

Multicenter Validation of the MassTrak™ Reagent Kit for the Quantification of Tacrolimus in Whole Blood Using HPLC/MS/MS

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Donald P Cooper¹, Kimberly L Napoli², Paul J Taylor³, Catherine Hammett-Stabler⁴, Kendon S Graham¹, Gareth W Hammond¹, Michael E Franklin³, Webb S Lowe⁴, Quynhmai Nguyen² and Michael R Morris¹

¹Waters Corporation, Manchester, United Kingdom; ²University of Texas Medical School at Houston, Houston, TX; ³Princess Alexandra Hospital, Brisbane, Australia; ⁴University of North Carolina Hospitals, Chapel Hill, NC.

OVERVIEW

- Validation of a new reagent kit for the LC/MS/MS analysis of Tacrolimus in whole blood is reported.
- The kit contains calibrators, controls, internal standard, chromatography column and detailed methods for sample preparation and analysis.
- The performance characteristics were found to be acceptable for the intended use of quantification of Tacrolimus (FK506; Prograf) in liver and kidney transplant patient whole blood samples as an aid in the management of Tacrolimus therapy.

INTRODUCTION

- HPLC with tandem mass spectrometric (MS/MS) detection is becoming increasingly common in clinical laboratories.
- The sensitivity and selectivity of HPLC/MS/MS allow accurate and precise quantification of parent drug in the presence of metabolites and other interferences.
- Currently, all quantitative HPLC/MS/MS Tacrolimus assays are "home brew" techniques requiring extensive development and validation before routine use.
- For some laboratories, this presents a major barrier to adoption. Furthermore, it contributes to lack of standardization which can be problematic when trying to compare results and performance.
- To overcome these limitations a reagent kit was developed based on a previously published method¹.
- We now present the results of a multicenter trial of the first therapeutic drug monitoring reagent kit designed specifically for use with HPLC/MS/MS technology.
- The MassTrak Immunosuppressant Kit has been cleared by the FDA and is CE marked per 98/79/EC for the quantification of Tacrolimus (FK506; Prograf) in liver and kidney transplant patient whole blood samples as an aid in the management of Tacrolimus therapy.



Figure 1: The MassTrak™ Immunosuppressants TDM Kit.

METHODS

- Whole blood calibrators, quality controls and internal standard (Figure 1) were prepared by redissolving in the appropriate solvent as described in the MassTrak user manual.
- Calibrators, QCs and patient samples were prepared for analysis as outlined in Figure 2.
- Samples were analysed using the MassTrak TDM C18 2.1mm x 10mm column eluted with a step gradient (Table 1).
- Tacrolimus and Ascomycin (internal standard) were detected using a Quattro micro tandem mass spectrometer operated in MRM mode (Table 2).
- Tacrolimus concentrations in test samples were determined by reference to the calibration curve.

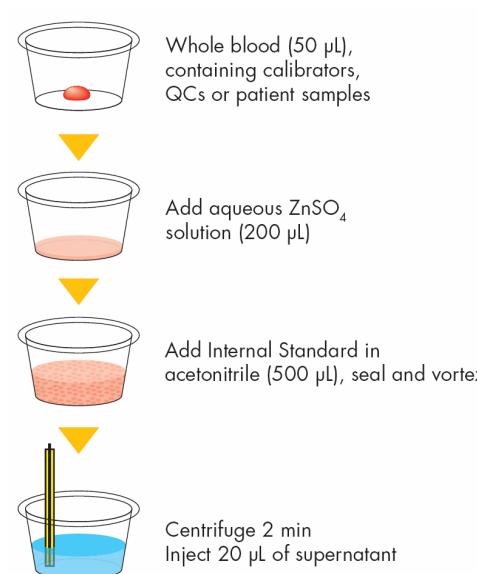


Figure 2: Sample pre-treatment using the MassTrak Reagents.

Time (min)	%A	%B	Flow (mL/min)	Curve
0	50	50	0.6	1
0.4	0	100	0.6	11
1.0	50	50	0.6	11

Table 1: Step gradient used to elute the MassTrak TDM column. A=water and B=methanol, both containing 2mM ammonium acetate and 0.1% formic acid.

Analyte	MS 1 (m/z)	MS 2 (m/z)	Cone Voltage (V)	Collision Energy (eV)
Tacrolimus	821.5	768.5	28	22
Ascomycin	809.5	756.5	28	22

Table 2: Typical optimised MS/MS Settings for the analysis of Tacrolimus and the internal standard, Ascomycin.

The following assay characteristics were assessed at three evaluation centres using protocols based on the appropriate CLSI²⁻⁴ and US FDA⁵ guidelines: precision, accuracy, recovery and method comparison.

RESULTS

- Within run and total imprecision evaluated over 20 days at three separate test centers were <10% over the range 2.0–29.9ng/mL (Table 3).
- When compared to independent, validated HPLC/MS/MS methods at three test centers using a minimum of 50 samples from liver transplant patients and 50 samples from kidney transplant patients (ie >300 individual samples) the MassTrak reagent kit provided results that differed by ≤10% at the lower and upper medical decision points (Table 4 & Figure 3).
- Accuracy was assessed by analyzing Tacrolimus IPT samples (71) at two centers. All results were within the acceptable limits of the scheme (mean +/- 3 SD). Passing-Bablok analysis gave a slope and intercept that were not statistically significantly different than 1.0 and 0, respectively (Figure 4).
- The assay was linear over the range 1ng/mL–31ng/mL (Figure 5).
- Mean recovery from drug-free whole blood and from spiked patient samples was between 97% and 109% (Data not shown).

Method Comparison

Test Center	N	Deming Regression (value ± 95% CI)		Medical Decision Point 5ng/mL (95% CI)	Medical Decision Point 15ng/mL (95% CI)
		Slope	Int.		
Houston	109	1.092 (1.080 to 1.104)	-0.165 (-0.266 to -0.065)	5.3 (5.2 – 5.4)	16.2 (16.1 – 16.4)
		0.9965			
		1.107 (1.081 to 1.133)	-0.148 (-0.357 to 0.061)		
Brisbane	100	0.9862		5.4 (5.3 – 5.5)	16.5 (16.2 – 16.7)
		1.017 (1.001 to 1.033)	-0.138 (-0.347 to 0.061)		
		0.993 (0.974 to 1.012)	-0.06 (-0.21 to 0.09)		
Chapel Hill	100	0.9907		4.9 (4.8 – 5.0)	14.8 (14.7 – 15.0)
		0.993 (0.974 to 1.012)	-0.06 (-0.21 to 0.09)		
		0.9907			

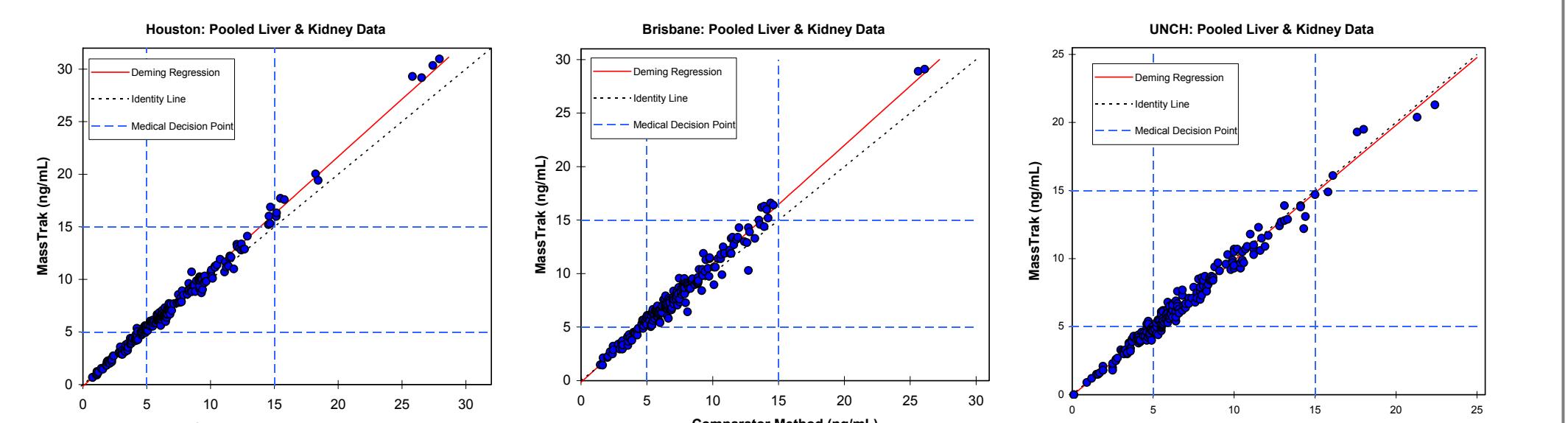


Table 4 & Figure 3: Deming regression analysis of pooled liver & kidney method comparison data from each of the three evaluation centers. Each patient sample was analysed in duplicate using the MassTrak method and a validated comparative HPLC/MS/MS method according to Reference 3. Upper & lower medical decision points of 5ng/mL and 15ng/mL were selected based on the current Tacrolimus monitoring consensus document⁶.

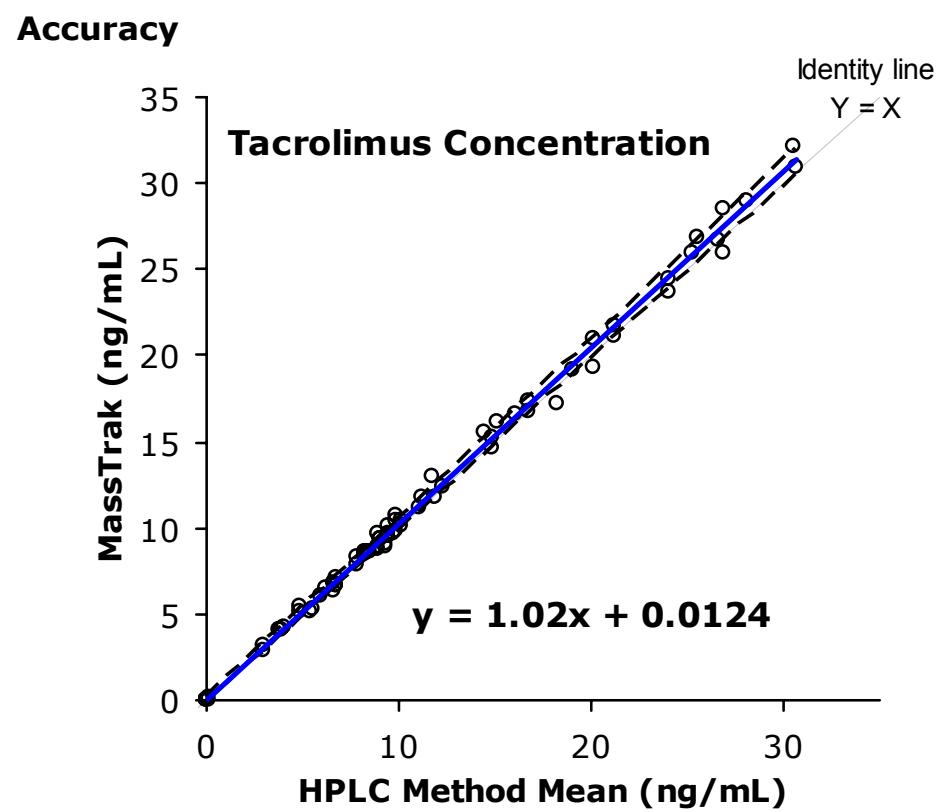


Table 3: Within run and total precision assessed with pooled patient samples analysed twice per day for 20 days as per CLSI EP5-A2².

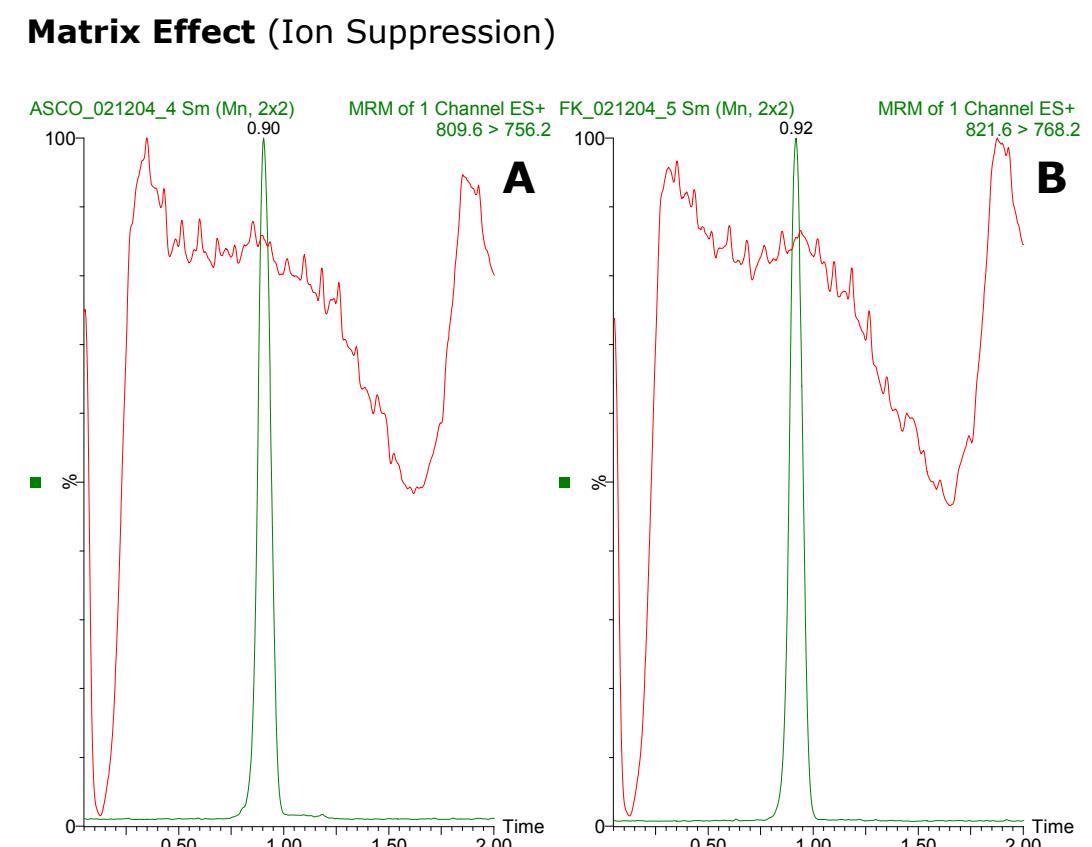


Figure 6: Extract prepared from drug-free whole blood was injected whilst the internal standard Ascomycin (A) or Tacrolimus (B) was infused post-column (red traces). In separate analyses, the elution positions of Ascomycin & Tacrolimus were determined (green traces). No significant matrix effect was seen at the elution position of the analyte or internal standard.

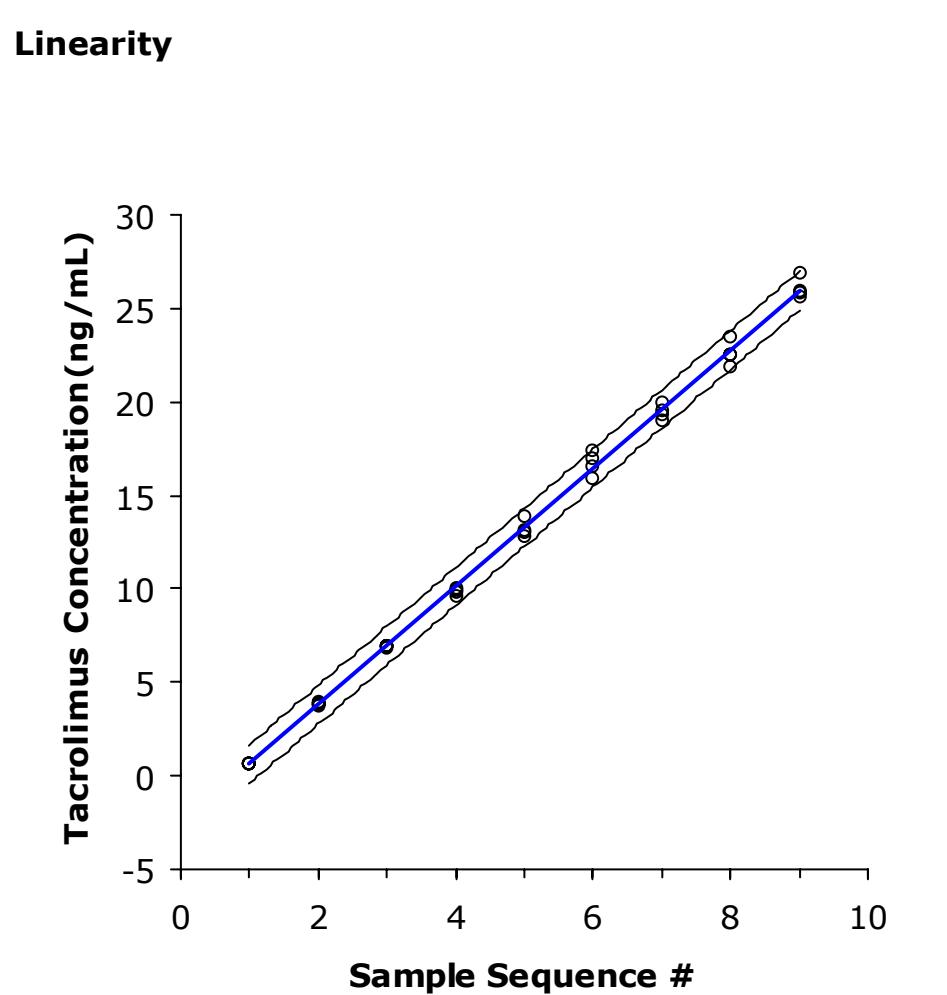


Figure 5: Example linearity data generated using CLSI EP6-A⁴. A "low" and "high" patient sample were mixed in defined proportions to create a sequence of 9 samples with Tacrolimus concentrations known to be related linearly. Polynomial regression coefficients for 2nd & 3rd order curve fits were not significantly different from zero, indicating linearity. The plot shows a linear fit (N=5) with 95% confidence interval.

CONCLUSIONS

- The performance characteristics of the MassTrak™ reagent kit are acceptable for the routine therapeutic drug monitoring of Tacrolimus in kidney and liver transplant recipients⁷.
- The reagent kit may provide the potential for harmonization of results between laboratories using HPLC/MS/MS.
- The MassTrak kit provides a turn-key solution for those laboratories wanting to analyse Tacrolimus in kidney and liver transplant patients using HPLC/MS/MS.

References

- B. G Keevil, S McCann, D P Cooper & M R Morris, "Evaluation of a rapid micro-scale assay for Tacrolimus by liquid chromatography-tandem mass spectrometry". *Annals of Clinical Biochemistry*, **39**, 487-492, 2002.
- CLSI Evaluation of Precision Performance of Quantitative Measurement Methods, EP5-A2, August 2004.
- CLSI Method Comparison and Bias Estimation Using Patient Samples, EP9-A2, September 2002.
- CLSI Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach, EP6-A, April 2003.
- Class II Special Controls Document: Cyclosporine and Tacrolimus Assays; Guidance for Industry, FDA, September 2002.
- M. Oellerich, V. W Armstrong, E Schutz, & L M Shaw "Therapeutic Drug Monitoring of Cyclosporine and Tacrolimus". *Clin. Biochem.*, **31**, 309 – 316, 1998.
- L M Shaw, T M Annesley, B Kaplan & L Brayman, "Analytic Requirements for Immunosuppressive Drugs in Clinical Trials". *Ther. Drug Monitoring*, **17**, 577–583, 1995.