Multi-site Analytical Evaluation of the Abbott ARCHITECT Tacrolimus Assay

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Abstract: The objective of this study was to evaluate the analytical performance of the Abbott ARCHITECT Tacrolimus immunoassay. Proficiency panels and specimens from a population of organ transplant recipients were analyzed in 6 clinical laboratories in Europe and the United States, and the results were compared with other methods. The ARCHITECT assay requires a whole blood specimen pretreatment step with methanol/zinc sulfate to precipitate protein and extract the drug, followed by a 30-minute immunoassay using anti-tacrolimus antibody–coated paramagnetic microparticles and an acridinium–tacrolimus tracer. The assay was free from hematocrit interference in the range 25%–55% and from interference by extremes of cholesterol, triglycerides, bilirubin, total protein, and uric acid. The total percent of coefficient of variations of the assay were 4.9%–7.6% at 3 ng/mL, 2.9%–4.6% at 8.6 ng/mL, and 3.1%–8.2% at 15.5 ng/mL. Limit of detection was ≤0.5 ng/mL and limit of quantification (LOQ) ranged from 0.69 to 1.07 ng/mL across the 6 sites (based on the upper 95% confidence interval concentrations). The 2007 European Consensus Conference on Tacrolimus Optimization recommended the use of assay methods with an LOQ around 1 ng/mL, based upon the need to measure trough tacrolimus blood concentrations precisely down to 3 ng/mL during low-dose tacrolimus regimens. Tacrolimus International Proficiency Testing Scheme samples were measured by the ARCHITECT immunoassay at 5 sites and showed an average bias of −0.28 to +0.85 ng/mL versus IMx Tacrolimus II immunoassay historical values and −0.21 to +0.68 ng/mL versus liquid chromatography/tandem mass spectrometry (LC-MSMS) Tacrolimus historical values. Method comparison studies were performed with the ARCHITECT Tacrolimus immunoassay on patient specimens with the following results: ARCHITECT Tacrolimus assay versus the Abbott IMx Tacrolimus II immunoassay (4 sites) yielded average biases between −0.94 and +0.26 ng/mL; ARCHITECT assay versus the Dade Dimension Tacrolimus immunoassay (2 sites) yielded average biases of −0.46 and +0.11 ng/mL; and ARCHITECT assay versus LC-MSMS methods at 2 sites yielded average biases of +0.51 and +1.63 ng/mL. Spearman correlation coefficients were ≥0.90 on all method comparisons. The ARCHITECT Tacrolimus assay is a semiautomated, robust, and highly sensitive immunoassay, representing an alternative approach for laboratories not equipped with LC-MSMS, and meets the 1 ng/mL recommendation of LOQ by the European Consensus Conference on Tacrolimus Optimization.

Key Words: tacrolimus, FK 506, immunoassay, LC-MSMS, method evaluation, therapeutic drug monitoring

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INTRODUCTION

Tacrolimus is an immunosuppressive drug discovered in 1984 by the Fujisawa Pharmaceutical Co Ltd and is marketed under the name Prograf, and more recently Advagraf, as a prolonged release formulation (Astellas Pharma Inc, Tokyo, Japan). It is a macrolide immunosuppressant that inhibits the intracellular calcineurin pathway required for T-lymphocyte activation. Therapeutic drug monitoring is required to maintain a blood concentration adequate to prevent organ transplant rejection but low enough to minimize its toxic side effects such as diabetes, neuropathy, and nephrotoxicity.[1]

The 2007 European Consensus Conference on Tacrolimus Optimization recommended the use of assay methods with a limit of quantification (LOQ) around 1 ng/mL, based upon the need to measure trough tacrolimus blood concentrations precisely down to 3 ng/mL during low-dose tacrolimus regimens. The ARCHITECT Tacrolimus immunoassay was developed to provide a semiautomated method with the sensitivity of liquid chromatography/tandem mass spectrometry (LC-MSMS) for monitoring blood specimens below 3 ng/mL tacrolimus and the convenience and ease of use of an automated immunoassay kit. Other immunoassays available from Abbott (IMx Tacrolimus II) and Dade Behring (Siemens, EMIT and Dimension RxL Tacrolimus assays) do not provide the required sensitivity, though the Dimension RxL assay can be run directly on whole blood specimens. The objective of the current multicenter study was to evaluate the clinical laboratory performance of the Abbott ARCHITECT Tacrolimus immunoassay with proficiency samples and clinical specimens from a mixed population of organ transplant patients.

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MATERIALS AND METHODS

A multi-site study of the ARCHITECT Tacrolimus assay was conducted to evaluate analytical performance at 5 European sites located in Austria (AU, Medizinische Universität Wien, Vienna, Austria); Belgium (BE, U.C.L. Cliniques universitaires Saint-Luc, Brussels, Belgium); France (FR, Lille Hospital, Loos, France); Germany (GE, Medizinische Hochschule Hannover, Hannover, Germany); Italy (IT, Molinette Hospital, Torino, Italy); and 1 site in the United States (US, 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.

Assay Method

The ARCHITECT Tacrolimus assay is based on the Chemiluminescent Microparticle Immunoassay methodology. In the ARCHITECT Tacrolimus assay, EDTA blood specimens were pretreated according to the manufacturer's instructions by rapidly vortex mixing 200 μL of EDTA blood with 200 μL of a precipitation reagent containing methanol and zinc sulfate. The resulting precipitate was centrifuged to remove insoluble protein and the clear supernatant was tested on the ARCHITECT instrument according to manufacturer's instructions. The instrument combined the extracted blood sample with paramagnetic microparticles coated with mouse anti-tacrolimus antibody, followed by addition of a tacrolimus–acridinium tracer. After incubation and particle washing, the chemiluminescent signal was measured and the tacrolimus concentration was calculated from the calibration information stored in the instrument memory. Tacrolimus calibrators were tested in duplicate at 0, 3, 6, 12, 20, and 30 ng/mL to establish the assay calibration.

Antibody Specificity

The mouse anti-tacrolimus antibody used in the ARCHITECT Tacrolimus assay was the same antibody used in the Abbott IMx Tacrolimus II assay (Abbott Laboratories, North Chicago, IL). To measure tacrolimus metabolite cross-reactivity, metabolites M-I (13-O-demethyltacrolimus), M-II (31-O-demethyltacrolimus), M-III (15-O-demethyltacrolimus), and M-IV(12-hydroxytacrolimus) were prepared in vitro by incubation of tacrolimus with liver microsomes prepared from phenobarbital-treated rats in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) generating system under aerobic condition or bioconverted by incubating tacrolimus with an actinomycete.[2–4] Oxidative metabolites formed in the reaction medium were isolated and identified. Purified samples were analyzed by high-performance liquid chromatography with mass spectrometric detection and by nuclear magnetic resonance spectroscopy. Metabolite cross-reactivity was measured using the ARCHITECT Tacrolimus assay (n = 5 replicates) at the US site, with 10 ng/mL metabolite spiked into whole blood samples containing a target background concentration of 5–22 ng/mL tacrolimus. The percentage cross-reactivity was calculated as the mean excess tacrolimus concentration (nanogram per milliliter) detected divided by the metabolite concentration added (10 ng/mL) × 100. The effect on analytical performance of patient specimen metabolites was not evaluated in this study.

Limit of Detection

Limit of detection (LOD), or analytical sensitivity, was evaluated at European sites on 1 ARCHITECT instrument with n = 4 runs, 10 replicates of Calibrator A (0 ng/mL) and 4 replicates of Calibrator B (3 ng/mL, prepared gravimetrically). In the US site, LOD was evaluated on 3 ARCHITECT instruments, with 8 runs per instrument (n = 24 runs) using 10 replicates of Calibrator A (0 ng/mL) and 4 replicates of Calibrator B (3 ng/mL).

LOD was calculated as follows[5]:

$$\mathrm{LOD} = 2 \times \mathrm{SD}_{\text{A calibrator signal}} \times (3\ \mathrm{ng/mL} / (\text{A calibrator signal} - \text{B calibrator signal}))$$

Limit of Quantification

LOQ was calculated as functional sensitivity using a series of centrally prepared whole blood specimens spiked with tacrolimus to achieve approximate concentrations from 0.2 to 4.4 ng/mL. Each replicate tested was pretreated separately before running the assay. The European sites ran 5 of the specimens in replicates of 10 on 2 separate days (n = 20 replicates). The US site ran all 7 specimens in replicates of 10, 2 runs per day on 5 separate days (n = 100 replicates). LOQ was calculated as the tacrolimus concentration at 20% coefficient of variation (CV), using a linear curve fit of CV% versus 1/concentration for the mean, the 5% and 95% confidence interval concentration limits.[5] The LOQ of the Dade Dimension tacrolimus immunoassay (Siemens Healthcare Diagnostics, Inc, Tarrytown, NY) was also evaluated at the BE site using 6 independently prepared specimens ranging from 0.35 to 6.0 ng/mL tacrolimus. This assay is based on the Antibody Conjugated Magnetic Immunoassay methodology, which does not require analytical pretreatment.

Dilution Linearity

The ARCHITECT Tacrolimus 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 tacrolimus blood specimens in the range of 27.8–29.4 ng/mL, diluted with ARCHITECT Tacrolimus Calibrator A to 90%–10% of their original concentrations. The concentration of tacrolimus in the original samples was back calculated for each dilution, and percent recovery was calculated as calculated concentration/original concentration × 100.

Potentially Interfering Endogenous Substances

Whole blood specimens with tacrolimus concentrations of approximately 5.5 and 18.0 ng/mL were supplemented with the following potentially interfering endogenous substances, using concentrated stock solutions of bilirubin, cholesterol, and uric acid, a commercially available egg triglyceride fraction to supplement with triglycerides and human serum albumin to supplement with protein: high triglycerides, 8 g/L; high bilirubin, 400 mg/L; low and high total protein, 30–120 g/L;

high cholesterol, 5 g/L; and high uric acid, 200 mg/L. The percentage interference was calculated from the difference in mean tacrolimus concentration between samples containing interferent (test) or diluent (control) using replicates of 2. Effects of low and high hematocrit, 25%–55%, were also investigated using whole blood samples, which had been adjusted by addition or removal of the plasma fraction. The study, which was performed at the US site, was based on guidance from the clinical and laboratory standards institute (CLSI) document EP7-A2.[6]

Assay Imprecision

Assay imprecision was evaluated following CLSI protocol EP5-A2[7] using 4 replicates per day for each of 3 Lyphochek Whole Blood Immunosuppressant Controls, Levels 1, 2, and 3 (Bio-Rad Laboratories, Hercules, CA). Each replicate was pretreated separately. The European sites ran the study over 5 days (n = 20 per control). The US site ran the study over 20 days (n = 80 per control). The total CV% includes variance components due to with-run, between-run, and between-day assay imprecision.

Proficiency Testing Protocol

Single replicates of proficiency samples from the Tacrolimus International Proficiency Testing Scheme (TIPTS) were tested at 5 sites (AU, BE, FR, GE, and IT). Proficiency samples were supplied blinded to the sites. Results were compared with historical LC-MSMS Tacrolimus and IMx Tacrolimus II immunoassay concentrations reported for individual samples (Analytical Services International Ltd; www.bioanalytics.co.uk, November 2006). Tacrolimus concentrations in the samples varied from approximately 0–24 ng/mL. The majority of samples (60%) were made from drug-free blood spiked with tacrolimus, with the remainder derived from pools of blood samples from transplant patients receiving tacrolimus.

Method Comparison

Surplus patient whole blood specimens were obtained at the European and US sites using local ethics procedures and tested on the ARCHITECT instrument versus the LC-MSMS methods at the AU and US sites, the IMx Tacrolimus II immunoassay (Abbott Diagnostics, Abbott Park, IL) at 4 sites (BE, FR, GE, and US), and the Dade Behring Dimension Tacrolimus assay (Siemens Healthcare Diagnostics Inc, Tarrytown, NY) at the AU and BE sites.

Statistical Methods

Functional sensitivity analysis was performed with Sigma Plot analysis software (version 6.0; Systat Software Inc, San Jose, CA). Correlation, assay imprecision, and bias statistics were performed with Analyse-it statistical analysis software (version 1.73, Analyse-it Software Ltd, Leeds, UK) using Spearman correlation coefficients and Passing–Bablok regression.[8] Bland–Altman bias analysis[9] was used for bias plots.

RESULTS

Antibody Specificity

Metabolites that showed significant cross-reactivity in the ARCHITECT Tacrolimus assay were M-II (94%) and M-III (45%). Cross-reactivity was less than the LOD for metabolites M-I and M-IV.

Limits of Detection and Quantification

ARCHITECT assay LOD results were ≤0.5 ng/mL at all sites. LOQ measurements are shown in Table 1. The LOQ measured using the upper 95% concentration limits ranged from 0.69 to 1.07 ng/mL at the 6 evaluation sites. Figure 1 shows graphical plots comparing the ARCHITECT and Dade Dimension assays at the BE site. LOQ values calculated from the mean tacrolimus concentration data (solid line) were 0.61 ng/mL for the ARCHITECT assay and 3.12 ng/mL for the Dade Dimension assay. The LOQ calculated from the 5% and 95% confidence limit concentrations are shown by the dotted lines.

Dilution Linearity

Mean tacrolimus recovery for the 3 different elevated blood specimens diluted with Calibrator A was 102% (range 95%–108%). Results are shown in Table 2. When compared with the percent dilution factor of the neat specimen, the correlation (R^2) of observed values ranged from 0.998 to 1.000.

Endogenous Interferences

The average recovery observed during the interference study ranged from 96% to 105%, indicating no significant analytical interference from hematocrit (25%–55%), total protein (30–120 g/L), triglycerides (up to 8 g/L), cholesterol (up to 5 g/L), bilirubin (up to 400 mg/L), or uric acid (up to 200 mg/L).

Assay Imprecision

Assay imprecision observed at the 6 evaluation sites is shown in Table 3. The total CV% for the low control (3 ng/mL) ranged from 4.9% to 7.6% across the 6 sites. The CV% for the medium control (8.6 ng/mL) ranged from 2.9% to 4.6% and the CV% for the high control (15.5 ng/mL) ranged from 3.1% to 8.2% across the 6 sites.

Proficiency Testing

Proficiency testing results on a panel of 53 samples from the TIPTS showed strong correlation to historical proficiency survey mean results reported for LC-MSMS and the IMx Tacrolimus II assay. Results across the 5 evaluation sites were

TABLE 1. ARCHITECT Tacrolimus Assay's LOQ

Clinical Site	Predicted (ng/mL)	5% Confidence Interval (ng/mL)	95% Confidence Interval (ng/mL)
AU	0.69	0.58	0.84
BE	0.61	0.46	0.86
FR	0.59	0.44	0.84
GE	0.72	0.58	0.94
IT	0.64	0.43	1.07
US	0.56	0.46	0.69

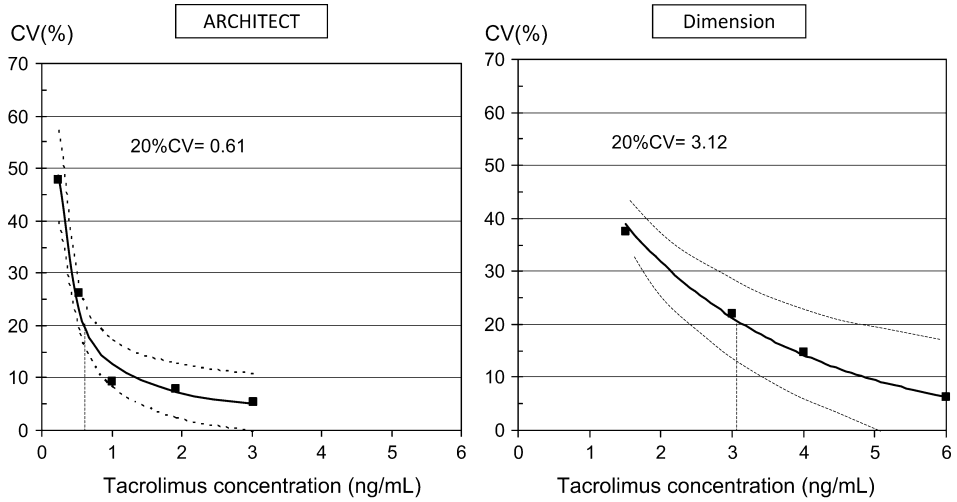

FIGURE 1. LOQ of the ARCHITECT Tacrolimus assay and the Dade DIMENSION Flex-Tacrolimus assay at the BE site.

sufficiently reproducible to allow pooling of data into a single bias plot as shown in Figure 2. The correlation to historical IMx values had an *r* value of 0.96 and showed a positive slope (1.14) and negative intercept (−1.0 ng/mL). The correlation to historical LC-MSMS values showed an *r* value of 0.98 with a slope of 1.00 and an intercept of 0.3 ng/mL.

TABLE 2. ARCHITECT Tacrolimus Assay's Dilution Linearity Results

Specimen	Dilution Factor (%)	Observed Concentration (ng/mL)	Expected Concentration (ng/mL)	Recovery (%)
1	100.0	29.4	29.4	100
	90.1	26.7	29.6	101
	80.0	23.0	28.8	98
	69.9	20.7	29.6	101
	59.9	17.3	28.9	98
	40.0	11.7	29.3	99
	20.0	6.0	30.0	102
	10.0	2.8	28.0	95
2	100.0	27.8	27.8	100
	90.1	25.6	28.4	102
	80.0	23.3	29.1	105
	69.9	20.1	28.7	103
	59.9	17.9	29.9	108
	40.0	11.9	29.8	107
	20.0	5.8	29.0	104
	10.0	2.9	29.0	104
3	100.0	28.1	28.1	100
	90.1	25.3	28.1	100
	80.0	22.8	28.5	101
	69.9	20.1	28.7	102
	59.9	17.2	28.7	102
	40.0	11.7	29.3	104
	20.0	5.9	29.5	105
	10.0	2.8	28.0	100

Method Comparison to LC-MSMS and Immunoassay on Patient Specimens

Results for the ARCHITECT assay comparison testing on clinical specimens at the 5 sites are summarized in Table 4. The ARCHITECT assay showed a positive bias versus the AU and US LC-MSMS methods with correlation slopes >1 and an average bias of +1.63 ng/mL at the AU site and +0.51 ng/mL at the US site. Bland–Altman bias plots of the ARCHITECT and LC-MSMS data (percent concentration difference between methods plotted versus the mean concentration of the methods) are shown in Figures 3 and 4. They demonstrate a combined average 14% bias (17% for the AU site and +11% for the US site) across the range of tacrolimus concentrations tested.

The IMx method comparison data showed ARCHITECT assay with a trend toward negative bias (correlation slopes <1 and average bias between +0.3 and −0.9 ng/mL at 4 sites). Average bias versus the Dade Dimension assay was +0.1 and −0.5 ng/mL at 2 evaluation sites).

Total Error

Total error (TE) was calculated at the AU and US sites using the imprecision data in Table 3 for each control and the

TABLE 3. ARCHITECT Tacrolimus Assay Imprecision Study Using MCC

	MCC Level 1		MCC Level 2		MCC Level 3	
Clinical Site	Mean (ng/mL)	CV Total (%)	Mean (ng/mL)	CV Total (%)	Mean (ng/mL)	CV Total (%)
AU	2.8	7.6	8.8	4.6	15.5	3.1
BE	3.2	7.2	8.5	2.9	15.2	8.2
FR	3.2	6.3	9.0	3.3	15.9	3.1
GE	3.1	6.6	9.3	4.6	16.3	4.5
IT	2.9	5.6	8.2	4.4	15.4	3.5
US	3.0	4.9	7.8	3.6	14.5	3.5

MCC, multiconstituent controls.

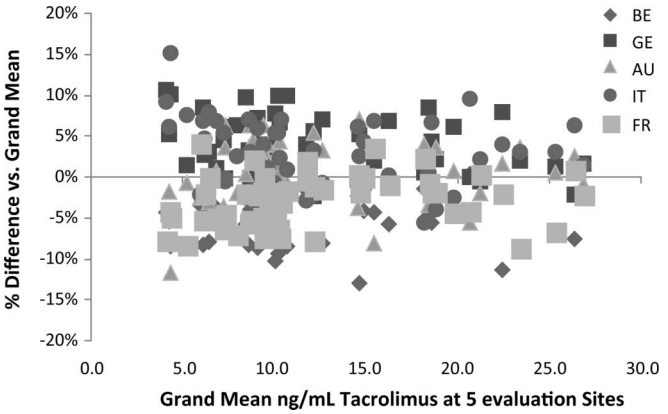

FIGURE 2. Bias plot of TIPTS proficiency data at 5 European evaluation sites.

regression equations for comparison of ARCHITECT to LC-MSMS assays in Table 4 as follows[10]:

$$\mathrm{TE} = \mathrm{Bias} + 2(\%\mathrm{CV}),$$

$$\text{where Bias} = 100 \times [\text{ARCHITECT (ng/mL)} - \text{LC-MSMS (ng/mL)}]/[\text{LC-MSMS (ng/mL)}].$$

TE ranged from 26.3% to 28.8% at the AU site and from 16.0% to 25.2% at the US site.

DISCUSSION

The clinical outcome of transplant patients on long-term immunosuppressive therapy with calcineurin inhibitors (cyclosporine or tacrolimus) depends not only on organ rejection but also on the side effects of these drugs, such as diabetes, neuropathy, and nephrotoxicity.[11] Recent clinical studies have shown significant improvement in organ transplant patient outcomes using a low-dose tacrolimus treatment regimen, which also included mycophenolate mofetil, daclizumab, and steroids.[12] Due to the need to monitor therapeutic blood concentrations down to 3 ng/mL in such patients, the 2007 European Consensus Conference on Tacrolimus Optimization recommended at the 10th IATDMCT (International Association of Therapeutic Drug Monitoring and Clinical Toxicology) meeting in Nice, France, that analytical methods measuring whole blood concentrations of tacrolimus need a lower LOQ, ideally, of 1 ng/mL.[13] Assay methods using LC-MSMS offer an LOQ as low as 0.25 ng/mL.[14] The ARCHITECT Tacrolimus evaluation described here showed assay LOQ between 0.69 and 1.07 ng/mL across 6 different evaluation sites and conforms to the 2007 European Consensus Conference on Tacrolimus Optimization recommendation of 1 ng/mL. This low LOQ was due in part to the excellent assay precision shown by ARCHITECT immunoassay technology and might be related to an efficient and reproducible whole blood extraction procedure. It should be noted that the Dade Behring Dimension Flex-tacrolimus assay, which offers the advantage of avoiding extraction pretreatment, had a much higher LOQ than is being recommended for monitoring low-dose tacrolimus.

The excellent reproducibility of the ARCHITECT assay results on proficiency samples across all sites suggests that rebaselining of patient tacrolimus blood concentrations when tested by the ARCHITECT method at different laboratory sites, for example, transplant center hospital laboratory versus local laboratory near the patient's home, would not be necessary. The ARCHITECT results of clinical specimens from a mixture of organ transplant types also correlated well with results from LC-MSMS and 2 widely used immunoassay methods (Table 4). Minimal or slightly negative bias was observed between ARCHITECT results and the IMx and Dade immunoassays, whereas a modest positive bias was seen between ARCHITECT results and LC-MSMS (+11% for the US data and +17% for the AU data). Both the US and the AU Bland–Altman bias plots versus LC-MSMS show a 2 SD range of approximately ±30% around the mean bias, indicative of variability between specimens. The lack of apparent method bias versus LC-MSMS on the TIPTS proficiency samples is probably related to the low concentration of any tacrolimus metabolites in the majority of samples supplied.

It is unclear how much of the ARCHITECT assay bias might be ascribed to a difference in tacrolimus concentration standardization between methods. It is known that LC-MSMS can show method bias due to internal standard instability described by Napoli,[15] ion suppression effects,[16] or calibrator matrix effects.[17] Such interlaboratory variability has been seen, for instance, in the concentration data collected on proficiency samples in tacrolimus quality assurance surveys (www.bioanalytics.co.uk, November 2006). An improvement was observed in the 2007 and 2008 tacrolimus surveys, which was possibly due to the emergence of commercial calibrators for

TABLE 4. ARCHITECT Assay Patient Specimen Correlation Results Versus Other Assay Methods

Clinical Site	Assay Method	n	*r*(Spearman)	Slope (Passing–Bablock)	Intercept (Passing–Bablock)	Average Bias (ng/mL)
AU	LC-MSMS	139	0.95	1.24	−0.33	1.63
US	LC-MSMS	125	0.92	1.07	0.22	0.51
BE	IMx	305	0.91	0.89	0.32	−0.64
FR	IMx	102	0.95	1.00	−0.25	−0.30
GE	IMx	113	0.94	0.93	1.00	0.26
US	IMx	124	0.90	0.81	0.37	−0.94
AU	Dimension	139	0.91	1.06	−0.24	0.11
BE	Dimension	197	0.95	0.98	−0.27	−0.46

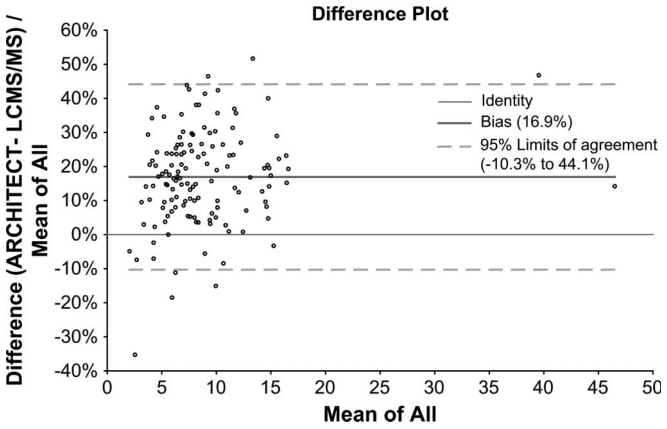

FIGURE 3. Bland–Altman bias plot for the ARCHITECT assay versus LC-MSMS correlation data from the AU site.

tacrolimus. A plausible explanation for the positive bias of the ARCHITECT assay relative to LC-MSMS is metabolite cross-reactivity inherent in all immunoassay methods. The ARCHITECT method showed cross-reactivity to tacrolimus metabolites M-II (94%) and M-III (45%). In steady-state blood samples from renal transplant patients, these 2 metabolites show, respectively, mean blood concentrations of approximately 15% and 6% compared with the parent drug.[18] Metabolite M-III is reported to be biologically inactive, whereas M-II has a biological activity approximately the same as tacrolimus.[19] Cross-reactivity of M-II could then explain up to 15% bias in measured tacrolimus concentration, with M-III accounting for as much as 3%. The ARCHITECT method bias due in part to metabolites should not represent clinically misleading results as the total immunosuppressive bioactivity of tacrolimus would be the sum of the parent drug plus M-II metabolite whole blood concentrations. Interference from typical blood constituents was not seen with the ARCHITECT Tacrolimus method. The ARCHITECT assay specimen pretreatment procedure releases tacrolimus from endogenous binding proteins and precipitates plasma proteins that might be sources of nonspecific binding interference. Notably, the ARCHITECT method is also insensitive to variations in

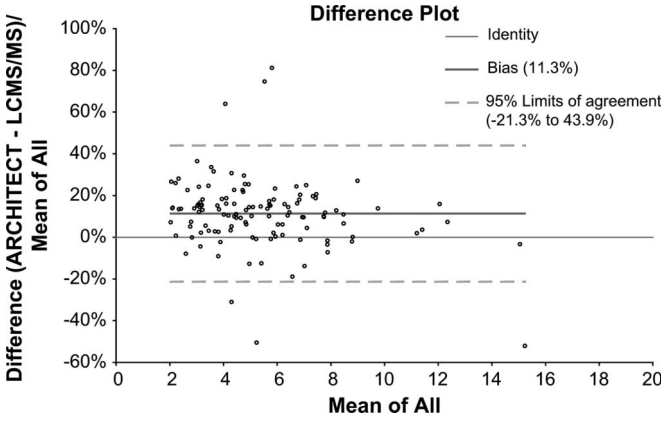

FIGURE 4. Bland–Altman bias plot for the ARCHITECT assay versus LC-MSMS correlation data from the US site.

hematocrit between 25% and 55%, an improvement over the IMx Tacrolimus II assay, which has shown a hematocrit bias.[20]

At present, there are no established regulatory guidelines on TEa limits that need to be protected for tacrolimus. The ARCHITECT Tacrolimus assay was designed to meet a TEa of 30% to prevent the release of results that could impact on patient management. In our method comparison study of ARCHITECT assay versus LC-MSMS at 2 sites, we found that TE $<$ TEa across the clinically important range of the assay, that is, 2.8–15.5 ng/mL. The calculated TE for the ARCHITECT assay overestimates the clinically relevant TE due to the fact that the M-II tacrolimus metabolite, which is detected by immunoassay, has a similar biological activity as the parent compound but is not detected by the reference method LC-MSMS.

CONCLUSIONS

The data from these evaluation studies demonstrated that the ARCHITECT Tacrolimus assay has the precision, freedom from interferences, and LOQ required for monitoring whole blood concentrations of tacrolimus in transplant recipients. In addition, the assay met the 1 ng/mL LOQ recommendation from the 2007 European Consensus Conference on Tacrolimus Optimization. The ARCHITECT Tacrolimus assay provides a semiautomated, robust, and highly sensitive immunoassay based on Chemiluminescent Microparticle Immunoassay technology, representing an alternative approach to laboratories not equipped with LC-MSMS.

REFERENCES

1. Wallemacq P, Reding R. FK506 (Tacrolimus), a novel immunosuppressant in organ transplantation: clinical, biomedical and analytical aspects. *Clin Chem*. 1993;39:2219–2228.
2. Chen TS, Arison BH, Wicker LS, et al. Microbial transformation of immunosuppressive compounds I. Desmethylation of FK506 and immunomycin (FR 900520) by *Actinoplanes* sp. ATCC 53771. *J Antibiotics*. 1992;45:118–123.
3. Chen TS, Arison BH, Wicker LS, et al. Microbial transformation of immunosuppressive compounds II. Specific desmethylation of 13-methoxy group of FK506 and FR 900520 by an unidentified Actinomycete ATCC 53828. *J Antibiotics*. 1992;45:577–580.
4. Perotti BYT, Okudaira N, Prueksaritanont T, et al. FK506 metabolism in male and female rat liver microsomes. *Drug Metab Dispos*. 1994;22: 85–89.
5. Westgard JO. *Basic Method Validation*, 3rd ed. Madison, WI: Westgard QC, Inc; 2008, 168–174.
6. Clinical and Laboratory Standards Institute. *Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition*. CLSI document EP7-A2. Wayne, PA: Clinical and Laboratory Standards Institute, 2005.
7. National Committee for Clinical Laboratory Standards. *Evaluation of Precision Performance of Quantitative Measurement Methods*; *Approved Guideline—Second Edition*. NCCLS document EP5-A2. Wayne, PA: NCCLS, 2004.
8. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. *J Clin Chem Clin Biochem*. 1983;21:709–720.
9. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*. 1986;1:307–310.
10. Radomski KM, Bush BA, Ensom MHH. Collaboration between pharmacy and laboratory: defining total allowable error limits for therapeutically monitored drugs. *Ann Pharmacother*. 1998;32:170–175.
11. Cosio FG, Amer H, Grande JP, et al. Comparison of low versus high tacrolimus levels in kidney transplantation: assessment of efficacy by protocol biopsies. *Transplantation*. 2007;83:411–416.

12. Ekberg H, Tedesco-Silva H, Demirbas A, et al. The ELITE–Symphony study. Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med*. 2007;357:2562–2575.
13. Wallemacq P, Armstrong VW, Brunet M, et al. Opportunities to optimize tacrolimus therapy in solid organ transplantation: report of the European consensus conference. *Ther Drug Monit*. 2009;31:139–152.
14. Streit F, Armstrong VW, Oellerich M. Rapid liquid chromatography-tandem mass spectrometry routine method for simultaneous determination of sirolimus, everolimus, tacrolimus and cyclosporine A in whole blood. *Clin Chem*. 2002;48:955–958.
15. Napoli K. Organic solvents compromise performance of internal standard (ascomycin) in proficiency testing of mass spectrometry-based assays for tacrolimus. *Clin Chem*. 2006;52:765–766.
16. Taylor PJ. Matrix effects: the Achilles heel of quantitative high-performance liquid chromatography-electrospray-tandem mass spectrometry. *Clin Biochem*. 2005;38:328–334.
17. Vogeser M. Instrument specific matrix effects of calibration materials in the LC-MS/MS analysis of tacrolimus. *Clin Chem*. 2008;54:1406–1408.
18. Murthy JN, Davis DL, Yatscoff RW, et al. Tacrolimus metabolite cross-reactivity in different tacrolimus assays. *Clin Biochem*. 1998;31:613–617.
19. Iwasaki K, Shiraga T, Matsuda H, et al. Identification and biological activities of the metabolites oxidized at multiple sites of FK506. *Drug Metab Dispos*. 1995;23:26–34.
20. Goffinet JS, Mullier F, Capron A, et al. Evaluation of three immunoassays to quantify tacrolimus: impact of hematocrit (Abstract). *Ther Drug Monit*. 2007;29:508.

