Review Article

Clinical laboratory diagnostics of immunosuppressants: One laboratory's journey

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Abstract

Laboratory support for the organ transplant patient is the provision of the best testing technology for a specific and accurate determination of immunosuppressive drug level. This translates to better management with the therapeutic cocktail of immunosuppressive drugs used with a lesser incidence of organ rejection and side effects. Over the years, development of automated immunoassay/chemistry test platforms with standardization of test protocols has demonstrated great improvements. The present clinical laboratory services with tandem mass spectrometry in our hospital present more precise and specific therapeutic drug monitoring so necessary still for the patient. This article follows the evolving testing technologies over the years for immunosuppressive drug monitoring following the organ transplants programme (renal in general) in Singapore General Hospital.

Keywords

Clinical transition, immunosuppressants, immunoassay, mass spectrometry

Introduction

Organ transplants remain the only good treatment modality for the afflicted person with kidneys, liver, heart and pancreas being the failed organs. As an external entity, the organ transplanted is subject to host rejection. Graft survival and acute rejection can be mitigated with the use of various immunosuppressants. However, it was only with the introduction of better immunosuppressive drugs that the rate of organ transplantations rose steeply in the 1980s with the discovery and utilization of cyclosporine for the management of organ rejection. Since then, other immunosuppressive drugs have been introduced but cyclosporine remains first in the front line of powerful immunosuppressive drugs to be used.[1–5] These drugs act primarily on the signal transduction pathways in activating T-cell immune response. Over the years, the availability of immunosuppressants has led to dramatically reduced rejection rates.

Today the Clinical Biochemistry Laboratories of the Department of Pathology in Singapore General Hospital (SGH) have test assays for most of the common immunosuppressive drugs available – cyclosporine, tacrolimus, sirolimus, everolimus and mycophenolic acid (MPA) – whose introductions in SGH followed soon after worldwide release (Table 1).

Laboratory services for immunosuppressants monitoring

The wide inter- and intra-individual variable pharmacokinetics observed, compliance issues and also the narrow drug therapeutic range for effectiveness have required regular drug monitoring. With this need for drug monitoring and with the advent of immunoassays in the 1980s, the in-vitro diagnostics market for immunosuppressive drugs monitoring grew.[6,7]

In 1989, Pathology, then a Ministry of Health department, joined SGH. In the same year, cyclosporine testing by a fluorescence polarization immunoassay (FPIA) with whole blood was made available in its Clinical Biochemistry Laboratories.[8] Workload increased rapidly from 752 (1989) to 5070 (1995). The next immunosuppressant test that followed was tacrolimus, or FK506, in 1998. For the next six years, these were the only two tests available. Sirolimus and

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Table 1. Immunosuppressant tests in Singapore General Hospital.

Immunosuppressant	Class	Year drug available worldwide	Year test available in SGH and immunoassay type	Current test technology in SGH
Cyclosporine	Calcineurin inhibitor	1983 Sandimmune	1989 FPIA (fluorescence polarization)	Tentative 2015 LC-MS/MS
Tacrolimus	Calcineurin inhibitor	1994 FK506	1998 MEIA (microparticle enzyme-linked)	Tentative 2015 LC-MS/MS
Sirolimus	Mammalian target-of-rapamycin inhibitor	1999 Rapamune	2004 MEIA	April 2010 LC-MS/MS
Mycophenolic acid	Anti-proliferative agent	2004 Cellcept	2004 EMIT (enzyme-multipled)	2012 EIA (enzyme inhibition assay)
Everolimus	Mammalian target-of-rapamycin inhibitor	2004 Certican	2006 FPIA	November 2009 LC-MS/MS

SGH: Singapore General Hospital; LC-MS/MS: liquid chromatography-tandem mass spectrometry
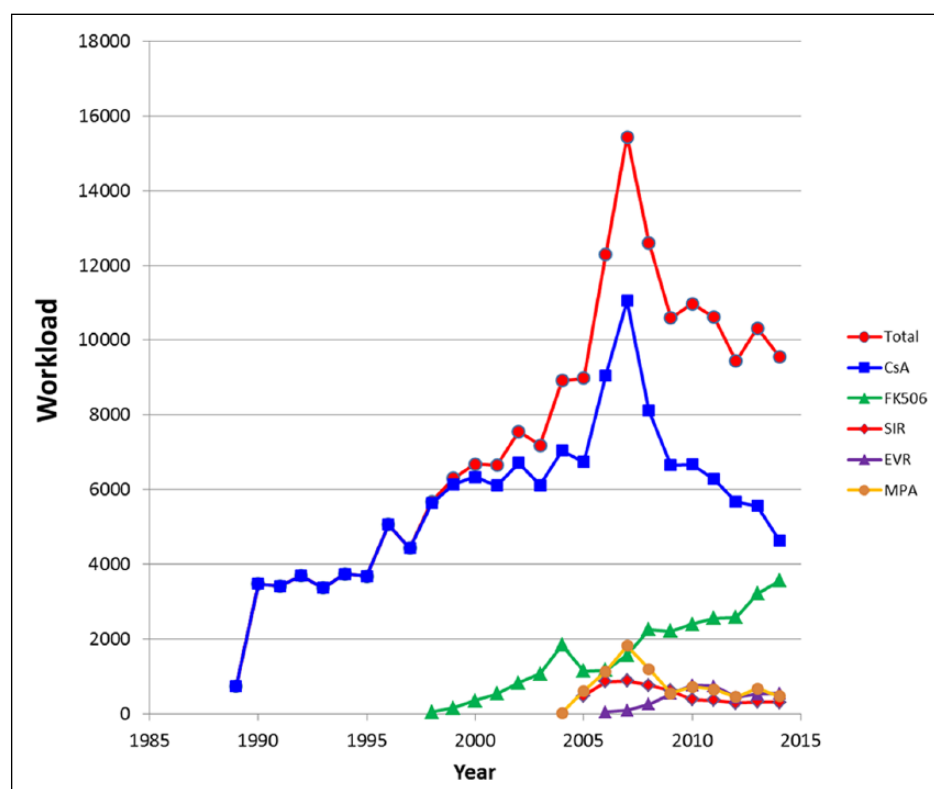

Figure 1. Annual workload of immunosuppressant testing. Test introduced: 1989 cyclosporine (CsA); 1998 tacrolimus (FK506); 2004 sirolimus (SIR), mycophenolic acid (MPA); 2006 everolimus (EVR).
Source: Pathology Annual Reports.

MPA (through its pro-drug mycophenolate mofetil), introduced for use worldwide in 1999 and 2004 respectively, were offered for drug testing in SGH in 2004.[9–11] The next breakthrough immunosuppressive drug, everolimus, released in 2004, joined the test repertoire in 2006. By this time, the annual combined workload (cyclosporine, tacrolimus, MPA and sirolimus) had reached 12,299 (Figure 1). Subsequent years saw a sharp decline attributed to a significant drop in cyclosporine testing in tandem with increase FK506 testing. Overall continued growth in

immunosuppressants testing would likely be due to increase transplants being performed.

Mass spectrometry for the better

All immunoassays are affected to some degree by cross-reactivity of similar compounds and especially metabolites of the parent drug. The cross-reactivity of metabolites can be sufficiently substantial to cause drug dosing inaccuracies. Hence, very early on, the metabolites of cyclosporine given to the

Table 2. Immunosuppressants and their metabolites.

Immunosuppressant	Metabolites	Studies (reference number)
Cyclosporine	Major: AM1, AM9 Minor: AM4N, AM19, AMc9 AM1a, AM11d	15, 16, 27, 28
Tacrolimus	M-I (13-O-demethyl) M-II (31-O-demethyl) bioactive M-III (15-O-demethyl) M-IV (12-hydroxy)	18, 19, 29
Sirolimus	Hydroxy, dihydroxy demethyl, didemethyl	11, 22, 23, 24
Everolimus	Major: 46-hydroxy, 24-hydroxy 25-hydroxy Minor: 45-hydroxy, 12-hydroxy 11-hydroxy	25, 26

Study numbers as in the References.

patient were recognized and studied. This is also shown for tacrolimus, sirolimus and everolimus.[12–26] Cyclosporine was particularly affected, with the major metabolites identified and widely known (Table 2).[11,15,16,18,19,22–29] Trough levels of AM1, the major metabolite and pharmacologically active, can equal or exceed that of the parent drug level. The same limitation can also be demonstrated for tacrolimus, sirolimus and everolimus, albeit to a lesser degree as compared with cyclosporine.

Improved immunoassays in recent developments did not totally remove the issue of active metabolite cross-reactivity as shown for cyclosporine and tacrolimus immunoassays.[27,29,30] The new improved assay for tacrolimus (a next generation assay on the Abbott ARCHITECT test system) showed a bias against LC-MS/MS, which can be accounted for by the two main metabolites, M-II and M-III, which can have 15% and 3% contribution respectively to results (in steady-state blood from renal patients) as the monoclonal antibodies used still have significant reactivity with tacrolimus M-II (94%) and M-III (45%).[29]

Other shortcomings of immunoassays include effect of haematocrit on recoveries (tacrolimus, sirolimus), overestimation compared with high performance liquid chromatography (HPLC) (sirolimus) and a recent note on matrix-comparability for QC material (everolimus).[31–39] Hence, it was noteworthy to consider more specific test methods for measuring the immunosuppressive drugs[28] and the idea of liquid chromatography-tandem mass spectrometry (LC-MS/MS) for immunosuppressant measurements in clinical practice took off.[40–43]

Clinical practice and standardization efforts

In 2007, we brought in an Agilent Technologies G6410 QQQ triple quadrupole system coupled with liquid chromatography to kick-start mass spectrometry for measuring immunosuppressants. A state-of-the-art technology then, the principle of
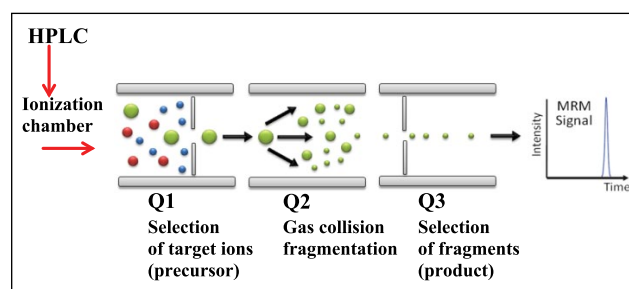

Figure 2. Tandem mass spectrometry schematics. Triple quadrupole segments (Q1–Q3) illustrating ion filtering and detection.
HPLC: high performance liquid chromatography; MRM: multiple reaction monitoring

measurement follows ionization of small molecules (as for the immunosuppressants) with the mass ions passing through three stages: the first to accurately select target analyte (precursor) mass ions, followed by fragmentation in a gas collision chamber and finally the expected fragment masses (product) identified and quantitated (Figure 2). What is novel about this technology is that for each pass of liquid chromatographically separated analytes, the instrument has the ability to perform 'multiple reaction monitoring' of various target ion pairs (precursor/product) with the system being quick enough to scan, identify and quantitate them. This was effectively a multiplexed analysis of each sample in one assay run. The subsequent quantitation technique follows the classic way by extrapolation of the unknown analyte level to a calibration curve of set values.

As no commercial assay kit was available at that time but based on published work on LC-MS/MS, our own development and establishment of a simultaneous measurement of all five current immunosuppressants (with commercially available calibrators/controls) on the LC-MS/MS test platform was presented at the 17th SGH Annual Scientific Meeting in April 2008.[44] A series of other studies – on measures taken for

controlling assay variation, analyte stability and target therapeutic levels from proposed transition of immunoassay to tandem mass spectrometry – were later presented locally and abroad. In 2009 and 2010, we also worked with fellow transplant teams (pharmacists and renal physicians) on clinical correlation of test results by LC-MS/MS following the proposed transition from immunoassay to tandem mass spectrometry technique in determining the levels for everolimus, sirolimus and tacrolimus.[45,46] The decision to move the tests to the LC-MS/MS platform was strengthened by many studies expounding the benefits of increase specificity and potential cost savings although negating factors were available staff skills, initial capital outlay and maintenance costs. Everolimus and sirolimus were ported over to the LC-MS/MS platform in November 2009 and April 2010 respectively. Currently tacrolimus and cyclosporine are slated to port over to LC-MS/MS within this year. During the in-between years, we built up staff experience and training.

Although we had also established testing for mycophenolic acid (MPA) on the LC-MS/MS with validation studies, the test remains on the immunoassay platform today in view of the specimen type (plasma instead of whole blood), workload and service requirements. The latest and current version of MPA assay in SGH is reported to align more with LC-MS/MS determinations and also less be impacted by the glucuronide form which earlier assays had cross-reactivity issues with.[47]

Our laboratory performance amongst peer groups worldwide through the CAP (College of Pathologists) EQA (External Quality Assurance) Surveys have indicated satisfactory returns for the tests (sirolimus and everolimus) that have been ported over to the LC-MS/MS methods and also for tests (tacrolimus, cyclosporine and MPA) that have remained on immunoassay platforms. More laboratories have reported with LC-MS/MS technology over the years. Results from the 2015 CAP CSM-B challenges indicated there are now 83/543 laboratories reporting cyclosporine, 85/321 reporting sirolimus and 91/573 reporting tacrolimus with LC-MS/MS. There are also 43/63 laboratories reporting everolimus with LC-MS/MS, compared with its debut in the 2013 Survey with 23/35 laboratories. This is in contrast to five years ago, in 2010, when there were 51/465 laboratories (cyclosporine), 56/220 (sirolimus) and 53/434 (tacrolimus) with LC-MS/MS. Although there is increased adoption of LC-MS/MS, interlaboratory standardization is not yet within sight as most LC-MS/MS techniques are laboratory-developed. The recent availability of commercial kits is a development that could reduce this gap. Recent CAP Surveys have shown acceptable CVs for the LC-MS/MS group as compared with all-labs CVs, with the widest variation seen for everolimus (14.7% compared with 24.5% in all-labs – 2015) and a similar picture for the other three immunosuppressants. Higher CVs variation seen in the all-labs group is also a reflection of the dominant number of various immunoassay platforms in use. While it is still a difficult-to-acquire technology for any hospital, reports have marked LC-MS/MS as the right choice to institute for (at least) immunosuppressants.[48,49] A difficulty with most research-originated techniques is that typically such rapidly developed procedures are in many cases ahead of the availability of reference materials. Standardization of reference material has yet to be available

although calibrators/controls are commercially available. In addition, matrix effects are well-known caveats in any test assay and this issue is not fully resolved although the use of appropriate internal standards has mitigated this to a large extent. Several consensus meetings have also convened to establish standardization of drug monitoring mode, test optimizations, protocols and target levels and there is still much work to do at this moment.[50–53]

Challenges near and far

From a research setting to clinical practice, our technology quotient and the reliance of LC-MS/MS as a possibly disruptive technology in medical diagnostics will be put to the test. At the 2007 European Consensus Conference on Tacrolimus Optimization at the 10th IATDMCT (International Association of Therapeutic Drug Monitoring and Clinical Toxicology) meeting, a lower limit of quantitation (LOQ) was recommended to ideally be 1.0 ng/ml so as to allow confident measurements (down) to 3.0 ng/ml, important in low-dose tacrolimus treatment regimens. The LC-MS/MS technology in SGH reports levels down to 2.0 ng/ml, and with newer instruments the lower LOQ of 1.0 ng/ml would be achievable.

While test load growth can be gradual and concomitant with the organ transplant programme as in most centres, the expectations of test requests are likely to increase especially with enhanced use of drug monitoring in the outpatient clinics. For the physicians, their clinical expectation for test turnaround time (TAT) is one to consider. The current LC-MS/MS run dates are twice a week with same day TAT. There are occasions when the laboratory performs 'urgent' requests by appointment on other weekdays and weekends. A potential rate limiting step in providing STAT TAT is that sample preparation for both the LC-MS/MS technique and current immunoassays requires pre-treatment before analysis. Reducing the whole work process further to 1–2 h TAT (for all four immunosuppressive drugs) would stretch the laboratory's capability as on a typical busy morning clinic day 50–70 specimens are received. To overcome this potential bottleneck, one approach is to increase capacity. As the local medical fraternity pushes for all things in a 'hub-'n'-spoke' model, it can only mean a more demanding need for test results from this hub to be ready in short times. Will the test be available 24/7 for a hospital with an active transplant programme? Can the average medical technologist in turn be able to handle the complexity of 'research-grade' instruments round the clock? The desired outcome is a 'Yes' to these expectations.

Conclusions

Better management of immunosuppressant therapy has depended to a large extent on accurate measurement of the effective parent drug while minimizing measurement of its metabolites. In the quest for tight titration norms, it is generally accepted that mass spectrometry is the way to proceed for drug testing. In a high volume demand situation like a central transplant hub in a tertiary acute hospital (such as SGH), operational challenges to the laboratory are in terms of both hardware availability and also skilled technologists to be able to provide full seven-day service and report the results in a

timely manner. It will require full management support and encouragement to meet future expectations.

Drug developments such as the biologics present the next frontier for immunosuppression advances. Being antibody-based, manufactured in a customized form to reduce host (patient) reaction and more effective in stopping the immune transduction network, these biologically engineered bio-agents will be a challenge to measure by the clinical laboratory. Although available for many years, the number of biologics is also increasing, for example, alemtuzumab (CD52; Campath-IH), muromoab (CD30, OKT3), basiliximab and diachizumab (IL2R antagonist), and they are now heading for mainstream use. Will they ever need to be specifically monitored and what metric is best to measure their effectiveness?[54–56] The laboratory will need to define the metric for measurement and the technology to determine it and report results that are appropriate for clinical management and monitoring purposes.

Conflict of interest

Funding

References

1. Watson CJE and Dark JH. Organ transplantation: historical perspective and current practice. *Br J Anaesth* 2012; 108(Suppl.1): i29–i42.
2. Taylor AL, Watson CJ and Bradley JA. Immunosuppressive agents in solid organ transplantation: mechanisms of action and therapeutic efficacy. *Crit Rev Oncol Hematol* 2005; 56: 23–46.
3. Pillai AA and Levitsky J. Overview of immunosuppression in liver transplantation. *World J Gastroeneterol* 2009; 15: 4225–4233.
4. Halloran PF. Immunosuppressive drugs for kidney transplantation. *N Engl J Med* 2004; 351: 2715–2729.
5. Helderman JH, Bennett WM, Cibrik DM, et al. Immunosuppression: practice and trends. *AM J Transplant* 2003; 3(Suppl. 4): 41–52.
6. Johnston A and Holt DW. Therapeutic drug monitoring of immunosuppressant drugs. *J Clin Pharmacol* 1999; 47: 339–350.
7. Shaw LM, Holt DW, Keown P, et al. Current opinions on therapeutic drug monitoring of immunosuppressive drugs. *Clin Ther* 1999; 21: 1632–1652.
8. Sanghvi A, Diven WF, Warty VS, et al. Cyclosporine measurement by fluorescent polarization immunoassay utilizing Abbott TDx instrument. *Transplant Proc* 1989; 21: 898–900.
9. Greiner FC, Luczkiw J, Bergmann M, et al. A whole blood FK506 assay for the IMx analyser. *Transplant Proc* 1991; 23: 2748–2749.
10. Wallemacq PE and Reding R. FK506 (tacrolimus), a novel immunosuppressant in organ transplantation: clinical, biomedical and analytical aspects. *Clin Chem* 1993; 39: 2219–2228.
11. Stenton SB, Partovi N and Ensom MH. Sirolimus: the evidence for clinical pharmacokinetic monitoring. *Clin Pharmacokinet* 2005; 44: 769–786.
12. Wallemacq PE, Lee SC, Lhoest G, et al. Cross-reactivity of cyclosporine metabolites in two different radioimmunoassays in which the same specific monoclonal antibody is used. *Clin Chem* 1990; 36: 385.
13. Lensmeyer GL, Wiebe DA, Carlson IH, et al. Three commercial polyclonal immunoassays for cyclosporine in whole blood compared: 2. Cross-reactivity of the antisera with cyclosporine metabolites. *Clin Chem* 1990; 36: 119–123.
14. Yatscoff RW, Rosano TG and Bowers LD. The clinical significance of cyclosporine metabolites. *Clin Biochem* 1991; 24: 23–35.
15. Bleck JS, Schlitt HJ, Christians U, et al. Ciclosporin metabolite pattern in blood and urine of kidney graft patients in relation to liver function. *Eur J Clin Pharmacol* 1991; 40: 565–569.
16. Christians U, Kohlhaw K, Surig T, et al. Parallel blood concentrations of second-generation cyclosporine metabolites and bilirubin in liver graft recipients. *Ther Drug Monit* 1995; 17: 487–498.
17. Steimer W. Evaluation of the AxSYM CyA assay compared with HPLC, TDx monoclonal and EMIT with both pretreatments. *Transplant Proc* 1998; 30: 4045–4047.
18. Gonschior AK, Christians U, Winkler M, et al. Tacrolimus (FK506) metabolites patterns in blood from liver and kidney transplant patients. *Clin Chem* 1996; 42: 1426–1432.
19. Iwasaki K, Shiraga T, Matsuda H, et al. Further metabolism of FK506 (tacrolimus). Identification and biological activities of the metabolites oxidized at multiple sites of FK506. *Drug Metab Dispos* 1995; 23: 28–34.
20. Tredger JM, Gilkes CD and Gonde CE. Therapeutic monitoring of Tacrolimus (FK506) with the first- and second-generation microparticle enzyme immunoassays: performance and results in four patient populations. *Ther Drug Monit* 1998; 20: 266–275.
21. Murthy JN, Davis DL, Yatscoff RW, et al. Tacrolimus metabolite cross-reactivity in different tacrolimus assays. *Clin Biochem* 1998; 31: 613–617.
22. Streit F, Christians U, Schiebel HM, et al. Sensitive and specific quantification of sirolimus (rapamycin) and its metabolites in blood of kidney graft recipients by HPLC/electrospray-mass spectrometry. *Clin Chem* 1996; 42: 1417–1425.
23. Kirchner GI, Jacobsen W, Deters M, et al. Fast quantification method for sirolimus and its major metabolites. *Transplant Proc* 2001; 33: 1091–1092.
24. Holt DW, McKeown DA, Lee TD, et al. The relative proportions of sirolimus metabolities in blood using HPLC with mass-spectrometric detection. *Transplant Proc* 2004; 36: 3223–3225.
25. Strom T, Haschke M, Zhang YL, et al. Identification of everolimus metabolite patterns in trough blood samples of kidney transplant patients. *Ther Drug Monit* 2007; 29: 592–599.
26. Strom T, Haschke M, Boyd J, et al. Crossreactivity of isolated everolimus metabolites with the Innofluor Certican immunoassay for therapeutic drug monitoring of everolimus. *Ther Drug Monit* 2007; 29: 743–749.
27. Wallemacq P, Maine GT, Berg K, et al. Multisite analytical evaluation of the Abbott ARCHITECT cyclosporine assay. *Ther Drug Monit* 2010; 32: 145–151.
28. Steimer W. Performance and specificity of monoclonal immunoassays for cyclosporine monitoring: how specific is specific? *Clin Chem* 1999; 45: 371–381.
29. Wallemacq P, Goffinet JS, O'Morchoe S, et al. Multi-site analytical evaluation of the Abbott ARCHITECT tacrolimus assay. *Ther Drug Monit* 2009; 31: 198–204.
30. Wallemacq PE and Alexandre K. Evaluation of the new AxSYM cyclosporine assay: comparison with TDx monoclonal whole blood and Emit cyclosporine assays. *Clin Chem* 1999; 45: 432–435.
31. Schmid RW, Lotz J, Schweigert R, et al. Multi-site analytical evaluation of a chemiluminescent magnetic microparticle immunoassay (CMIA) for sirolimus on the Abbott ARCHITECT analyser. *Clin Biochem* 2009; 42: 1543–1548.

32. Taylor PJ and Morris RG. Tacrolimus measurement by microparticle enzyme immunoassay II. (Editorial.)*Ther Drug Monit* 2003; 25: 259–260.

33. Kuzuya T, Ogura Y, Motegi Y, et al. Interference of haematocrit in the tacrolimus II microparticle enzyme immunoassay. *Ther Drug Monit* 2002; 24: 507–511.

34. Brown NW, Gonde CE, Adams JE, et al. Low haematocrit and serum albumin concentrations underlie the overestimation of tacrolimus concentrations by microparticle enzyme immunoassay versus liquid chromatography-tandem mass spectrometry. *Clin Chem* 2005; 51: 586–592.

35. Morris RG, Salm P, Taylor PJ, et al. Comparison of the reintroduced MEIA assay with HPLC-MS/MS for the determination of whole-blood sirolimus from transplant recipients. *Ther Drug Monit* 2006; 28: 164–168.

36. Salm P, Taylor PJ and Pillans PI. The quantification of sirolimus by high-performance liquid chromatography-tandem mass spectrometry and microparticle enzyme immunoassay in renal transplant recipients. *Clin Ther* 2000; 22(Suppl. B): B71–B85.

37 Westley IS, Morris RG, Taylor PJ, et al. CEDIA sirolimus assay compared with HPLC MS/MS and HPLC-UV in transplant recipient specimens. *Ther Drug Monit* 2005; 27: 309–314.

38 Park HI, Oh EJ, Park YJ, et al. Failure of systematic error detection with internal quality control program. (Letter to Editor.) *Ann Clin Lab Sci* 2008; 38: 410–413.

39. Moes DJ, Press RR, de Fijter JW, et al. Liquid chromatography-tandem mass spectrometry outperforms fluorescence polarization immunoassay in monitoring everolimus therapy in renal transplantation. *Ther Drug Monit* 2010; 32: 413–419.

40. Keevil BG, Tierney DP, Cooper DP, et al. Rapid liquid chromatography-tandem mass spectrometry method for routine analysis of cyclosporine A over an extended concentration range. *Clin Chem* 2002; 48: 69–76.

41. Keevil BG, McCann SJ, Cooper DP, et al. Evaluation of a rapid micro-scale assay for tacrolimus by liquid chromatography-tandem mass spectrometry. *Ann Clin Biochem* 2002; 39: 487–492.

42. Wallemacq PE, Vanbinst R, Asta S, et al. High-throughput liquid chromatography-tandem mass spectrometric analysis of sirolimus in whole blood. *Clin Chem Lab Med* 2003; 41: 921–925.

43. Deters M, Kaever V and Kirchner GI. Liquid chromatography/mass spectrometry for therapeutic drug monitoring of immunosuppressants. *Anal Chim Acta* 2003; 492: 133–145.

44. Ng WY, Chong SC and Jacob E. Simultaneous analysis of immunosuppressants with liquid chromatography-tandem mass spectrometry. In: *Proceedings of the SGH 17th Annual Scientific Meeting, Singapore*, 25–26 April 2008.

45. Yong HS, Fan PYW, Lee PH, et al. Clinical outcomes of kidney transplant recipients during transition from immunoassay to tandem mass spectrometry based therapeutic drug monitoring of everolimus. 13th International Congress of the Transplantation Society, Vancouver, Canada, 15–19 August 2010.

46. Yeo YT, Cao JY, Mallya D, et al. Evaluation of clinical impact of switching from fluorescent polarization immunoassay (FPIA) to high performance liquid chromatography-tandem mass spectrometry (HPLC-TMS) for therapeutic drug monitoring of sirolimus in renal transplant recipients. 12th Congress of Asian Society of Transplantation, 25–28 September 2011, Seoul, Korea.

47. Lim KSL, Ng WY, Yeo CP, et al. Technical evaluation of Roche total MPA assay with comparison to the Siemens EMIT 2000 MPA assay. SingHealth – Duke-NUS Scientific Congress, 3–4 August 2012, Singapore.

48. Adaway JE and Keevil BG. Therapeutic drug monitoring and LC-MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci* 2012; 883–884: 33–39.

49. Aucella F, Lauriola V, Vecchione G, et al. Therapeutic drug monitoring (TDM) in renal transplant (RT): reasons to choose the LC-MS/MS method. *J Chromatogr Sep Tech* 2012; 3: e101.

50. Annesley TM, McKeown DA, Holt DW, et al. Standardisation of LC-MS for therapeutic drug monitoring of tacrolimus. *Clin Chem* 2013; 59: 1630–1637.

51. 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.

52. Kahan BD, Shaw LM, Holt D, et al. Consensus document: Hawk's Cay meeting on therapeutic drug monitoring of cyclosporine. *Clin Chem* 1990; 36: 1510–1516.

53. Kuypers DRJ, Le Meur Y, Cantarovich M, et al. Consensus report on therapeutic drug monitoring of mycophenolic acid in solid organ transplantation. *Clin J Am Soc Nephrol* 2010; 5: 341–358.

54. Budde K, Matz M, Durr M, et al. Biomarkers of over-immunosuppression. *Clin Pharmacol Ther* 2011; 90: 316–322.

55. Kumar V and Gaston RS. Advances in kidney transplant immunosuppression: emerging biologics. *Dialysis Transplant* 2011; 40: 30–32.

56. Page EK, Dar WA and Knechlte SJ. Biologics in organ transplantation. *Transplant Int* 2012; 25: 707–719.