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# Performance of the Roche Total Mycophenolic Acid® assay on the Cobas Integra 400®, Cobas 6000® and comparison to LC-MS/MS in liver transplant patients

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## Abstract

**Background:** Mycophenolic acid (MPA) is an immunosuppressant for which therapeutic drug monitoring (TDM) is performed for optimal prophylaxis and avoidance of toxicity in transplant patients. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is ideally suited for TDM of MPA. There have been several method comparisons of the Roche Total MPA assay, but none have been performed with respect to liver transplant patients.
**Methods:** We validated the Roche Total MPA assay on the Cobas Integra 400 and Cobas 6000 and compared it to a validated LC-MS/MS (API 2000™) method. Fifty-five EDTA plasma samples from liver transplant patients were measured with the Roche assay on these platforms and compared to the LC-MS/MS results.
**Results:** Validation of the LC-MS/MS, Cobas Integra 400 and 6000 was performed with good results. The LC-MS/MS/Integra 400/Cobas 6000 were linear up to 30, 15 and 17 mg/L, respectively. Imprecision was $<10\%$ for LC-MS/MS and $<7\%$ for the Roche assay on both platforms. The samples showed good agreement with LC-MS/MS. Passing-Bablok regression analysis showed Cobas Integra (mg/L) = 1.02×LC-MS/MS (mg/L)–0.50 and Cobas 6000 (mg/L) = 0.98×LC-MS/MS–0.47.
**Conclusions:** The Roche Total Mycophenolic Acid-assay is suitable for measuring total MPA in plasma from liver transplant patients and is a good alternative for LC-MS/MS.

**Keywords:** enzyme assay; inosine monophosphate dehydrogenase; liquid chromatography-tandem mass spectrometry; liver transplantation; mycophenolic acid.

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## Introduction

In transplant patients, mycophenolic acid (MPA) has a prominent role in the prophylaxis of acute rejection. The drug is administered to kidney, heart, liver, lung, bowel, pancreas and bone marrow transplant patients. MPA is commonly used in combination with corticosteroids and calcineurin inhibitors, such as tacrolimus and cyclosporine, or in combination with the macrolide antibiotic sirolimus (1).

Two forms are widely available: the prodrug mycophenolate mofetil (MMF) and the enteric-coated mycophenolate sodium (EC-MPS). MMF and EC-MPS are absorbed from the gastrointestinal tract and hydrolyzed, resulting in a high MPA bioavailability ($>90\%$). MPA is the active metabolite of MMF. MPA is metabolized in the liver to the pharmacologically inactive phenolic glucuronide (MPAG), a phenolic glucoside and the in vitro active acyl glucuronide (AcMPAG) (2, 3). The pharmacokinetics of MPA are complicated by the enterohepatic circulation, giving rise to a second peak of MPA 6–12 h following oral administration. Furthermore, the pharmacokinetics are influenced by the high protein binding of MPA; 97% of MPA is bound to plasma albumin and only the free fraction has immunosuppressive action (4). Therapeutic drug monitoring (TDM) of MPA may minimize the risk for rejection after transplantation. Despite insufficient evidence to provide unequivocal guidelines on the requirement for MPA monitoring in liver transplantation, the latest Consensus Report indicated six conditions where TDM of MPA is recommended (1). Monitoring the area under the curve (AUC) has been recommended, an $AUC_{0-12\ h}$ $>30$ mg/L/h and $<60$ mg/L/h provides optimal prophylaxis (1, 5).

The standard procedures to quantify MPA currently used are high performance liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). The use of the Enzyme Multiplied Immunoassay Technique (EMIT®) and Cloned Enzyme Donor Immunoassay method (CEDIA®) for MPA have not gained widespread use, mainly because of the cross-reactivity with the metabolite AcMPAG (6–12). The use of the Roche Total Mycophenolic Acid® assay has previously been compared with HPLC and LC-MS/MS methods for renal and cardiac transplant patients (13–16). However, to our knowledge there have been no studies about the performance in liver transplant patients. In this study, we evaluated the Roche Total Mycophenolic Acid® assay (Roche Diagnostics, IN, USA) on the COBAS Integra® 400 and Cobas 6000® (Roche Diagnostics, IN, USA) and compared it with LC-MS/MS.

## Materials and methods

### Sample collection

Fifty-five EDTA plasma samples from liver transplant patients at our university hospital were collected. These samples were analyzed on the three platforms on the same day. The samples were anonymized leftover samples from routine analysis.

### Enzyme assay

The Roche Total Mycophenolic Acid® assay was performed on the Cobas Integra® 400 and Cobas 6000® (Roche Diagnostics, IN, USA) according to the manufacturer's instructions. Reactions conditions are detailed in Figure 1. We used serum-based total MPA calibrators and total MPA controls from Roche Diagnostics for all assays. The calibrators consisted of six levels: 0, 1, 3, 5, 10 and 15 mg/L. The controls consisted of three levels: 0.869 mg/L (range 0.695–1.043 mg/L), 3.58 mg/L (range 2.86–4.30 mg/L) and 12.5 mg/L (range 10.1–14.9 mg/L).

### LC-MS/MS

LC-MS/MS was performed with an API 2000™ (Applied Biosystems, Foster City, CA, USA) with an electrospray ionization (ESI) inlet. The mobile phase was a gradient of 2 mmol/L ammonium acetate (Fluka, Buchs, Switzerland) in water and methanol (Biosolve BV, Valkenswaard, The Netherlands). The column used was an Agilent© Zorbax RP-C18, 2.1×30 mm (Agilent Technologies Inc., Santa Clara, CA, USA). Precipitation fluid (200 μL) containing 3.33 mg/L internal standard mycophenolic acid carboxyether (Roche Bioscience, Palo Alto, CA, USA) in methanol (Biosolve BV, Valkenswaard, The Netherlands) and zinc sulphate 0.3 mol/L (Merck nv, Overijse, Belgium) (70/30 vol:vol) was added to the EDTA plasma samples (50 μL). After vortexing for 1 min, 5 min centrifugation at 12,000 rpm, the supernatant (60 μL) was transferred into a vial. The injection volume was 3 μL. The system consisted of an Agilent© 1100 LC Quaternary Pump, thermostated to 40°C by an Agilent Column Oven. During the acquisition there was a flow rate of 500 μL/min using solvent A (2 mmol/L $NH_4^+$ in water) and solvent B (2 mmol/L $NH_4^+$ in methanol). An eluent gradient was used, starting with 1.5 min 90% A and 10% B, 9.5 min 5% A and 95% B and ending with 12 min 90% A and 10% B. We used a multiple reaction monitoring (MRM) acquisition method of 12 min. The following transitions were monitored: 438.3/303.0 ($NH_4^+$-MPAC), 421.1/303.2 (MPAC), 338.2/207.0 ($NH_4^+$-MPA, quantifier transition), 321.1/303.1 (MPA) and 321.1/159.0 (qualifier transition). The ammonium adduct of MPA and MPAC was used for sample quantification.

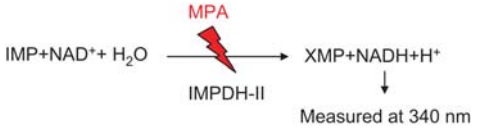


**Figure 1** Schematic overview of the enzymatic reaction of the Roche Total Mycophenolic Acid® assay.
IMP, inosine monophosphate; NAD, nicotinamide adenine dinucleotide; MPA, mycophenolic acid; IMPDH-II, type 2 inosine monophosphate dehydrogenase; XMP, xanthosine monophosphate. IMP is enzymatically converted into XMP through the enzyme IMPDH-II. This step requires conversion of NAD to NADH. MPA blocks the enzymatic process of IMP into XMP. When MPA is present, there is no formation of XMP and hence no release of NADH. In the assay from Roche, the formation of NADH is measured at 340 nm. NADH production is inversely proportional to the concentration of MPA.

### Statistical analysis

We used MedCalc® (MedCalc Software, Mariakerke, Belgium) and Microsoft Office Excel® (Microsoft Corporation, Redmond, WA, USA). A Grubbs test, a one sided F-test and a one-way ANOVA test were used in the LC-MS/MS method validation. Passing-Bablok regression analysis was performed in the method comparison.

### LC-MS/MS method validation

The LC-MS/MS method for the quantification of total mycophenolate (tMPA) in plasma, was validated according to a published experimental design (17). This design includes a pre-validation and a main validation phase.

In the pre-validation phase, absence of interferences (selectivity), matrix effects, processed sample stability and linearity were investigated. Ion suppression/enhancement (ME), extraction efficiency (RE) and process efficiency (PE) were measured as described by Matuszewski et al. (18). Linearity was assessed after removing outliers with the Grubbs test (19), and the check for homogeneity of variance was performed with a one-sided F-test between the variances at the highest and lowest concentration values (17). A p-value $<0.05$ was considered significant.

For the main validation, the Roche total MPA calibrators and control material were run over an 8 day period in duplicate, with a new calibration curve obtained each day. Using a one-way ANOVA with each day as the grouping variable allowed the calculation of intra- and inter-assay precision, and total assay precision expressed as percent coefficient of variation (CV) (17). In order to measure the variability of the calibration curve, the imprecision and accuracy characteristics were determined, as well as the lower limit of quantification (LLOQ). The imprecision and accuracy was deemed acceptable, at respectively, 10% and 15%, and the CV and bias at LLOQ at 20% (20). The dilution experiment was performed with a serial dilution (dilution 2, 4, 8) with bovine serum on a spiked plasma pool (60 mg/L). The plasma pools underwent three freeze/thaw cycles.

### Method validation on Cobas Integra 400 and Cobas 6000

In order to assess linearity, 11 dilutions were made spanning 0%–100% of a high concentration pool (28.5 mg/L). The lower limit of detection (LLOD) was measured by running 21 replicates of three blank samples on three different days. The LLOQ was determined using a functional sensitivity approach. Ten aliquots of five patient samples with total MPA concentrations between 0.45 and 0.85 mg/L were analyzed on 10 different days. Imprecision characteristics were determined by analyzing the Roche total MPA controls, Roche total MPA calibrator B (1 mg/L) and three total MPA plasma pools (0.8, 2.5 and 9.0 mg/L), in triplicate for 21 days. The criteria from Roche Diagnostics were used to evaluate the imprecision: SD $\leq$0.07 up to a concentration of 1 mg/L and CV $\leq$7.0% at concentrations $>$1 mg/L.

### Method comparison

Only measurements up to 15 mg/L could be used for comparison because the Cobas Integra reports all results $>$15 mg/L as

'>15 mg/L'. Higher concentrations can be diluted with blank plasma or diluent, but we did not evaluate this. Forty-seven of 55 samples were <15 mg/L, the other eight samples were excluded from the method comparison.

For evaluating the degree of agreement, Passing-Bablok regression analysis was used to determine proportional and constant bias by checking of the 95% confidence intervals (CI) of the slope and the intercept. If one was not included in the 95% CI of the slope, there is constant error, and if zero was not included in the 95% CI of the intercept, proportional error is present.

## Results

### LC-MS/MS method validation

**Pre-validation** No interferences were detected with 10 different blank plasma samples and two zero samples. Possible interference from cyclosporine, tacrolimus, sirolimus and everolimus was tested at concentrations of, respectively, 514, 16.7, 20.4 and 8.48 μg/L. The compounds did not cause any interference.

The ME, RE and PE were, respectively, 87%, 97% and 85% for the low concentration, and 85%, 98% and 83% for the high concentration. Our method showed similar results as Shen et al. (ME 90.7%–94.3%; RE 90.6%–93.4%) (21).

There were no obvious changes in stability of processed samples during the analysis. The within-group CV at 0.63 mg/L was 5.2%, and at 3.14 mg/L was 4.4%. Ranging from 0 to 30 mg/L, the linearity was determined after identifying one outlier and performing a one-sided F-test between the variances at the 6 mg/L pool and the 30 mg/L pool. The variance ratio was 2.36 ($p=0.363$), the variance over the calibration range is not homogeneous and therefore a weighted regression model was used (weighting factor $1/x$, $r=0.99$).

**Main validation** Variability of the calibration curve: two concentration levels in our six-point calibration curve were too high. The target concentrations of 1 mg/L and 5 mg/L were not within the 95% confidence interval of our measured mean value, respectively [1.0068–1.0832] and [5.0073–5.2802]. The blank calibrator was excluded from this experiment, the other five concentration levels were: 1, 3, 5, 10, 15 mg/L, and the between run CVs were respectively: 4.4, 2.6, 3.2, 2.9, 1.9%. The within group CV was 1.6%. Bias ranged from 0.04% to 4.5%.

Results of accuracy and imprecision are given in Table 1. At each QC level, the CV and bias was <10%. The LLOQ was determined by analyzing two spiked plasma pools at two different concentrations (0.18–0.36 mg/L). The criterion of <20% CV was met for both concentrations. The LLOQ was therefore set to 0.18 mg/L.

The within group CV at dilution 1 (60 μg//L), 2 (30 μg/L), 4 (15 μg/L), 8 (7.5 μg/L) were respectively 6.3%, 8.8%, 6.2%, 8.4%. Linearity was good up to 30 mg/L. Above this concentration, the test reached a plateau, with a high negative bias (19%) at 60 mg/L. The 24 results at each concentration all gave <10% CV.

### Method validation on Cobas Integra 400 and Cobas 6000

The linearity could only be measured partially on the Cobas Integra 400, where all results >15 mg/L are expressed as '>15'. The bias was <10% for all measured concentrations and linearity was good between 0.4 and 15 mg/L. On the Cobas 6000 the method was not linear up to 28.5 mg/L, but up to 17 mg/L only. Analyzing blank samples on the Cobas Integra yielded an LLOD that was <0.4 mg/L. On the Cobas 6000, all results were lower than the LLOQ (0.45 mg/L) with a mean LLOD of 0.06 mg/L. The functional sensitivity was evaluated using five samples with mean concentrations ranging from 0.45 to 0.85 mg/L, the CV for the Cobas Integra ranged between 5.3% and 12.8% and for the Cobas 6000 between 5.1% and 13.8%. The LLOQ was 0.45 mg/L for the Cobas Integra and 0.48 mg/L for the Cobas 6000.

The Cobas Integra and the Cobas 6000 met the Roche imprecision criteria for all levels, except for calibrator B on the Cobas 6000 ($SD=0.13$). There were no significant differences in CV or bias between Cobas Integra 400 and Cobas 6000.

### Method comparison

Figures 2 and 3 show good the agreement between LC-MS/MS and the Cobas Integra 400 and Cobas 6000. The range of measurement was 1.19–14 mg/L (median, 5.35 mg/L) for LC-MS/MS, 0.72–14.68 mg/L (median, 5.02 mg/L) for Integra and 0.63–14.16 mg/L (median, 4.89 mg/L) for Cobas 6000. Passing-Bablok regression analysis yielded an equation of Cobas Integra 400 (mg/L) = 1.02 × LC-MS/MS (mg/L)

**Table 1** Imprecision and accuracy characteristics of the LC-MS/MS method. Quality control materials were run over 8 days in duplicate (a and b).

| | Low | | Medium | | High | |
|---|---|---|---|---|---|---|
| | QCIa | QCIb | QCIIa | QCIIb | QCIIIa | QCIIIb |
| Target, μg/mL | 0.87 | 0.87 | 3.58 | 3.58 | 12.50 | 12.50 |
| Mean, μg/mL (n=8) | 0.84 | 0.86 | 3.50 | 3.64 | 12.26 | 12.59 |
| Bias, % | –4.0 | –0.5 | –2.2 | 1.8 | –1.9 | 0.7 |
| Intra-assay CV, % | 3.5 | | 5.5 | | 3.1 | |
| Inter-assay CV, % | 2.9 | | 0.0 | | 0.0 | |
| Total precision CV, % | 4.6 | | 5.3 | | 2.7 | |

SD, standard deviation; CV, coefficient of variation.

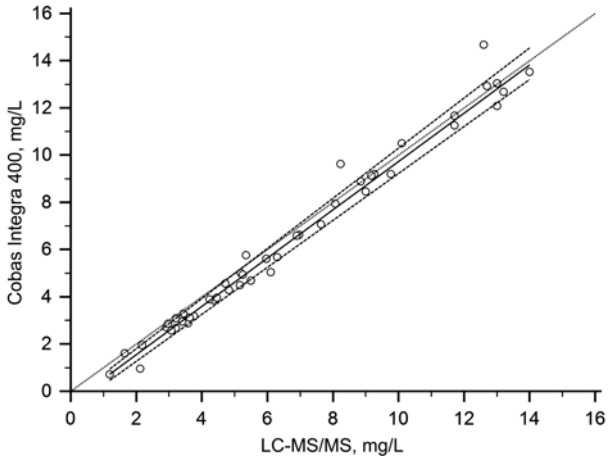


**Figure 2** Passing-Bablok regression analysis of the Cobas Integra 400 with LC-MS/MS.
Cobas Integra 400 (mg/L) = 1.02 × LC-MS/MS (mg/L)–0.50 ($r^2$ = 0.98). The full line is the regression-line. The two dashed lines show the 95% confidence interval and the point line is slope = 1.

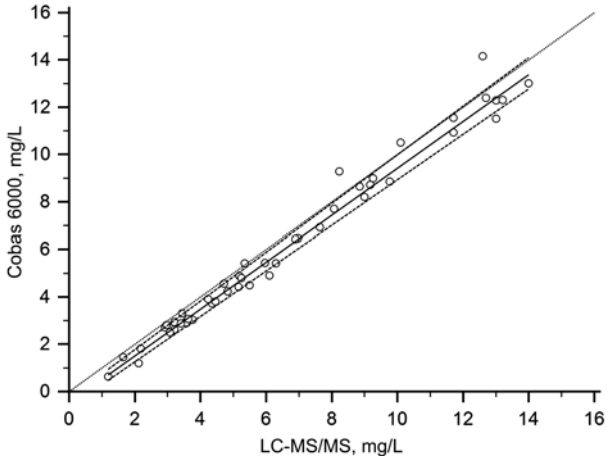


**Figure 3** Passing-Bablok regression analysis of Cobas 6000 with LC-MS/MS.
Cobas 6000 (mg/L) = 0.98 × LC-MS/MS (mg/L)–0.47 ($r^2$ = 0.98). The full line is the regression-line. The two dashed lines show the 95% confidence interval and the point line is slope = 1.

–0.50 (95% CI intercept: –0.66 to –0.281; 95% CI slope: 0.96–1.03) (Figure 2) and Cobas 6000 (mg/L) = 0.98 × LC-MS/MS (mg/L)–0.47 (95% CI intercept: –0.72 to –0.324; 95% CI slope: 0.99–1.06) (Figure 3). The mean absolute difference was, respectively, 0.45 mg/L and 0.26 mg/L (Figures 4 and 5). These results indicate that there is constant error, but no proportional error.

## Discussion

There is a good agreement between results of the LC-MS/MS and Cobas Integra or Cobas 6000. As indicated in Table 2, our regression analysis is similar to other studies (13–15,

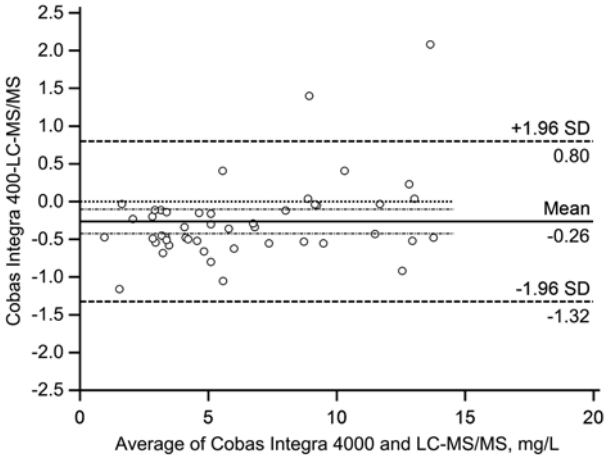


**Figure 4** Bland-Altman plot of the Cobas Integra compared to LC-MS/MS.
The Bland-Altman plot of the Cobas Integra compared to LC-MS/MS demonstrates that Cobas Integra 400 measures approximately 0.26 mg/L lower than LC-MS/MS. The two point-dashed lines are the 95% confidence interval of mean of differences. The point line is the line of equality.

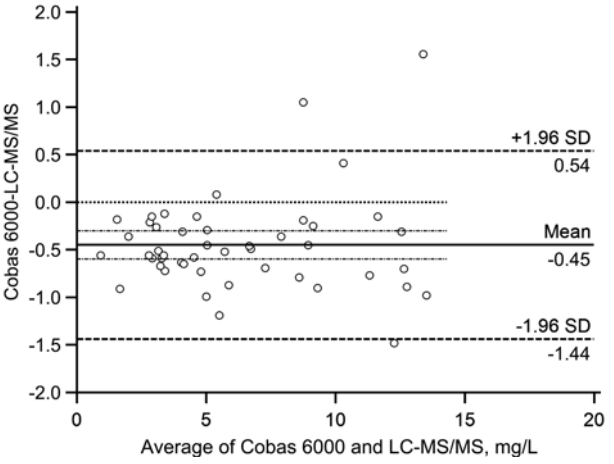


**Figure 5** Bland-Altman plot of the Cobas 6000 compared to LC-MS/MS.
Bland-Altman plot of the Cobas 6000 compared to LC-MS/MS demonstrates that the Cobas 6000 measures approximately 0.45 mg/L lower than LC-MS/MS. The two point-dashed lines are the 95% confidence interval of mean of differences. The point line is the line of equality.

22, 23). However, in our study, a higher intercept was obtained. The intercepts of the Cobas Integra 400 (–0.50) and the Cobas 6000 (–0.47) were similar. Problems with LC-MS/MS, such as falsely lowered results due to ion suppression, or falsely elevated results by in-source fragmentation of the phenolic glucuronide have been described (24). The Bland-Altman plots in Figures 4 and 5 indicate that the enzymatic assays yield lower results than the LC-MS/MS, for the Cobas 6000 approximately 0.45 mg/L and the Cobas Integra 0.26 mg/L. However, the observed percent ion suppression (14%) in our study could not explain the lower intercept, and

the in-source fragmentation of the phenolic glucuronide was not present in our method. Moreover, MPA and MPAG were baseline separated. Besides this constant error, the slopes and coefficients of the regression analysis were comparable with other studies. Together with other studies, summarized in Table 2, there is sufficient proof that the Roche assay can quantitatively measure total MPA in liver transplant patients. However, further studies are needed to confirm the negative bias.

A well described problem for the EMIT and CEDIA method is the overestimation of, respectively, 25% and 36% of the MPA concentration at the LLOQ concentration. This could partly be explained by cross-reactivity with AcMPAG (1). For the IMPDH (type 2 inosine monophosphate dehydrogenase)-based enzyme inhibition enzyme assays (Roche), MPA overestimation by AcMPAG was low (<5%) and was considered as clinically irrelevant (13). In our study, too few samples at such concentrations were included in the study to confirm these findings.

The most important advantage of chromatography is the possibility to measure the MPA metabolites. Measurement of the metabolites is a growing concern, especially in liver and renal compromised patients. The largest CVs for AUC and predose concentrations were found in postoperative liver transplant patients and in renal transplant patients with early graft dysfunction. For liver transplant patients this is probably due to hyperbilirubinemia, uremia and hypoalbuminemia. MPA is 97% bound to albumin, while MPAG is only 82% bound to albumin. MPAG itself is not pharmacologically active, the formation of an O- or N-glucuronides is a common pathway in phase II drug metabolism and is considered a detoxification mechanism (25). However, high MPAG concentrations can augment the free fraction of MPA by displacement effects on albumin (26). In case of liver transplant patients, measuring high MPAG can indicate an increase in free MPA. Renal insufficiency is characterized by a higher free MPA fraction and lower total MPA due to restrictive clearance (27). Besides the need for measuring both MPA and MPAG, AcMPAG should also be measured. The immunosuppressant action of MPA is an uncompetitive and reversible inhibition of IMPDH, resulting in a decreased de novo synthesis of guanine nucleotides and impaired nucleic acid synthesis (25). AcMPAG seems to inhibit IMPDH in vitro, but its possible immunosuppressive activity needs to be further investigated. In contrast, AcMPAG may be involved in the development of gastrointestinal side-effects associated with MPA. Wieland et al. demonstrated in vitro that an acyl glucuronide has the potential to induce a proinflammatory reaction in human leukocytes. As diarrhea and intestinal ulceration may result from an inflammatory reaction, and AcMPAG would promote release of cytokines in vivo, AcMPAG could contribute to these toxic actions of MPA (2, 25). Many chromatographic methods to measure free MPA, MPAG and AcMPAG have been described (21, 26, 28–33). The downside is that they all require a significant amount of knowledge, sophisticated equipment and trained technicians, and are therefore expensive and time-consuming.

The latest consensus report on TDM of MPA advises measurement of total MPA in limited sampling strategies for solid organ transplants. Measuring free MPA or metabolites

**Table 2** Summary of published method comparison studies between measurement of total MPA with the enzymatic assay and chromatographic methods.

| Author | Year | Group specifications | n | Linear regression | Correlation | Reference method | Analyzer |
|---|---|---|---|---|---|---|---|
| Domke et al. (22) (abstract) | 2005 | Total MPA | 96 | 1.061x + 0.11 | r = 0.99 | LC-MS/MS | Cobas Integra |
| Marquet et al. (14) (abstract) | 2006 | Adult renal + tacrolimus | 270 | 1.044x + 0.03 | $r^2$ = 0.97 | LC-MS/MS | Cobas Integra |
| | | Adult lung + tacrolimus | 109 | 1.025x + 0.059 | $r^2$ = 0.96 | LC-MS/MS | Cobas Integra |
| | | Adult renal + sirolimus | 240 | 1.105x–0.144 | $r^2$ = 0.97 | LC-MS/MS | Cobas Integra |
| | | Pediatric renal + cyclosporin | 115 | 1.0x + 0.06 | $r^2$ = 0.96 | LC-MS/MS | Cobas Integra |
| | | Adult renal + cyclosporin | 119 | 1.208x–0.109 | $r^2$ = 0.98 | LC-MS/MS | Cobas Integra |
| Luthe et al. (23) (poster) | 2006 | Renal/cardiac transplant | 87 | 1.055x–0.010 | r = 0.98 | LC-MS/MS | Cobas Integra 800 |
| | | Renal/cardiac transplant | 87 | 1.045x + 0.1011 | r = 0.97 | LC-MS/MS | Cobas C501 |
| | | Renal/cardiac transplant | 65 | 1.12x + 0.009 | r = 0.98 | LC-MS/MS | Cobas Integra 400 |
| Brandhorst et al. (13) | 2008 | Renal transplant | 174 | 1.09x–0.13 | r = 0.99 | HPLC-UV | Cobas Integra 800 |
| | | – | 87 | 1.06x–0.01 | r = 0.98 | LC-MS/MS | Cobas Integra 800 |
| | | Renal transplant | 190 | 1.01x + 0.04 | r = 0.99 | LC-MS/MS | Cobas C501 |
| | | Renal transplant | 65 | 1.12x + 0.01 | r = 0.98 | LC-MS/MS | Cobas Integra 400 |
| | | Renal/cardiac transplant | 265 | 1.01x + 0.06 | r = 0.99 | HPLC-UV | Cobas Integra 400 |
| | | Renal/cardiac transplant | 60 | 1.17x–0.17 | r = 0.98 | HPLC-UV | Cobas Integra 400 |
| | | Tacrolimus | 158 | 1.01x + 0.08 | r = 0.99 | – | – |
| | | Cyclosporine | 32 | 0.99x + 0.01 | r = 1.00 | – | – |
| Van Gelder et al. (16) | 2009 | Renal transplant | 1986 | 1.009x–0.05 | r = 0.99 | LC-MS/MS | Cobas Integra 800 |
| | | Cyclosporine | 242 | 1.037x–1.046 | r = 0.99 | LC-MS/MS | Cobas Integra 800 |
| | | Tacrolimus | 462 | 1.020x–0.626 | r = 0.99 | LC-MS/MS | Cobas Integra 800 |
| Marquet et al. (15) | 2009 | Renal transplant | 694 | 1.079x–0.031 | r = 0.98 | LC-MS/MS | Cobas Integra 400 |
| | | Lung transplant | 109 | 1.045x + 0.048 | r = 0.99 | LC-MS/MS | Cobas Integra 400 |

Linear regression: y, Roche Total MPA assay on mentioned automatic platforms; x, LC-MS/MS or HPLC-UV. Cobas C501, Cobas 6000.

of MPA is so far not included in the consensus report (1). Concentration-controlled MPA dosing is only needed for patients who are at immunologic risk, patients with altered renal, hepatic or bowel function and patients undergoing minimization or withdrawal of therapy. However, there is still no evidence that TDM of MPA provides benefit in graft outcome or patient survival (1).

In conclusion, the Roche assay is a good alternative for LC-MS/MS or HPLC-UV method if total MPA dosing is sufficient. The observed constant bias of 0.5 mg/L needs to be confirmed in further studies. We confirmed that the total MPA assay of Roche performs as well as LC-MS/MS, and is therefore a reliable method to measure total MPA in liver transplant patients.

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## Conflict of interest statement

**Authors' conflict of interest disclosure:** The authors stated that there are no conflicts of interest regarding the publication of this article. Research support played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.
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