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Multi-center analytical evaluation of a novel automated tacrolimus immunoassay☆

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ABSTRACT

Background: Tacrolimus (TAC) is a post-transplantation immunosuppressant drug used in patients for whom careful monitoring of TAC concentration is essential. A new semi-automated immunoassay for TAC measurement, the Elecsys Tacrolimus assay, is available and has been assessed in a multi-center evaluation.

Methods: Residual whole blood samples from patients undergoing TAC therapy after organ transplant were used in assay evaluation at five clinical laboratories in Europe. Experiments included imprecision according to CLSI EP5-A2 (within-run and intermediate), functional sensitivity, linearity according to CLSI EP6-A, and recovery from external quality assessment scheme (EQAS) samples. The assay was compared to LC–MS/MS used routinely at each investigational site, and to the Abbott Architect immunoassay.

Results: Linearity from 0.5 to 40 μg/L was observed and functional sensitivity of 0.3 μg/L (CV ≤ 20%) was determined. Within-run imprecision was ≤5.1% on cobas e 602 (5.1% at 1.5 μg/L) and ≤8.9% (8.9% at 0.8 μg/L) on cobas e 411. The intermediate imprecision for TAC concentrations ≥6.8 μg/L was ≤6.5%. At lower therapeutic concentrations (to 1.5 μg/L) it was consistently ≤10%. Deming regression analysis of method comparison to LC–MS/MS yielded slopes of 1.07 (95%CI: 1.05/1.10) for heart transplant samples, 1.13 (95%CI: 1.09/1.16) for kidney, and 1.05 (95%CI: 1.02/1.08) for lung transplant samples.

Conclusions: The Elecsys Tacrolimus assay has good linearity, functional sensitivity and intermediate imprecision and is comparable to LC–MS/MS methods. The over-all performance of ECLIA demonstrates a modern generation TAC assay that meets the demands of monitoring drug concentrations in current immunosuppressive regimens.

Introduction

The discovery of the second generation calcineurin inhibitor tacrolimus (TAC) in the early 1990s has greatly enhanced the therapeutic success of organ transplantation initiated by cyclosporine A as immunosuppressive therapy [1]. Therapeutic drug monitoring (TDM) was recommended with the introduction of TAC, and undoubtedly contributed to this success. According to the 2011 OPTN/SRTR annual report [2], over 80% of solid organ recipients received TAC. However, the calcineurin inhibitor toxicity profile remains a major concern and may affect long-term outcome for many patients, which is today the main challenge for transplantation medicine [1,3].

New strategies to improve the long-term preservation of organ function and to reduce the incidence of accompanying diseases (e.g. infections, renal insufficiency, cardiovascular disease and malignancy)

Abbreviations: TAC, tacrolimus; TDM, therapeutic drug monitoring; OPTN/SRTR, Organ Procurement and Transplantation Network and Scientific Registry of Transplant Recipients; LOQ, limit of quantification; LC–MS/MS, liquid chromatography tandem mass spectrometry; IPT, International Proficiency Testing Scheme; CMIA, chemiluminescent microparticle immunoassay; MCE, multi-center evaluation; ECLIA, electrochemiluminescence immunoassay; QC, quality control; EQAS, external quality assessment scheme; HTx, heart transplant; KTx, kidney transplant; LTx, liver transplant; IRB/EC, Internal Review Board or Ethics Committee; GCP, good clinical practice; CV, coefficient of variation; ACMIA, antibody-conjugated magnetic immunoassay.

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Table 1
Comparison of LC–MS/MS measurement methods used by investigational sites in the tacrolimus MCE.

Site	Extraction[a,d]	Calibrators	LC/MS manufacturer/model[b,c]	Analytical column	Method working range	Within-lab imprecision
s-1 [32,33]	PPT	Chromsystems 6PLUS1	Waters Alliance 2695 HPLC/Quattro micro API	MZ-Analysentechnik MZ Aqua Perfect C18 150 × 3.0 mm, 5 μm	0.5–50 μg/L	<7%
s-2	PPT + on-line SPE	Chromsystems 6PLUS1	Waters Alliance 2795 HPLC/Quattro Ultima Pt	Waters Sunfire C18 2.1 × 100 mm, 5 μm	2.1–42.4 μg/L	<5%
s-3	PPT	RECIPE ClinCal	Agilent Infinity 1290 HPLC/Agilent 6460	Agilent Zorbax Eclipse XDB C18 4.6 × 50 mm, 1.8 μm	1.0–46 μg/L	<4%
s-4	PPT	Chromsystems MassCheck	Waters ACQUITY UPLC/TQD	Waters MassTrakTM TDM C18 2.1 × 10 mm	0.5–30.3 μg/L	<8%
s-5	PPT	RECIPE ClinCal	Waters ACQUITY UPLC/TQD	Waters MassTrakTM TDM C18 2.1 × 10 mm	0.6–44.7 μg/L	<6%

[a] All LC–MS/MS laboratories used ascomycine as an internal standard except site Barcelona [13Cd2-tacrolimus].
[b] All laboratories used step gradient with mobile phases consisting of ammonium acetate and formic acid in water or methanol for LC except for Munich [methanol/2 mM ammonium acetate].
[c] All laboratories used electrospray ionization in the positive mode (ESI+) for mass spectrometry.
[d] PPT, precipitation with organic solvent mixture and centrifugation; SPE, solid phase extraction.

associated with immunosuppression are increasingly important. One strategy is combining TAC with drugs displaying a different mode of action, minimizing dose requirement and related side effects [4–6]. Target TAC concentrations are now 5–10 μg/L (heart and liver) and 3–7 μg/L (kidney) for stable transplant recipients with current therapeutic protocols [7]. Dose minimization means concentration minimization, creating new challenges for the laboratory. The recommended lower limit of quantification (LOQ) for TAC is ~1 μg/L, as agreed at the 2007 European Consensus Conference on TAC optimization [7].

Controversial results have been derived from clinical studies investigating the concentration–effect relationship for TAC, in contrast to a better defined concentration–toxicity relationship. These discrepant results could be related to limited analytical performance of the assays used in the studies, poor assay standardization and lack of traceability to a single reference material [7–10]. Therefore, recent efforts have focused on assay improvement and standardization. An important step forward was the development of an exact-matching isotope-dilution mass spectrometry method and a certified reference material (ERM-DA110a) by LGC (Teddington, UK) [9,11].

Analysis of TAC in whole blood is performed either by immunoassays or by LC–MS/MS. Results from the Tacrolimus International Proficiency Testing Scheme (IPT) organized by Analytical Services International (ASI) indicate that of the 429 participating laboratories, approximately 60% of the participants use an immunoassay, and 40% use an LC–MS/MS method [12]. LC–MS/MS methods offer favorable analytical specificity and sensitivity with LOQs below 1 μg/L as well as multiplex testing capabilities. However, drawbacks such as instrument costs, lack of automation or 24/24 h technical support, and need for qualified staff render LC–MS/MS unattractive for many small laboratories [13]. Immunoassays offer around-the-clock results, operational flexibility and relative ease of incorporation into existing automation systems and laboratory workflow, including Laboratory Information System connection [13]. However, reagent costs are relatively high and many assays have limited analytical performance, particularly regarding analytical sensitivity (LOQ between 2 and 4 μg/L) and specificity (cross-reactivity with TAC metabolites) [7]. Immunoassays are susceptible to interferences like cross-reactivity with other drugs and metabolites, reaction with heterophilic antibodies, and influence of endogenous factors like hematocrit or albumin [7,13]. Method imprecision at the lower target therapeutic concentration range is often unsatisfactory, and calibration bias compromises performance. Only two available immunoassays (chemiluminescent microparticle immunoassay (CMIA), Abbott Diagnostics and Quantitative Microsphere System (QMS™), Thermo-Fisher) have a functional sensitivity below 1 μg/L, and were reported to offer adequate accuracy and precision [14–17]. CMIA is developed for the Architect platform. The QMS-based assay can be run on selected open clinical chemistry systems; however, it is very new and more data documenting its analytical performance is needed.

The purpose of the present multicenter evaluation (MCE) study was to evaluate the performance of the new electrochemiluminescence immunoassay (ECLIA) developed by Roche Diagnostics for use on cobas e immunoassay analyzers. Five European laboratories with experience in TDM of immunosuppressive drugs participated in the MCE. Interlaboratory comparability of the TAC results, agreement with LC–MS/MS (considered reference method in this study), and agreement with the most commonly used commercial immunoassay CMIA were points of particular focus in the MCE.

Table 2
Within-run and intermediate imprecision and bias.

Sample	Target (μg/L)	Site	Instrument	Mean (μg/L)	Within-run imprecision CV (%)	Intermediate imprecision CV (%)	Bias (%)
QC 1	2.5	1	cobas e 411	2.5	4.5	8.1	2.4
	2.6	4	cobas e 602	2.7	3.0	6.9	5.1
QC 2	10.4	1	cobas e 411	10.7	4.0	5.0	2.9
	10.4	4	cobas e 602	10.6	2.4	3.7	1.9
QC 3	19.8	1	cobas e 411	20.6	3.3	5.2	4.0
	19.9	4	cobas e 602	20.1	2.4	3.6	1.0
HSP 1		1	cobas e 411	0.8	8.9	21.0	
HSP 2		4	cobas e 602	1.5	5.1	10.0	
HSP 3		1	cobas e 411	2.5	4.3	8.5	
HSP 4		4	cobas e 602	2.8	3.7	6.5	
HSP 5		4	cobas e 602	4.2	2.9	5.4	
HSP 6		1	cobas e 411	5.5	3.9	6.4	
HSP 7		4	cobas e 602	6.8	2.1	4.4	
HSP 8		1	cobas e 411	9.4	3.1	6.5	
HSP 9		4	cobas e 602	13.0	1.9	3.6	
HSP 10		1	cobas e 411	28.6	2.9	5.9	

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