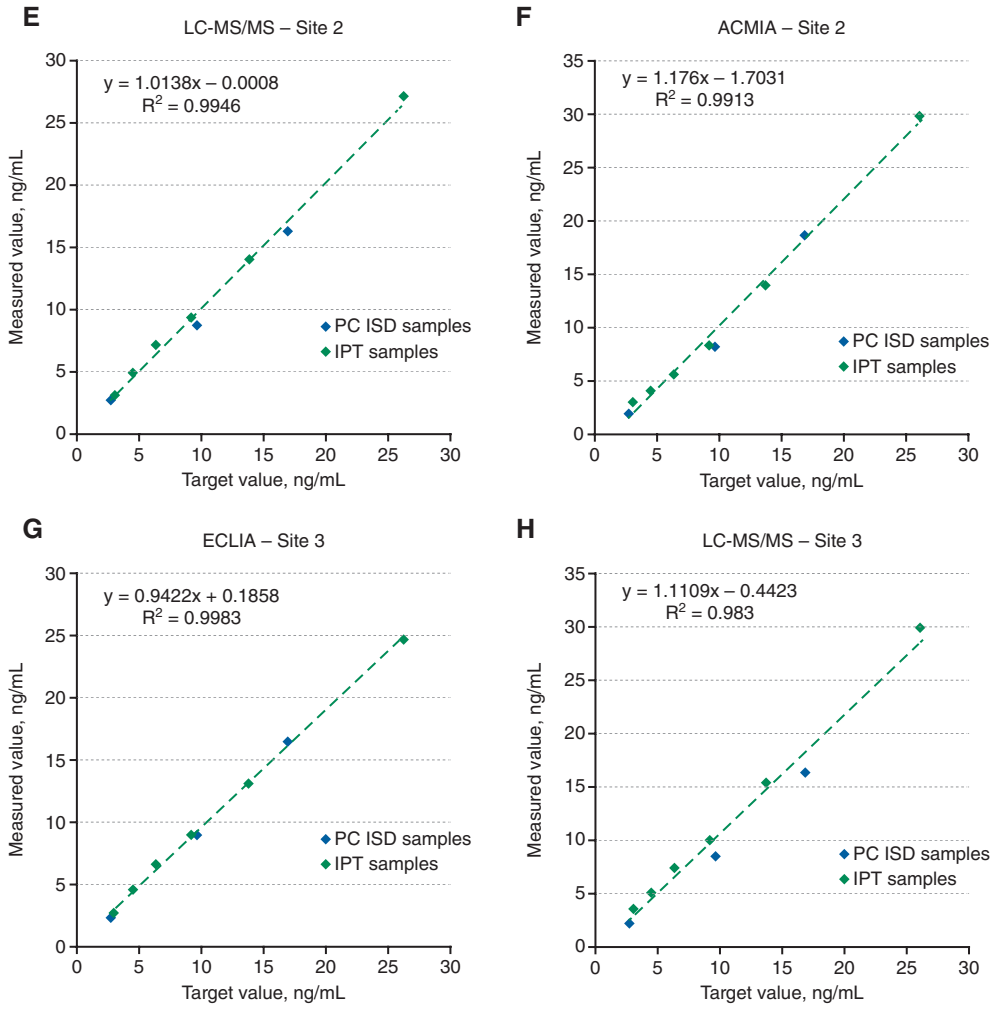

Figure 1 (continued)

CVs at 1.0 µg/L and 1.5 µg/L (i.e. LoQ) were similarly low, with values of 8.6% and 7.0%, respectively.

Method comparison experiments

For the ECLIA and LC-MS/MS method comparison experiments, a total of 402 EDTA whole blood samples from kidney transplant patients were analyzed. Weighted Deming regression analysis of the ECLIA vs. LC-MS/MS based on these 402 samples yielded a slope of 1.05 (95% CI, 0.981–1.12; Figure 3A), an intercept of 0.154 µg/L (95% CI, −0.253–0.561) and a Pearson's correlation coefficient (r) of 0.94. Bland-Altman analysis showed a combined mean bias of 0.41 µg/L (±2 SD, −1.96–2.68) (Figure 3B).

These results demonstrate a high level of agreement between ECLIA and LC-MS/MS, which was the reference method used in this study. Method comparison results for the ECLIA vs. LC-MS/MS from each individual site are displayed in Supplementary Figure 1A–E. The sample size of the individual analysis was not always sufficient for robust regression analysis, but general trends are visible.

The therapeutic range for sirolimus is between 4 and 12 µg/L for patients receiving CNIs and between 12 and 20 µg/L for those without CNIs. The estimated relative biases at these medical decision points were 9.6% (95% CI, 7.2–13.7) at 4 µg/L, 6.3% (95% CI, 2.1–8.8) at 12 µg/L and 5.7% (95% CI, −0.2–8.9) at 20 µg/L.

The ECLIA was compared with the CMIA with EDTA whole blood samples from n = 137 kidney transplant patients at site 1. The comparison yielded a slope of 0.91 (95% CI, 0.825–0.997), an intercept of 0.112 µg/L (95% CI, −0.428–0.652) and a Pearson's correlation coefficient (r) of 0.89 (Figure 3C). The Bland-Altman difference plots showed a mean bias of −0.63 µg/L (±2 SD, −3.59–2.33) (Figure 3D).

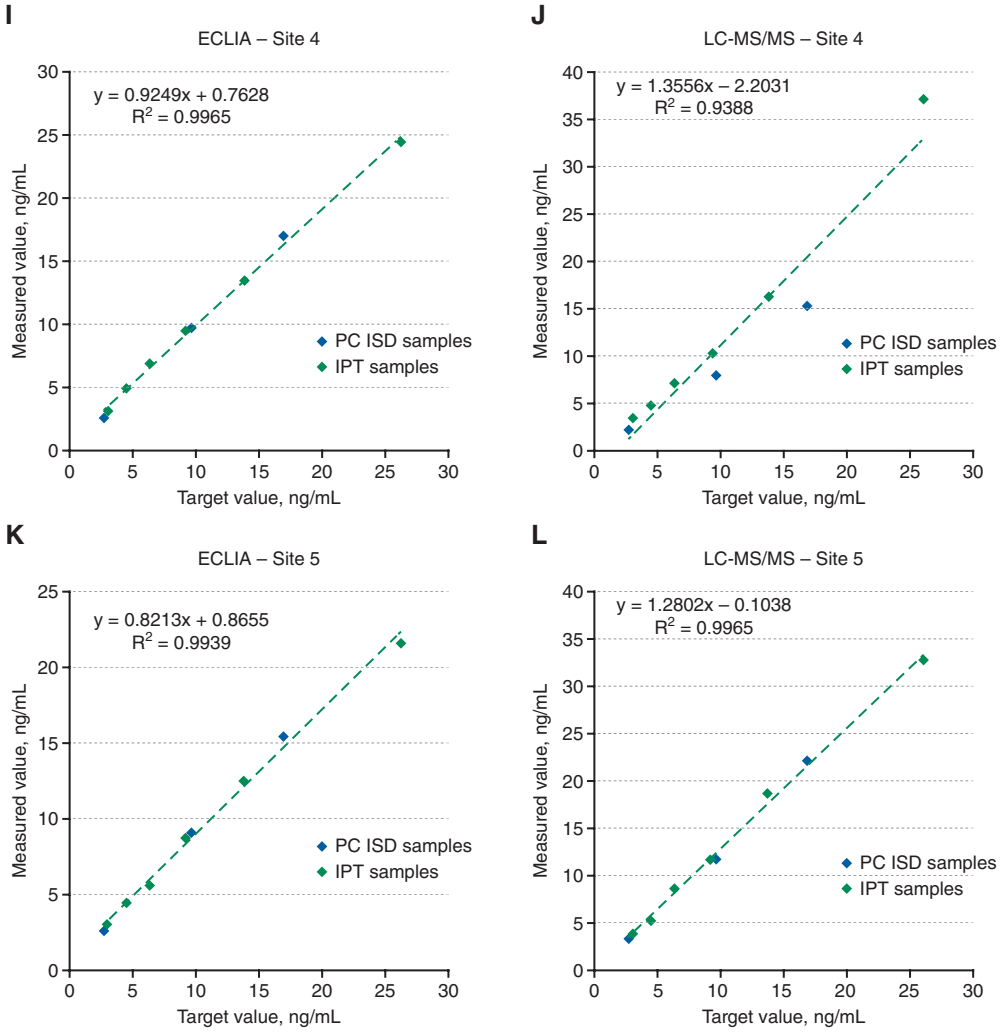

Figure 1 (continued)

Table 3: Repeatability and intermediate precision of the sirolimus ECLIA for all centers.

Sample	Mean conc., µg/L (measured range)	Repeatability, SD/CV (measured range)	Intermediate precision, SD/CV (measured range)
PC ISD L1 (target 2.66 µg/L)	2.679 (2.092–2.775)	0.163 µg/L (0.151–0.185)	0.264 µg/L (0.227–0.326)
PC ISD L2 (target 9.6 µg/L)	9.824 (9.718–9.962)	4.3% (3.1–5.0)	7.1% (4.2–9.6)
PC ISD L3 (target 16.9 µg/L)	17.378 (16.952–17.825)	3.4% (2.6–4.3)	6.3% (4.7–8.1)
HSP2 (target range 0.5–3 µg/L)	2.202 (2.092–2.284)	0.173 µg/L (0.110–0.228)	0.288 µg/L (0.187–0.437)
HSP3 (target range 3–8 µg/L)	5.560 (5.451–5.695)	6.4% (3.9–8.9)	10.7% (5.7–14.7)
HSP4 (target range 8–16 µg/L)	13.652 (13.425–13.847)	4.8% (3.3–6.0)	7.9% (4.5–11.1)
HSP5 (target range 16–24 µg/L)	21.416 (20.291–22.593)	4.8% (3.5–6.0)	8.9% (6.4–10.8)
HSP6 (target range 24–30 µg/L)	27.935 (26.094–28.872)[a]	5.0% (2.7–6.9)[a]	9.2% (7.1–11.3)[a]

[a]One site measured 16/84 samples as >30 µg/L, and these data could not be evaluated and could not be used for the pooled analysis.
HSP, human sample pool; PC ISD, PreciControl ISD.

Comparison of the ECLIA with the ACMIA using EDTA whole blood native samples from n = 119 kidney transplant patients was performed at site 2. The experiments yielded a slope of 0.99 (95% CI, 0.859–1.13), an intercept of 0.319 µg/L (95% CI, –0.491–1.13) and a Pearson's correlation coefficient of 0.97 (Figure 3E). The mean bias was 0.18 µg/L (±2 SD, –1.78–2.14) using Bland-Altman analysis (Figure 3F).

Table 4: Lot-to-lot and site-to-site variability and reproducibility of the sirolimus ECLIA at three participating centers.

Sample	Mean conc., µg/L	Lot-to-lot [SD/CV]	Site-to-site [SD/CV]	Reproducibility [SD/CV]
PC ISD L1 [target 2.66 µg/L]	2.770	0.038 µg/L (0.035 µg/L)[a]	0.234 µg/L (0.230 µg/L)[a]	0.311 µg/L (0.313 µg/L)[a]
PC ISD L2 [target 9.6 µg/L]	9.541	<0.1% (<0.1%)[a]	6.5% (6.5%)[a]	9.2% (9.3%)[a]
PC ISD L3 [target 16.9 µg/L]	16.874	<0.1% (<0.1%)[a]	6.9% (7.2%)[a]	9.7% (10.3%)[a]
HSP2 (range 0.5–3 µg/L)	2.218	0.038 µg/L	0.202 µg/L	0.286 µg/L
HSP3 (range 3–8 µg/L)	5.424	<0.1%	10.2%	12.7%
HSP4 (range 8–16 µg/L)	13.093	<0.1% (<0.1%)[a]	5.4% (6.1%)[a]	8.9% (12.9%)[a]
HSP5 (16–24 µg/L)	20.397	<0.1% (<0.1%)[a]	6.1% (6.1%)[a]	9.9% (10.0%)[a]
HSP6 (24–30 µg/L)	26.391	<0.1% (<0.1%)[a]	6.4% (6.2%)[a]	11.3% (11.3%)[a]

[a]Initial value with outlier. HSP, human sample pool; PC ISD, PreciControl ISD.

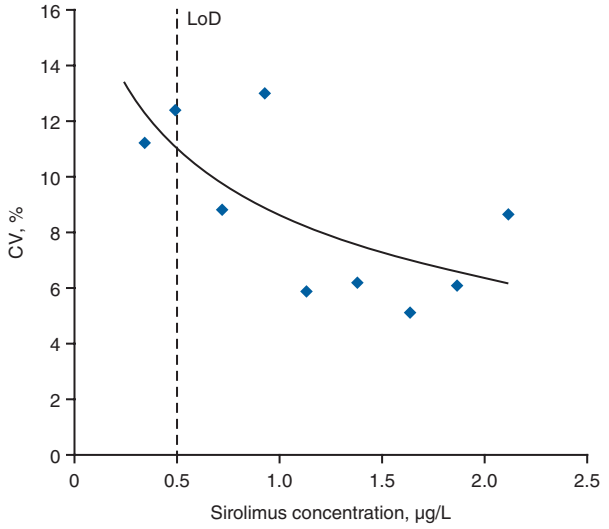

Figure 2: Functional sensitivity experiments. CVs of the sirolimus ECLIA are displayed across a range of sirolimus concentrations. CV, coefficient of variation; ECLIA, electrochemiluminescence assay; LoD, limit of detection.

Discussion

This was a multicenter evaluation of the analytical performance of an ECLIA for measurement of sirolimus whole blood concentrations to determine its suitability for routine TDM in the transplant setting.

For the inaccuracy experiments, the ECLIA met the acceptability criteria of a linear regression slope within ±10% of the theoretical value of 1.0, and the linear regression intercept did not differ significantly from zero. These results are in line with the recent Immunosuppressive Drug Scientific Committee of the International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT)-specified guidance [7] (Figure 1). When observing the regression analyses of the individual centers, the slopes were slightly higher than ±10% from 1.0 for some of the LC-MS/MS tests and the ACMIA. However, it must be noted that the IATDMCT guidelines refer to native samples, and we used spiked materials (PC ISD and IPT samples) for these comparisons. In addition, each center used a different LC-MS/MS system – with some differences between calibrators, column and spectrometer set-up; therefore, differences in recovery rates were not unexpected.

According to recommendations recently issued by the IATDMCT, assay precision is vital to support consistent dosing, and so a CV of ≤10% or even ≤6% should be achieved with assays for immunosuppressive drugs [7]. The therapeutic range for sirolimus is generally between 4 and 12 µg/L when given with ciclosporin or tacrolimus and between 12 and 20 µg/L when used alone [3]. Results from the present study showed that, generally, measurements with the ECLIA within these therapeutic ranges (4–20 µg/L) comfortably met these criteria. For the control samples with target concentrations within this therapeutic range, the repeatability results were all <6%, and the intermediate precision results were all <10%. In the cases where the repeatability or intermediate precision results exceeded the IATDMCT recommendations, these were the samples in lower concentrations outside the normal sirolimus therapeutic range.

Results from the functional sensitivity experiments showed that the measurement range of the ECLIA was appropriate for the span of drug concentrations observed in samples typically seen in routine clinical practice. In its recent guidance, the IATDMCT stressed the importance of the functional sensitivity of assays for immunosuppressive drugs given the very low target ranges for drugs that are now used to reduce long-term toxicity [7]. The IATDMCT suggests that to detect inappropriate low dosing or patient non-adherence, assays should be able to measure sirolimus at a concentration of 1.0 µg/L to allow for meaningful TDM at 2–3 µg/L [7]. As this multicenter evaluation demonstrated, the ECLIA had CV values

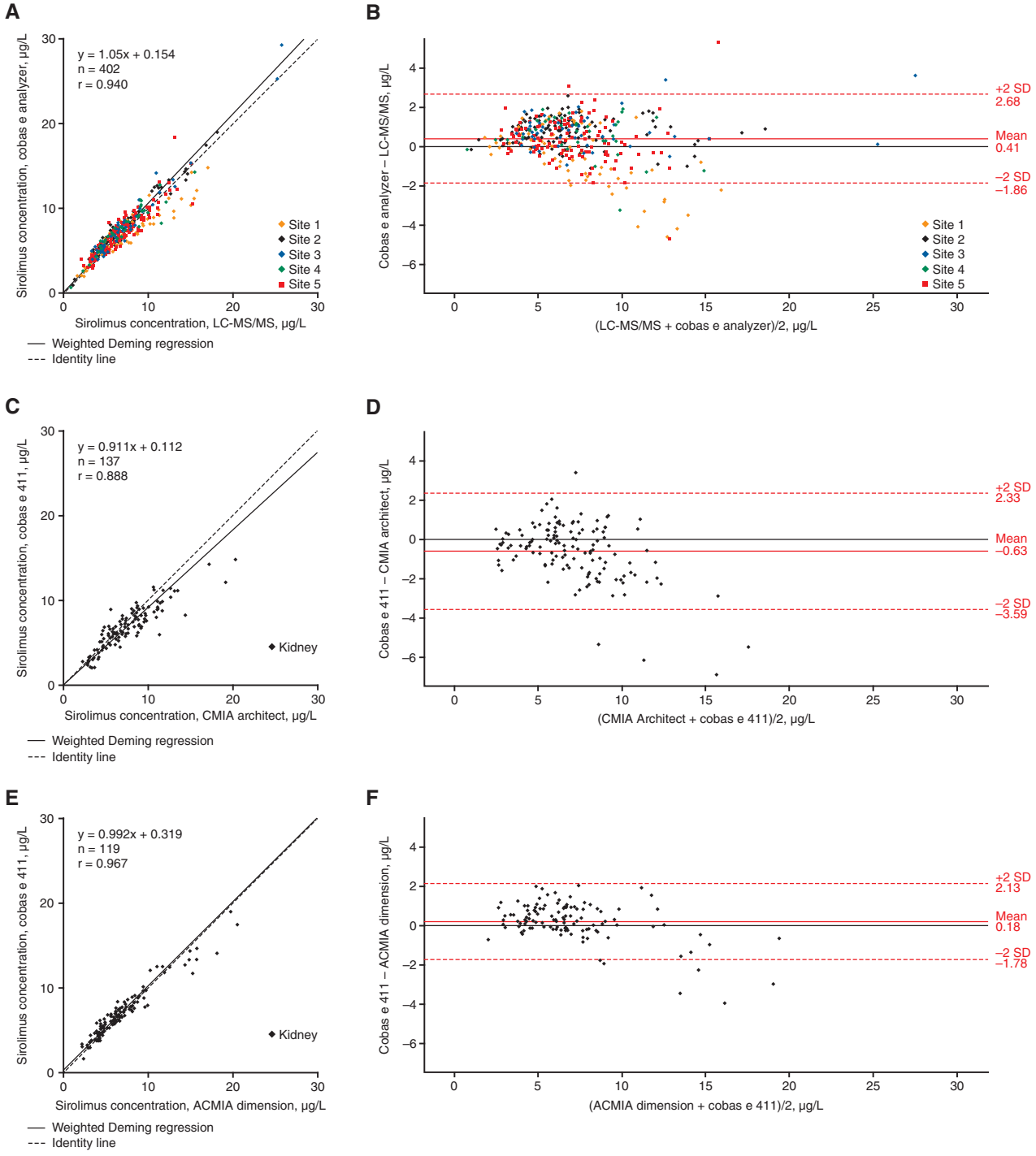

Figure 3: Weighted Deming regression and Bland-Altman plots of the ECLIA vs. LC-MS/MS methods at all participating sites (A and B), vs. CMIA at site 1 (C and D) and ACMIA at site 2 (E and F) using native samples from kidney transplant patients.
ACMIA, antibody-conjugated magnetic immunoassay; CMIA, chemiluminescent microparticle immunoassay; ECLIA, electrochemiluminescence assay; LC-MS/MS, liquid chromatography-tandem mass spectrometry.

of 10.9% at a sirolimus concentration of 0.5 µg/L and approximately 8.6% at a sirolimus concentration of 1.0 µg/L and, therefore, met these low detection limits.

Because the administered dose of sirolimus is a poor predictor of total drug exposure in any given transplant recipient, it is essential that concentrations in blood be

monitored on a regular basis. Highly sensitive analytical procedures are needed because of the relatively low whole blood concentrations obtained with the doses of sirolimus used for immunosuppression. Despite their complexity, LC-MS/MS methods have been widely adopted because they possess the requisite sensitivity and specificity. The weighted Deming regression analyses of the ECLIA in comparison with the reference LC-MS/MS showed excellent agreement between the two test procedures, based on pooled results from all five centers. LC-MS/MS is predominantly a laboratory-developed test, and as noted above, different calibrators and LC-MS/MS set-ups were used in each laboratory (e.g. liquid chromatography protocol, detectors, reagents, etc.) and may be responsible for the considerable interlaboratory variability with LC-MS/MS in the present study. Overall, the results of method comparisons with LC-MS/MS on samples from kidney transplant patients suggest that laboratories can have confidence in switching from LC-MS/MS to the new ECLIA with the proviso that potential sources of precision and accuracy are addressed.

Single-center comparisons between the ECLIA vs. CMIA and the ECLIA vs. ACMIA for sirolimus showed generally good comparability. Immunoassays are, to varying degrees, affected by cross-reactivity, especially from metabolites of the parent drug and structurally related compounds. This can lead to overestimation of drug levels and potential for dosing inaccuracies [8, 29]. The Architect CMIA for sirolimus, for example, has a positive bias of ~15%–20% relative to LC-MS/MS methods due to antibody cross-reactivity with sirolimus metabolites [16], whereas a positive bias has also been seen with the Dimension ACMIA for sirolimus [19]. We also observed a slope deviation for the immunoassays compared with LC-MS/MS, which was expected. Laboratories performing routine TDM need to be cognizant of the fact that assays used to measure trough concentrations of sirolimus are not always directly interchangeable. Switching to a new method for TDM of sirolimus should be done with care and following discussions with clinicians involved in the care of transplant patients. Clinicians should also be aware of the potential for cross-reactivity when patients are switched to sirolimus from a structurally related drug such as everolimus, which may initially lead to an overestimation of sirolimus levels [8].

In conclusion, the results of this large multicenter evaluation of a new ECLIA show that it can be used for TDM of sirolimus in kidney transplant recipients and offers practical advantages over LC-MS/MS, especially with respect to ease of performance and time taken to perform analyses. In its recent guidance, the IATDMCT suggests that a period of 3–6 h represents an ideal total turnaround time for routine TDM [7], something that can be readily achieved with the ECLIA.

Acknowledgments: The authors would like to acknowledge the technical support provided by Mrs. Sonja Rapp and Susanne Schneider, who performed the measurements in Stuttgart, and by Melanie Vantieghem, Lisa Florin and Grégoire Fauvarque, who performed the measurements in Ghent. Thanks also to Christian Schneider and Verena Hoofman, Roche Diagnostics GmbH, for their review of the manuscript and for their contribution to the study.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: The participating sites received funding from Roche Diagnostics to perform this study. This study was funded by Roche Diagnostics GmbH. Medical writing support was provided by Connect2 CME Limited, Emma McConnell of Gardiner-Caldwell Communications (GCC) and Kim Brown of Roche Diagnostics International Ltd and was funded by Roche Diagnostics.

Employment or leadership: A. Schuetzenmeister is an employee of Roche Diagnostics GmbH. C Schmiedel was a study manager at IST, which was contracted by Roche Diagnostics GmbH.

Honorarium: M. Vogeser has received honoraria from Roche Diagnostics for presentations with a commercial background.

Competing interests: The funding organization played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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Supplemental Material: The online version of this article offers supplementary material (https://doi.org/10.1515/cclm-2017-0583).