Multisite Analytical Evaluation of the Abbott ARCHITECT Cyclosporine Assay

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Abstract: The objective of this study was to evaluate the analytical performance of the Abbott ARCHITECT Cyclosporine (CsA) immunoassay in 7 clinical laboratories in comparison to liquid chromatography/tandem mass spectrometry (LC/MS/MS), Abbott TDx, Cobas Integra 800, and the Dade Dimension Xpand immunoassay. The ARCHITECT assay uses a whole blood specimen, a pretreatment step with organic reagents to precipitate proteins and extract the drug, followed by a 2-step automated immunoassay with magnetic microparticles coated with anti-CsA antibody and an acridinium-CsA tracer. Imprecision testing at the 7 evaluation sites gave a range of total % coefficient of variations of 7.5%–12.2% at 87.5 ng/mL, 6.6%–14.3% at 411 ng/mL, and 5.2%–10.7% at 916 ng/mL. The lower limit of quantification ranged from 12 to 20 ng/mL. Purified CsA metabolites AM1, AM1c, AM4N, AM9, and AM19 were tested in whole blood by the ARCHITECT assay and showed minimal cross-reactivity at all 7 sites. In particular, AM1 and AM9 cross-reactivity in the ARCHITECT assay, ranged from −2.5% to 0.2% and −0.8% to 2.2%, respectively, and was significantly lower than for the TDx assay, in which the values were 3.2% and 16.1%, respectively. Comparable testing of metabolites in the Dade Dimension Xpand assay at 2 evaluation sites showed cross-reactivity to AM4N (6.4% and 6.8%) and AM9 (2.6% and 3.6%) and testing on the Roche Integra 800 showed cross-reactivity to AM1c (2.4%), AM9 (10.7%), and AM19 (2.8%). Cyclosporine International Proficiency Testing Scheme samples, consisting of both pooled specimens from patients receiving CsA therapy as well as whole-blood specimens supplemented with CsA, were tested by the ARCHITECT assay at 6 sites and showed an average bias of −24 to −58 ng/mL versus LC/MSMS CsA and −2 to −37 ng/mL versus AxSYM CsA. Studies were performed with the ARCHITECT CsA assay on patient specimens with the following results: ARCHITECT CsA assay versus LC/MSMS, average bias of 31 ng/mL; ARCHITECT versus the Dade Dimension assay (4 sites), average biases of −7 to −228 ng/mL; ARCHITECT versus AxSYM and TDx, average biases of −4 and −53 ng/mL, respectively. Spearman correlation coefficients were ≥0.89. The ARCHITECT CsA assay has significantly reduced CsA metabolite interference relative to other immunoassays and is a convenient and sensitive semiautomated method to measure CsA in whole blood.

Key Words: cyclosporine, immunoassay, LC/MS/MS, method evaluation, therapeutic drug monitoring

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INTRODUCTION

Cyclosporine (CsA, also referred to as cyclosporin or ciclosporin) is a powerful immunosuppressive drug that has been widely used therapeutically to prevent solid organ rejection after transplantation. CsA has a narrow therapeutic range and many clinical laboratory assay methods have been developed to monitor the concentration of CsA in whole blood after transplant surgery and during lifetime immunosuppressive therapy.[1] Early nonspecific CsA immunoassay methods using polyclonal antibodies[2] showed extensive cross-reactivity with CsA metabolite present in trough blood samples and have not been well accepted for use in monitoring transplant patients, mainly due to the absence of significant clinical activity for most CsA metabolites. More specific liquid chromatography (LC) methods provided good separation of parent drug from metabolites, but were slow and labor intensive.[3] Improvements in blood extraction methods and automated tandem mass-spectrometry (MS/MS) detection[4,5] have reduced method complexity and now allow the rapid simultaneous measurement of multiple transplant drugs. LC/MS/MS methods for CsA are now used by approximately 19% of laboratories reporting in a large European CsA proficiency testing program[6] with CsA immunoassay methods making up the remaining ~81%. Several commercial monoclonal antibody methods with low to moderate CsA metabolite cross-reactivity have been developed using fluorescence polarization and enzyme activity.[7–10] The Abbott TDx Monoclonal and AxSYM Cyclosporine assays have been evaluated in clinical practice[11,12] and are widely used in clinical laboratories, despite cross-reactivity to 2 major metabolites, AM1 (M17) and AM9 (M1). An automated enzyme immunoassay method with lower CsA metabolite cross-reactivity, the

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Dade Dimension CsA assay, has also been evaluated[13] and is widely used.

The objective of the current multicenter study was to evaluate the clinical laboratory performance of the Abbott ARCHITECT CsA immunoassay with whole-blood samples spiked with cyclosporine metabolites, proficiency testing samples, and clinical specimens from a mixed population of organ transplant recipients.

MATERIALS AND METHODS

A multisite study of the ARCHITECT CsA assay was conducted to evaluate analytical performance at 6 European laboratory sites located in Austria (AU, Medical University, Vienna, Austria); Belgium (BE, U.C.L. Cliniques universitaires Saint-Luc, Brussels, Belgium); France (FR, CHU Limoges, Limoges, France); Germany (GE, Medizinische Hochschule, Hannover, Germany), Italy (Molinette Hospital, Torino, Italy); Switzerland (SW, Institute of Clinical Chemistry, Bern, Switzerland); and 1 site in the United States (Fujirebio Diagnostics, Malvern, PA). Patient specimen testing was performed using surplus samples under local institution ethics approval for patient informed consent and confidentiality. Test results were not used to monitor therapy.

Immunoassay Methods

In the ARCHITECT CsA assay methodology, blood specimens were pretreated according to the manufacturer's instructions by rapidly vortex mixing 200 μL of EDTA blood with 100 μL of a solubilization reagent and 400 μL of precipitation reagent containing methanol, saponin, and zinc sulfate, followed by centrifugation to remove precipitated protein and cell debris. The clear supernatant was tested on the ARCHITECT instrument according to the manufacturer's instructions. The instrument combines the blood extract with magnetic microparticles coated with mouse anti-CsA antibody, followed by a wash step and then incubation with a CsA-acridinium tracer. After a second wash step, the chemiluminescent signal is measured and the CsA concentration is calculated from calibration information stored in the memory of the instrument. CsA calibrators were tested in duplicate at 0, 40, 150, 400, 800, and 1500 ng/mL to establish the calibration information. The mouse monoclonal anti-CsA antibody used in this assay is the same one that is used in the Abbott TDx fluorescence polarization assay (Abbott Diagnostics, Abbott Park, IL). The TDx (Abbott Diagnostics), Cobas Integra 800 (Roche Diagnostics GmbH, Mannheim, Germany), and Dade Dimension Xpand (Siemens Healthcare Diagnostics, Tarrytown, NY) instruments and cyclosporine assays were used according to the manufacturer's instructions.

LC/MS/MS Methods

CsA determinations at the FR site were performed using turbulent flow chromatography– MS/MS (Limoges CHU, Limoges, France). Briefly, online extraction was performed at a high flow rate (1.25 mL/min) on a Cyclone P, 50-μm particle size (50 × 0.5 mm internal diameter [I.D.]) column (Cohesive technologies, Milton Keynes, UK) in alkaline conditions. Chromatographic separation was performed in acidic conditions using a Propel C18 MS, 5 μm (50 × 3.0 mm I.D.) column (Cohesive technologies, Milton Keynes, UK) kept at 60°C, with a constant flow rate of 300 μL/min. Detection was performed using a TSQ Quantum Discovery MS/MS system (Thermo-Fisher, Les Ulis, France) equipped with an orthogonal electrospray ionization source and controlled by the Xcalibur computer program. MS/MS detection was performed in the positive ion, multiple reaction monitoring mode following 3 transitions for CsA (m/z 1220 → 1203; m/z 1220 → 1185 and m/z 1220 → 425) and for the internal standard (IS) cyclosporine D (m/z 1234 → m/z 1234 → 1199). This method was fully validated for CsA determination in whole blood. The limit of quantitation (LOQ) was 20 μg/L and the calibration curves obtained using quadratic regression from the LOQ up to 2000 μg/L yielded correlation coefficients better than 0.99. The interassay bias was between −3.8% and 6.4% and the coefficient of variation between 4.9% and 7.1%.

CsA determinations at the US site were conducted following a method (eDOCS, CPWB) previously validated under Covance 2100-635 (Covance Laboratories, Madison, WI). Cyclosporin A and the IS were extracted from samples using liquid–liquid extraction. After evaporation under nitrogen, the residue was reconstituted and analyzed using LC/MS/MS. The standard curve range is from 5.00 to 2000 ng/mL for CsA, using a whole-blood sample volume of 0.100 mL. The interbatch accuracy and precision for 3 levels of quality controls were as follows: low (15.0 ng/mL), 95.3% and 10.1%; mid (150 ng/mL), 96.0% and 7.7%; high (1500 ng/mL), 98.7% and 9.9%, respectively. The LOQ for the method was 5.0 ng/mL. For linearity, the method is linear using $1/X^2$ regression and the correlation coefficient was 0.997–0.999.

Antibody Specificity

Metabolite cross-reactivity was tested using whole-blood specimens containing purified CsA metabolites AM1, AM1C, AM4N, AM9, and AM19 on the ARCHITECT, TDx, Cobas Integra 800, and Dade Dimension Xpand. CsA metabolites AM9 and AM4N were obtained from Sandoz Pharmaceuticals (now Novartis Pharma Inc, Basel, Switzerland). AM19 and AM1C metabolites purified from human bile were obtained from Dr Randy Yastcoff (University of Alberta, Edmonton, Canada). Metabolite AM1 was chemically synthesized as follows at Abbott Laboratories. CsA was acetylated at the position 1 hydroxyl group with acetic anhydride/dimethylaminopyridine to make cyclosporine acetate. The methyl group of the acetylated material was then brominated with N-bromosuccinimide/azobis-isobutyronitrile to make the allylic bromide. The bromide was reacted with tetraethylammonium acetate/potassium iodide/methylethylketone to form the acetate. Hydrolysis of the diacetate using sodium methoxide in methanol produced AM1. Metabolite concentrates prepared in organic solvent were diluted to 1000 ng/mL in whole-blood samples which had been supplemented with CsA at ~200 ng/mL. Metabolite and organic solvent control samples were tested in replicates of 5 on the ARCHITECT i2000. The measured CsA difference (ng/mL) between the mean values for the metabolite supplemented and control samples was divided by 1000 (the metabolite concentration in ng/mL) and multiplied by 100 to convert the result to percent

cross-reactivity. Stability and characterization data for the metabolite samples was unavailable.

Imprecision

Assay imprecision was evaluated following the Clinical and Laboratory Standards Institute protocol EP5-A2[14] using 3 Lyphochek Whole Blood Immunosuppressant Controls, Levels 1, 2, 3 (Bio-Rad Laboratories, Hercules, CA). Each replicate was pretreated separately. The European study sites performed testing on 4 replicates of each control over 5 days (n = 20 per control). The US study site ran the study over 20 days (n = 80 per control). The total % coefficient of variation (CV) includes variance components due to within-run, between-run, and between-day assay imprecision.

Limit of Detection

Limit of detection (LOD), or analytical sensitivity, was evaluated at each European site on 1 ARCHITECT instrument with n = 4 runs, 10 replicates of calibrator A (0 ng/mL) and 4 replicates of calibrator B (40 ng/mL, prepared gravimetrically). At the US site, LOD was evaluated on 3 ARCHITECT instruments, with 2 runs per instrument (n = 6 runs) using 10 replicates of calibrator A (0 ng/mL) and 5 replicates of calibrator B (40 ng/mL).

LOD was calculated as follows[15]:

$$\mathrm{LOD} = 2 \times \mathrm{SD}_{\text{A calibrator signal}} \times [40\,\mathrm{ng/mL}/(\text{A calibrator signal} - \text{B calibrator signal})]$$

Limit of Quantification

Limit of quantification (LOQ), or functional sensitivity, was calculated using a 7-member panel of whole-blood specimens prepared centrally; the samples were spiked with CsA to achieve nominal concentrations from 5 to 50 ng/mL. A CsA stock solution at 96.0 ng/mL was prepared in EDTA whole blood and was used to spike CsA-free EDTA whole blood. After the specimens were prepared they were frozen, thawed, and tested in replicates of 5 on an ARCHITECT Instrument. Each replicate for each specimen tested was pretreated separately before running the assay. The mean bias of measured over nominal concentration for the entire panel ranged from 90% to 120%. The European sites ran all 7 of the specimens in replicates of 10 on 2 separate days (n = 20 total replicates). The US site ran all 7 specimens in replicates of 10, 2 runs per day on 5 separate days (n = 100 total replicates). The %CV for each specimen was plotted versus concentration using a reciprocal curve fit. LOQ was calculated as the CsA concentration corresponding to a CV of 20%.

Dilution Linearity

The ARCHITECT CsA assay was designed to have a mean recovery of 100% ± 10% of the expected results for diluted samples. A dilution linearity study was performed at the US site by diluting 3 different CsA specimens in the range 241.2–1302.9 ng/mL, diluted with ARCHITECT CsA calibrator A, to approximately 60%–20% of their original concentrations. The concentration of CsA was back calculated for each dilution, and the percentage recovery was calculated as (calculated concentration/original concentration) × 100.

Potentially Interfering Endogenous Substances

Whole-blood specimens with CsA concentrations between 70 and 900 ng/mL were prepared with the following, potentially, interfering substances: high triglycerides, 15 g/L; low and high hematocrit, 25%–55%; high bilirubin, 400 mg/L; low and high total protein, 30–120 g/L; high cholesterol, 5 g/L; high uric acid, 200 mg/L. The study, performed at the US site, was based on guidance from CLSI document EP7-A2.[16]

Proficiency Testing Protocol

Single replicates of 35 samples from the Cyclosporine International Proficiency Testing Scheme (CIPTS, available from Analytical Services International Ltd, London, UK) were tested at all 6 European evaluation sites. CsA concentration results were compared with LC/MS/MS and AxSYM historical results available from previous proficiency surveys (www.bioanalytics.co.uk, accessed Nov 2006). CsA concentrations in the proficiency testing samples varied from approximately 250 to 1400 ng/mL. Some samples were derived from pools (n = 5) of blood from transplant recipients and contained CsA metabolites, but most were metabolite-free whole-blood specimens (n = 30) supplemented with CsA.

Method Comparison

Surplus patient EDTA whole-blood specimens (n = 848) were obtained at all sites using local ethics procedures and tested on the ARCHITECT versus an LC/MS/MS method at the FR site and the Dade Dimension Xpand method at the AU, BE, GE, and IT sites. Immunoassays were tested according to the manufacturer's procedures. The LC/MS/MS methods were performed by validated procedures determined by individual labs for use on transplant samples. CsA blood specimens from the FR and IT sites were evenly distributed across a concentration range of 20–1500 ng/mL. These specimens were sourced from the following allograft recipients: bone marrow, n = 83; heart, n = 138; kidney, n = 402; liver, n = 167; lung, n = 45; heart/lung, n = 1; kidney/pancreas, n = 1; kidney/liver, n = 6; and other, n = 5. Specimens at the AU, BE, and GE sites were clustered primarily in the trough concentration range (50–300 ng/mL). ARCHITECT test results were not used to monitor therapy or make clinical decisions.

Statistical Methods

The lower LOQ analysis was performed with Sigma Plot analysis software (version 6.0, Systat Software Inc, San Jose, CA). Correlation and bias statistics were performed with Analyse-it statistical analysis software (version 1.71, Analyse-it Software Ltd, Leeds, UK), using Spearman coefficients and Passing–Bablok correlations.[17]

RESULTS

Antibody Specificity

Table 1 compares metabolite cross-reactivity for the ARCHITECT, Dimension, Cobas, and TDx Cyclosporine immunoassays as determined by testing at the evaluation sites. The ARCHITECT assay showed minimal cross-reactivity to CsA metabolites with a mean % cross-reactivity ranging from

TABLE 1. Comparison of Cyclosporine Immunoassay Metabolite Cross-Reactivity*

Assay	Test Site	Metabolite % Cross-Reactivity				
		AM1	AM1c	AM4N	AM9	AM19
ARCHITECT	AU	−2.4	1.3	0.8	0.0	−0.2
ARCHITECT	BE	−0.3	−0.8	0.8	2.2	0.2
ARCHITECT	FR	0.2	−0.3	3.3	0.9	1.2
ARCHITECT	GE	−1.8	0.8	1.5	−0.1	1.3
ARCHITECT	IT	−1.8	−0.1	1.2	0.3	0.2
ARCHITECT	SW	−2.5	0.0	1.2	0.8	−0.2
ARCHITECT	US	−0.7	−0.8	−1.2	−0.8	0.7
Dimension	BE	−0.1	1.7	6.4	3.6	1.2
Dimension	GE	−1.5	−0.8	6.8	2.6	−0.1
Cobas	SW	0.9	2.4	−1.5	10.7	2.8
TDx	US	3.2	4.2	1.4	16.1	1.3

Specimens spiked with 200 ng/mL CsA were then spiked with solvent (matched control) or with 1000 ng/mL of each metabolite (panel) and run in replicates of 5. % Metabolite Cross-Reactivity = [(Panel mean) − (Matched control mean)/1000 ng/mL)] × 100.

–2.5% to 3.3% across 7 test sites. In contrast, metabolite cross-reactivity with the other immunoassays was higher relative to ARCHITECT, particularly for the metabolites AM1 (TDx, 3.2%), AM1c (TDx, 4.2%), AM4N [Dade Dimension (mean), 6.6%], AM9 [Dade Dimension (mean), 3.1%], and AM9 for Cobas and TDx, 10.7% and 16.1%, respectively.

Assay Imprecision

Assay imprecision observed at the 7 clinical sites is shown in Table 2. The total CV% for the low control (87.5 ng/mL) ranged from 7.5% to 12.2%, for the medium control (411 ng/mL) from 6.6% to 14.3%, and for the high control (916 ng/mL) from 5.2% to 10.7%.

Limits of Detection and Quantification

Analytical sensitivity (LOD) and functional sensitivity (LOQ) results are shown in Table 3. The LOD and LOQ for the ARCHITECT assay ranged from 5 to 12 ng/mL and 12 to 20 ng/mL, respectively, across 7 sites. Accuracy at the LOQ ranged from 89% to 107%.

TABLE 2. ARCHITECT Cyclosporine Assay: Imprecision Study Using Multiconstituent Controls (MCC)*

Clinical Site	MCC Level 1 (87.5 ng/mL)		MCC Level 2 (411 ng/mL)		MCC Level 3 (916 ng/mL)	
	Mean (ng/mL)	CV Total (%)	Mean (ng/mL)	CV Total (%)	Mean (ng/mL)	CV Total (%)
AU	70.3	7.5	382.9	14.3	855.2	9.5
BE	80.9	10.0	412.6	11.8	949.2	10.7
FR	74.9	7.8	384.8	9.7	846.8	8.4
GE	82.6	11.1	398.5	9.7	826.3	5.2
IT	82.6	8.5	343.4	6.6	764.4	6.8
SW	86.0	11.8	419.0	10.1	951.3	6.7
US	92.6	12.2	463.9	9.6	975.4	8.3

*The European sites performed testing on 4 replicates per day for 5 days (n = 20) and the US site ran the same study for 20 days (n = 80). CV total (%) includes variance components due to within-run, between-run, and between-day assay imprecision.

TABLE 3. ARCHITECT Cyclosporine Assay: LOD and LOQ

Site	LOD* (ng/mL)	LOQ† (ng/mL)
AU	8	20
BE	12	12
FR	8	12
GE	10	16
IT	12	18
SW	9	15
US	5	15

*LOD was evaluated at the European sites on 1 ARCHITECT instrument with n = 4 runs, 10 replicates of calibrator A (0 ng/mL, n = 40) and 4 replicates of calibrator B (40 ng/mL, n = 16). At the US site, LOD was evaluated on 3 ARCHITECT instruments, with 2 runs per instrument (n = 6 runs) using 10 replicates of calibrator A (0 ng/mL, n = 60) and 5 replicates of calibrator B (40 ng/mL, n = 30). LOD = 2 × $SD_{A\ calibrator\ signal}$ × [40 ng/mL/ (A calibrator signal − B calibrator signal)].

†LOQ was evaluated at the European sites by testing 7 specimens in replicates of 10 on 2 separate days (n = 20 total replicates). The US site tested 7 specimens in replicates of 10, 2 runs per day on 5 separate days (n = 100 total replicates). The %CV for each specimen was plotted versus concentration using a reciprocal curve fit. LOQ was calculated as the cyclosporine concentration corresponding to a 20% CV.

Dilution Linearity

Mean CsA recovery was 103% (range 97% to 114%) using 3 CsA whole-blood specimens in the range 241.2–1302.9 ng/mL, diluted with calibrator A (0 ng/mL). Results are shown in Table 4.

Endogenous Interferences

The average recovery observed during the interference study ranged from 97% to 108%, indicating no significant analytical interference from hematocrit (25%–55%), total

TABLE 4. ARCHITECT Cyclosporine Assay: Dilution/ Linearity Results*

Specimen	Dilution Factor	Observed Mean Concentration, ng/mL	Calculated Mean Concentration, ng/mL	% Recovery
1	Undiluted	1302.9	—	—
	1:1.67	799.3	1334.8	102
	1:2.00	652.0	1304.0	100
	1:2.50	562.8	1407.0	108
	1:5.00	297.1	1485.5	114
2	Undiluted	753.9	—	—
	1:1.67	448.6	749.2	99
	1:2.00	367.0	734.0	97
	1:2.50	313.7	784.3	104
	1:5.00	159.0	795.0	105
3	Undiluted	241.2	—	—
	1:1.67	146.3	244.3	101
	1:2.00	120.4	240.8	100
	1:2.50	96.7	241.8	100
	1:5.00	49.7	248.5	103

*Specimens were run in replicates of 5 either undiluted or diluted in calibrator A (0 ng/mL) and % recovery was calculated as follows: Calculated mean concentration = Observed mean concentration/Dilution factor.

% Recovery = (Calculated mean concentration/Undiluted observed mean concentration) × 100.

protein (30–120 g/L), triglycerides (15 g/L), cholesterol (5 g/L), bilirubin (400 mg/L), or uric acid (200 mg/L).

Proficiency Testing

ARCHITECT results for the 35 CIPTS samples tested at each of 6 sites are shown in Table 5 and are illustrated in Figure 1. The ARCHITECT assay results correlated well ($r \geq 0.98$) with previously reported historic values for LC/MSMS and AxSYM methods. Passing–Bablok correlation slopes ranged from 0.87 to 0.92 versus LC/MS/MS and from 0.94 to 1.00 versus AxSYM data. Intercept values ranged from −11 to 2 versus LC/MS/MS and from −20 to −5 versus AxSYM data. Average method biases ranged from −58 to −24 ng/mL for ARCHITECT versus LC/MS/MS and from −37 to −2 versus AxSYM.

Method Comparison

Results of ARCHITECT comparison testing on specimens from patients receiving CsA therapy at seven sites are shown in Table 6. A good correlation was observed between ARCHITECT, LC/MS/MS, TDx, AxSYM, and the Dade Dimension assays (range of Spearman coefficients r = 0.89–0.99). At the US site, where a comparison between ARCHITECT and TDx was performed, the population of patient samples was approximately divided equally between patients monitored at C_0 (trough blood concentrations) and C_2 (2 hours postdosing blood concentrations). Bland–Altman analysis of ARCHITECT versus TDx results on this mixed population, shown in Figure 2, which demonstrates clearly that the negative bias observed between ARCHITECT and TDx was more pronounced at lower whole-blood concentrations of CsA, that is, <500 ng/mL. Virtually no bias was observed between these assays above a concentration of 500 ng/mL.

Significant negative bias was observed at the IT site with the method comparison between ARCHITECT versus Dade Dimension: $y = 0.69$ * ARCHITECT + 26.1, $r = 0.93$, average bias = −76.2 ng/mL, n = 117. Unlike the AU, BE, and GE sites, the IT site ran a much larger number of C_2 specimens with a concentration >500 ng/mL. The nature of this high negative bias was further investigated by performing additional Passing–Bablok analyses on specimens with Dade Dimension results <200 ng/mL (n = 62), >200 ng/mL (n = 55), and >500 ng/mL (n = 32) at the IT site, as shown in Table 6. The high negative bias seen in the original Passing–Bablok analysis on all IT specimens (n = 117) seems to be attributable to specimens >200 ng/mL (average bias, −159 ng/mL) with even more negative bias observed in specimens >500 ng/mL (average bias, −228 ng/mL). In contrast, a negative bias was not observed in the method comparison of ARCHITECT versus LC/MS/MS performed at the FR site on a different set of patient specimens: $y = 1.10$ *ARCHITECT − 7, $r = 0.99$, average bias = 31 ng/mL, n = 122. Additional Passing–Bablok analyses on specimens with LC/MS/MS results <200 ng/mL (n = 50), >200 ng/mL (n = 72), and >500 ng/mL (n = 45) at the FR site are shown in Table 6. In contrast to the high negative bias results from the method comparison of ARCHITECT versus Dade Dimension at the IT site, a smaller positive bias was observed from the method comparison of ARCHITECT versus LC/MS/MS at the FR site.

TABLE 5. ARCHITECT Cyclosporine Assay: Comparison With Published CIPTS Proficiency Testing Results (ARCHITECT = Comparison Method*Slope + Intercept)

Site	Comparison	R	Slope	Intercept	Bias (ng/mL)
AU	LC/MS/MS	0.98	0.88	−11	−55
BE	LC/MS/MS	0.98	0.89	−10	−58
FR	LC/MS/MS	0.99	0.89	−1	−40
GE	LC/MS/MS	0.99	0.89	2	−35
IT	LC/MS/MS	0.99	0.87	−1	−43
SW	LC/MS/MS	0.99	0.92	−1	−24
AU	AxSYM	0.98	0.96	−20	−33
BE	AxSYM	0.98	0.96	−18	−37
FR	AxSYM	0.99	0.98	−8	−19
GE	AxSYM	0.98	0.97	−2	−14
IT	AxSYM	0.98	0.94	−4	−22
SW	AxSYM	0.99	1.00	−5	−2

DISCUSSION

CsA is a critical dose drug and therapeutic drug monitoring of patients on CsA therapy is required to achieve and maintain the delicate balance of toxicity versus efficacy required for safe and effective long-term immunosuppressive therapy. Measurement of the CsA concentration in whole blood is usually performed before the next dose is given (C_0) with a smaller number of laboratories performing C_2 monitoring. One of the challenges immunoassay based methods have had relative to LC/MS/MS methods, is the CsA metabolite cross-reactivity inherent with these assays when measuring trough concentrations in whole blood.[7–10] There are 3 lines of evidence presented in this work that demonstrate the improved specificity of the ARCHITECT CsA assay. First, using purified metabolites at several laboratory sites there was minimal cross-reactivity with these compounds in the ARCHITECT assay relative to the other immunoassays (see Table 1). Second, as shown in Figure 1, the negative bias of ARCHITECT to TDx results would be expected if the TDx assay cross-reacts with metabolites and ARCHITECT does not. The increasingly negative bias going from high to low CsA concentration probably reflects the higher relative concentration of metabolite to CsA often seen in trough blood specimens collected 12–24 hours after drug administration.[18] Third, only a small positive bias was observed between the ARCHITECT assay and LC/MS/MS assay methods during patient correlation studies (Table 6).

Assay imprecision studies (Table 2) and proficiency sample test results (Table 5) have demonstrated reproducibility of the ARCHITECT assay between 7 sites throughout Europe and the United States, and across 7 different instruments. The close agreement between proficiency test results at multiple testing sites is comparable with recent interlaboratory evaluations of the new ARCHITECT Tacrolimus and Sirolimus immunoassays[19,20] and demonstrates on the same instrument system the advantage of using common sets of standardized calibrators and pretreatment reagents across testing sites. Independently developed LC/MS/MS methods for TDM often

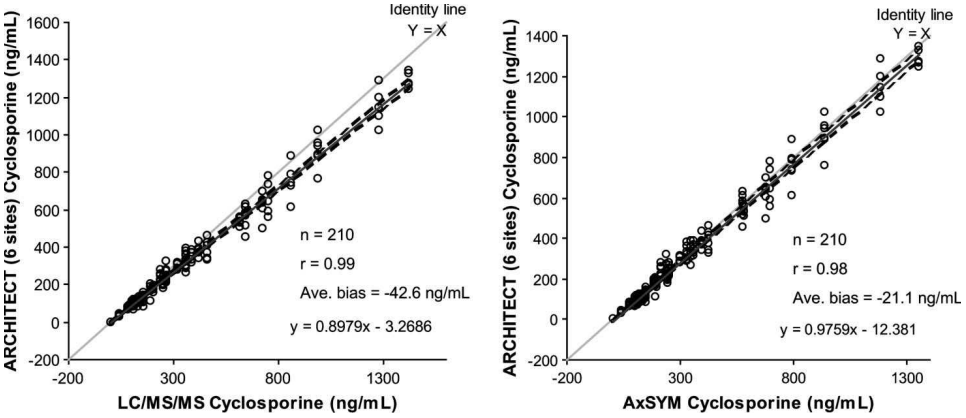

FIGURE 1. Comparative data for the ARCHITECT CsA assay and published mean results for the Ciclosporin International Proficiency Testing panel of samples by high-performance liquid chromatography/MS/MS and the AxSYM assays.

use different calibration standards, pretreatment methods, and IS, which has contributed to cases of poor reproducibility across sites.[21] Recent proficiency results from the European NEQAS program now indicate much better interlaboratory agreement between LC/MS/MS methods for immunosuppressive drugs, with interlaboratory CVs on proficiency samples comparable with or better than commercial immunoassays.[6]

Correlation of ARCHITECT test results with other CsA assay methods (LC/MS/MS, Dimension Xpand Plus immunoassay and AxSYM FPIA) confirms general method equivalency, even with methods that have different metabolite cross-reactivity patterns. This is due, in part, to variability in metabolite profiles and concentrations between specimens and the tendency to observe significant interference only in samples with a high metabolite to drug ratio, which can be as high as 5-fold.[10,22] Trough specimens, collected just before the next dose of drug, show more metabolite interference because of relatively low CsA and high metabolite concentrations compared with C_2 test samples, collected just 2 hours after the drug dose. The C_2 testing strategy for CsA demonstrates good correlation with area under the time–concentration curve measurements and predicts patient outcomes more reliably than trough concentrations.[1,23,24] However, the C_2 concentration is very sensitive to the timing between drug dosing and blood draw and has not been accepted universally. The use of cross-reactive immunoassays for CsA in trough sample testing has generated controversy in the TDM community[10,25] and is partly responsible for a trend toward CsA specific testing methods such as LC/MS/MS. CsA immunoassay methods with very low metabolite cross-reactivity, such as the ARCHITECT assay evaluated in this report, are an attractive option to LC/MS/MS since, in general use, random access immunoassay instruments often fit better into laboratory workflow schemes and can provide a much wider variety of tests.

TABLE 6. Patient Specimen Correlation Summary (ARCHITECT = Comparison Method* Slope + Intercept)

Clinical Site	Comparison Method	n	R (Spearman)	Slope (Passing-Bablok)	Intercept (Passing-Bablok)	Average Bias, ng/mL
FR	LC/MS/MS	122	0.99	1.10	−7	31
FR	LC/MS/MS <200 ng/mL	50	0.89	1.04	−3	5
FR	LC/MS/MS >200 ng/mL	72	0.97	1.10	−7	47
FR	LC/MS/MS >500 ng/mL	45	0.92	1.04	40	53
AU	Dimension	190	0.95	1.08	−20	−7
BE	Dimension	95	0.94	1.00	−20	−21
GE	Dimension	97	0.93	1.00	−5	−11
IT	Dimension	117	0.98	0.69	26	−76
IT	Dimension <200 ng/mL	62	0.91	0.92	6	−3
IT	Dimension >200 ng/mL	55	0.96	0.68	27	−159
IT	Dimension >500 ng/mL	32	0.89	0.84	−112	−228
SW	AxSYM	101	0.97	1.03	−9	−4
US	TDx	227	0.99	0.93	−25	−53

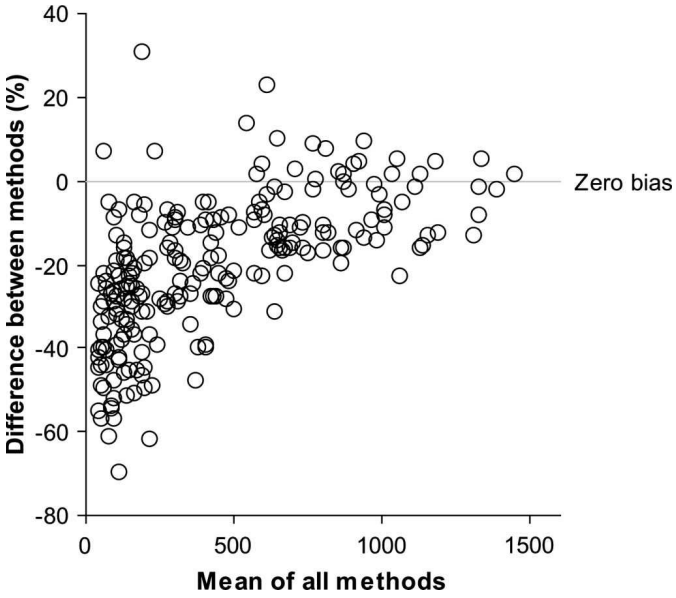

FIGURE 2. Bland–Altman bias plot for the ARCHITECT CsA Assay versus TDx correlation data from the US site.

There is currently no certified international reference standard designated for CsA by which to standardize and calibrate different assay methods for determination of CsA in whole blood. Thus, even though different assay methods for CsA can correlate well they do not necessarily give the same patient results due, in part, to calibration bias. This was particularly evident in the method comparison between ARCHITECT and the Dade Dimension assays with patient specimens at concentrations >500 ng/mL (see Table 6). Because these results are from patient specimens drawn at C_2, in which the concentration of CsA metabolites would be minimal, one possible explanation for these results is calibration bias between assay methods. This high negative bias for the ARCHITECT assay was not observed in a different transplant population monitored by both ARCHITECT and LC/MS/MS. Our study continues to highlight the need to calibrate all CsA assays to the same CsA certified reference standard[26] to remove the confounding variable of test method bias inherent in many multicenter drug regimen randomized trials.

The ARCHITECT family of instruments now include tests for the immunosuppressive drugs tacrolimus[19] and sirolimus.[20] The new, highly specific ARCHITECT Cyclosporine immunoassay evaluated in this study, completes the panel of the 3 most commonly used immunosuppressive drugs which require therapeutic drug monitoring.

CONCLUSIONS

The data from these evaluation studies demonstrate that the new ARCHITECT CsA immunoassay is relatively free of the metabolite interference previously observed with other immunoassay formats. It correlates well with both LC/MS/MS and other immunoassay methods, using whole-blood specimens drawn from transplant patients receiving CsA drug therapy, and demonstrates adequate interlaboratory reproducibility required for routine TDM in the clinical laboratory.

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