

THE MASSTRAK REAGENT KIT FOR TACROLIMUS QUANTIFICATION BY HPLC-MS/MS: COMPARISON FOR ACCURACY WITH THREE COMMERCIAL IMMUNOASSAY PROCEDURES

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INTRODUCTION

The method comparison test protocol used for establishing the performance of the MassTrak Immunosuppressants Kit was designed to compare the results obtained for patient samples using the MassTrak method with results obtained for the same samples using similar "home-brew" LC/MS/(MS)¹ methods.

This decision was based on the CLSI precision protocol, EP5A2², recommendation requiring that the comparator method should have similar or better precision than the test method (MassTrak). Although immunoassays clearly do not meet with the CLSI requirement, we hypothesized that proficiency testing results that included a combination of methods, e.g., chromatographic and immunoassay, could be used as a surrogate to derive such a comparison. For this study, we used results and samples from the Tacrolimus International Proficiency Testing (IPT) scheme (<http://www.bioanalytics.co.uk>) since materials were readily available and each laboratory had experience with the survey.

BACKGROUND

Each month, the Tacrolimus IPT scheme distributes three whole blood samples to each of the member laboratories. The samples consist of pooled whole blood from patients receiving tacrolimus (concentration unknown) or drug-free blood that has been spiked with tacrolimus to a specific target concentration. The laboratories analyse the three samples as part of their routine service without knowledge of the tacrolimus concentration. The results are reported back to the IPT scheme for method-specific statistical analysis. Table 1 shows the method groups currently used for the tacrolimus IPT scheme and the approximate number of participating laboratories in each group. The exact number of results returned in each group varies by month.

Analytical Method	Approximate Number of Participants
HPLC*	50
DiaSorin Pro-Trac-II Tacrolimus	4
Dade Behring Tacrolimus EMIT 2000	95
Abbott Tacrolimus IMx	200

Table 1: Analytical methods used by subscribers to the International Tacrolimus Proficiency Testing Scheme.

*Tacrolimus cannot be detected with sufficient sensitivity by UV so the HPLC analytical group is made up entirely of HPLC/MS and HPLC/MS/MS "home-brew" methods.

For each method group, the mean and standard deviation are calculated for each IPT sample. Results that fall within ± 3 SDs of the mean are accepted by the scheme and those outside the window are rejected. The means and standard deviations are recalculated using the accepted data only and a report distributed (see Figure 1). The data are also available for public viewing on the IPT web site (http://www.bioanalytics.co.uk/html/latest_results.html).

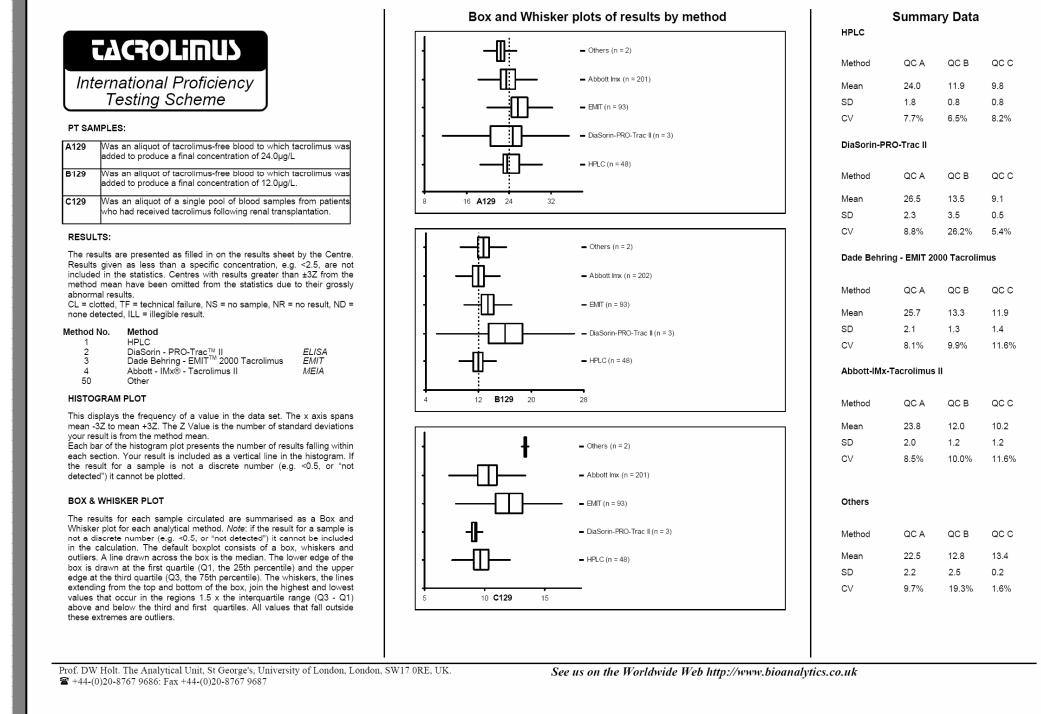


Figure 1: Example report from the Tacrolimus IPT scheme showing statistical analysis of the results returned for each method type. (www.bioanalytics.co.uk).

METHODS

Sample Analysis

A total of 71 Tacrolimus IPT samples were analysed at two test centres using the MassTrak LC/MS/MS kit during the validation phase³.



Statistical Analysis

The MassTrak results for the IPT samples were compared to the reported method means for three immunoassays: DiaSorin - PRO-Trac™ II (ELISA), Dade Behring EMIT™ 2000 Tacrolimus (EMIT) and Abbott IMx® Tacrolimus II (MEIA) for that same series of IPT samples. For control purposes, a comparison was also made to the HPLC group of laboratories. Data were processed using the Passing-Bablok Method Comparison algorithm provided in the Microsoft Excel add-in, Analyse-It Clinical Laboratory v1.73.

RESULTS

Figures 2–5 show comparisons of the existing MassTrak IPT results to the method mean results for the Abbott Tacrolimus IMx, Dade Behring EMIT 2000 MEIA, DiaSorin Pro-Trac II ELISA and the HPLC groups of laboratories respectively. In each case, comparisons for all samples, spiked samples only and patient pools only are presented. The Passing-Bablok Method Comparison parameters for each of the comparisons are summarised in Table 2.

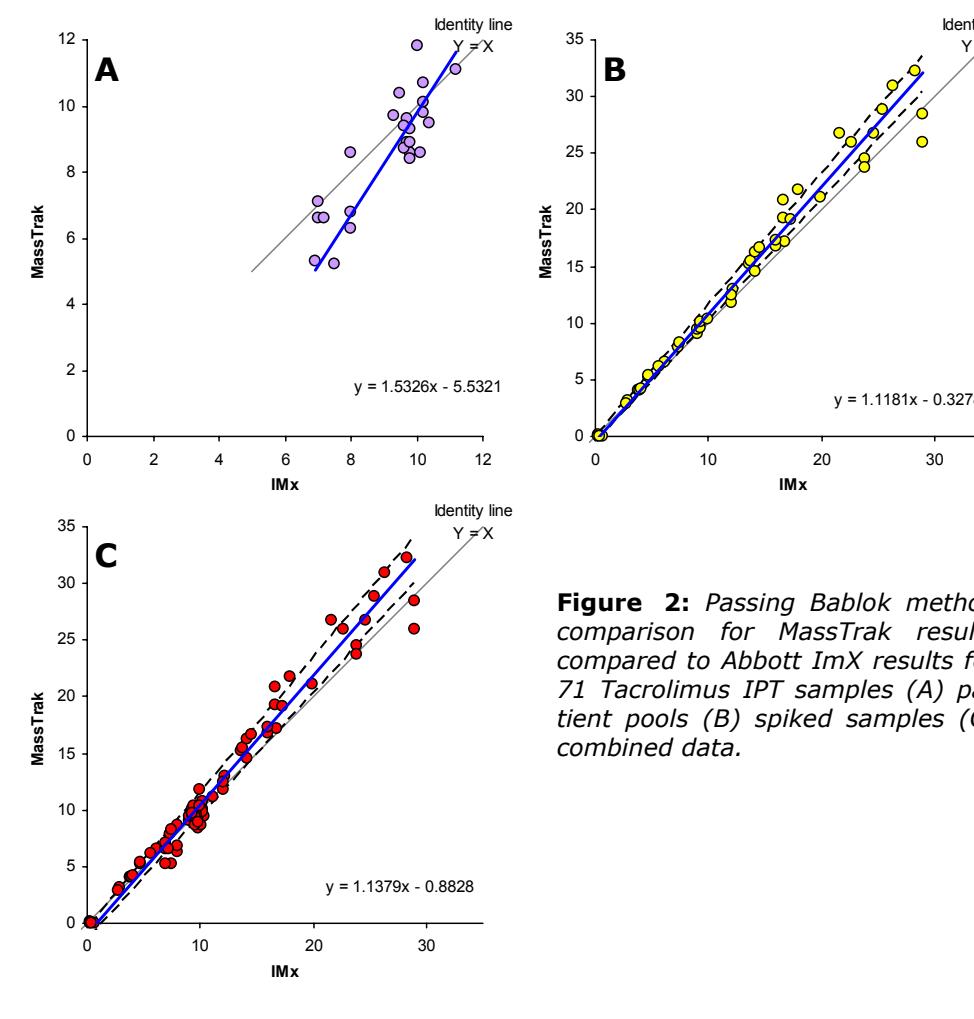


Figure 2: Passing Bablok method comparison for MassTrak results compared to Abbott IMx results for 71 Tacrolimus IPT samples (A) patient pools (B) spiked samples (C) combined data.

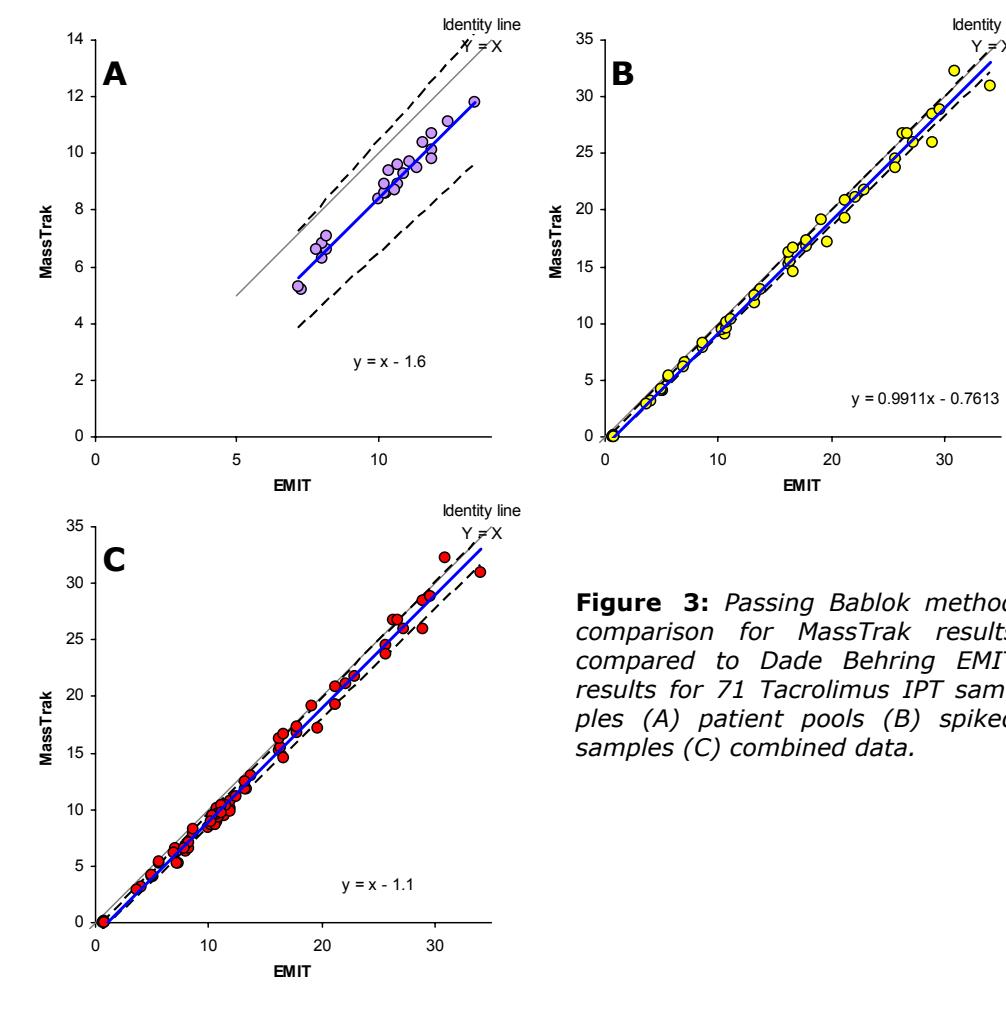


Figure 3: Passing Bablok method comparison for MassTrak results compared to Dade Behring EMIT results for 71 Tacrolimus IPT samples (A) patient pools (B) spiked samples (C) combined data.

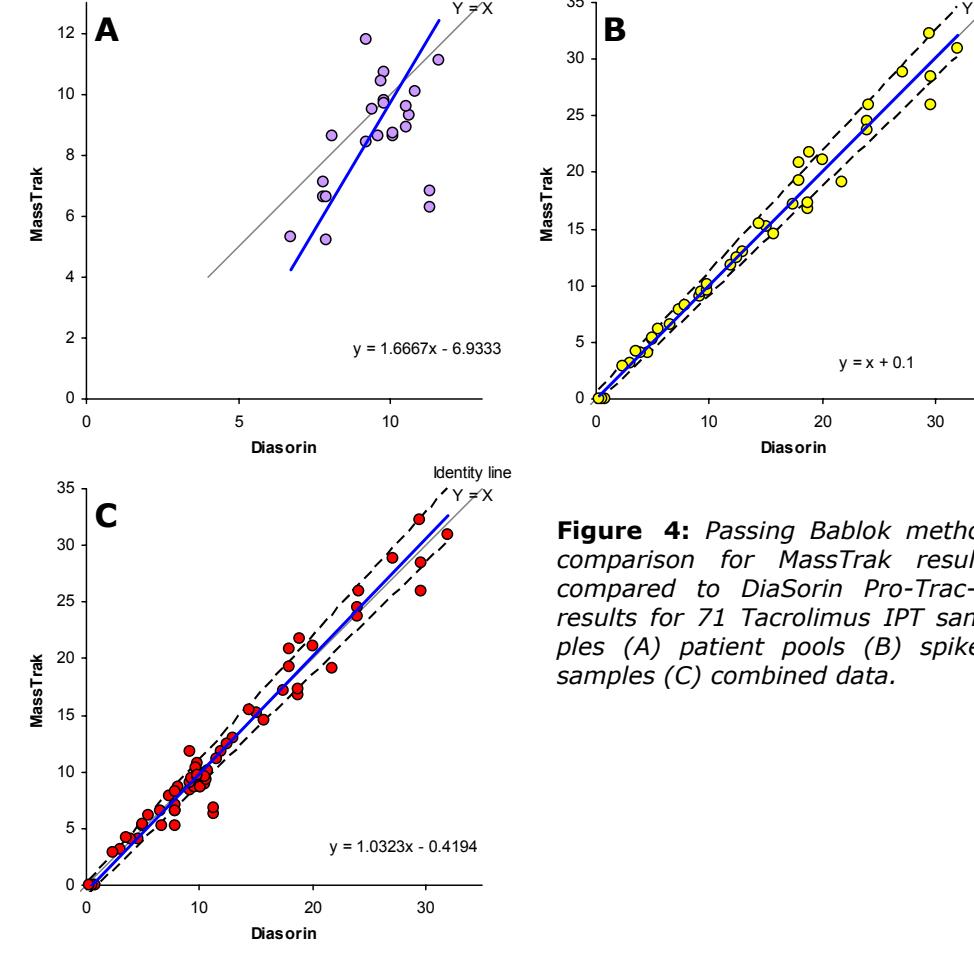


Figure 4: Passing Bablok method comparison for MassTrak results compared to DiaSorin Pro-Trac-II results for 71 Tacrolimus IPT samples (A) patient pools (B) spiked samples (C) combined data.

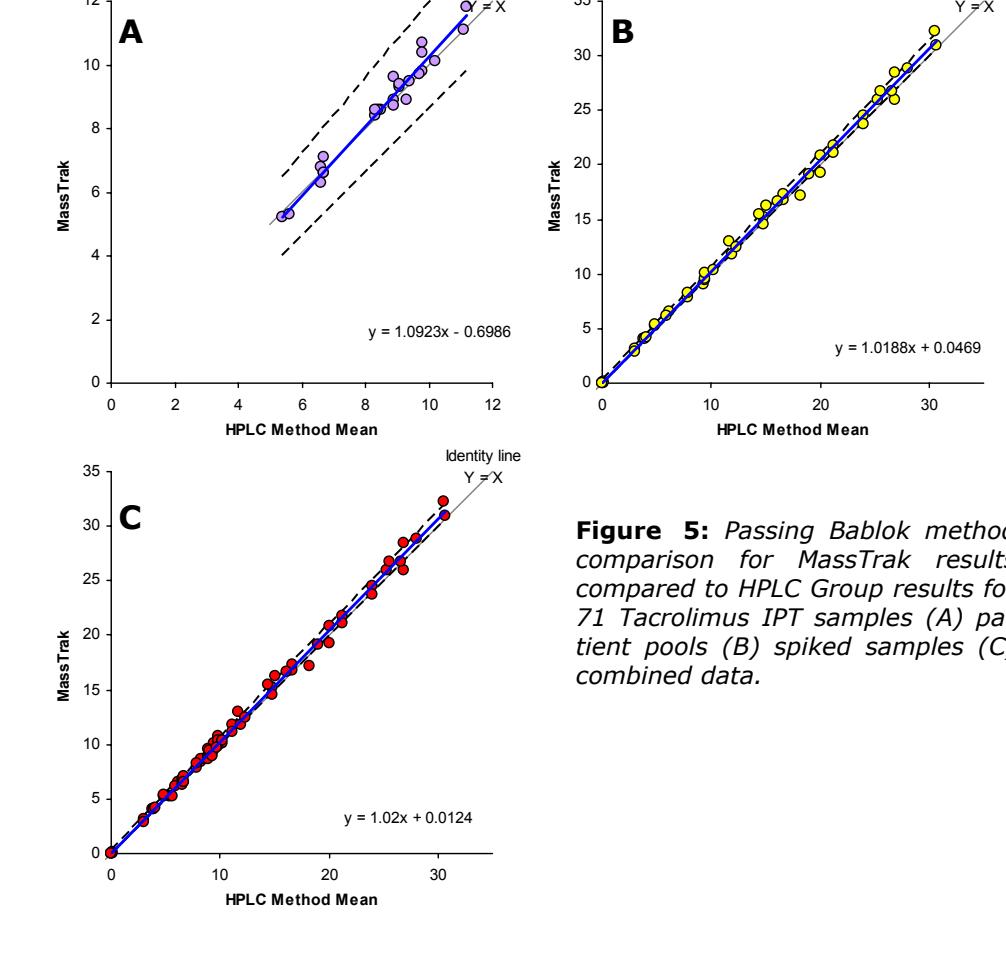


Figure 5: Passing Bablok method comparison for MassTrak results compared to HPLC Group results for 71 Tacrolimus IPT samples (A) patient pools (B) spiked samples (C) combined data.

Procedure	EMIT™ 2000 N = 90 (Range)			ProTrac II™ N = 3 (Range)			IMx® N = 200 (Range)			HPLC N = 50 (Range)		
Samples	Slope	Int	Slope	Int	Slope	Int	Slope	Int	Slope	Int	Slope	Int
All (n=71)	1.00 (0.968 to 1.023)	-1.100 (-1.385 to -0.774)	1.032 (0.977 to 1.089)	-0.419 (-0.971 to 0.168)	1.138 (1.083 to 1.188)	-0.883 (-1.406 to -0.358)	1.020 (1.000 to 1.036)	0.012 (-0.166 to 0.200)				
Spike (n=46)	0.991 (0.969 to 1.013)	-0.761 (-0.957 to -0.466)	1.000 (0.957 to 1.063)	0.100 (-0.447 to 0.561)	1.118 (1.067 to 1.156)	-0.328 (-0.531 to -0.001)	1.019 (1.000 to 1.036)	0.047 (-0.117 to 0.200)				
Patient Pools (n=25)	1.000 (0.930 to 1.128)	-1.600 (-2.882 to -0.888)	1.667 (1.132 to 3.833)	-6.933 (-27.77 to -2.226)	1.533 (1.167 to 2.200)	-5.532 (-11.74 to -2.100)	1.092 (1.000 to 1.182)	-0.699 (-1.320 to 0.100)				

Table 2: Summary of Passing-Bablok Method Comparison parameters for the comparison of combined MassTrak data from two test centres against IPT method means for a total of n samples from N laboratories. Slopes that are significantly different from 1.0 (95% CI) and intercepts that are significantly different from 0 (95% CI) are indicated in red font in the table.

DISCUSSION

Immunoassays are known to suffer from interference, such as crossreactivity from metabolites that will be present in the pooled patient IPT samples but not in the spiked IPT samples. The spiked samples are useful for determining the performance characteristics of an assay and the IPT Scheme manipulate the tacrolimus concentration in the spiked samples such that, over time, the entire analytical range of the assay is covered. While important, these samples do not accurately reflect the situation encountered with patient samples, it is important to stratify and analyze the IPT data with respect to the sample type and to focus on the patient pool samples. This inevitably reduces the range of the comparison to a relatively narrow band around the target therapeutic range.

A review of the data shows that for the spiked samples, the MassTrak results are comparable to those obtained using immunoassay. It should be noted that for these samples both the EMIT and IMx assays showed significant bias. This was less pronounced for the EMIT assay where there was a small constant bias (0.761ng/mL) that might be attributed to calibration differences. The IMx assay showed both proportional and constant bias that is more difficult to explain but might also be related to calibration.

When only the patient pools are considered the EMIT assay is in reasonable agreement with the MassTrak method, showing a relatively small constant bias. Both the IMx and ProTrac II assays show significant constant and proportional bias, presumably as a result of metabolite interference.

CONCLUSIONS

We report an indirect comparison of MassTrak using IPT data.

- The MassTrak results did not differ significantly from those reported by the HPLC group of laboratories for either the spiked samples or the patient pools.
- A significant bias was observed when the results obtained using immunoassay were analyzed for the patient pools reflecting cross-reactivity of the antibodies used in these methods for metabolites.
- We propose that this type of comparison provides a more accurate estimate than a comparison made using immunoassay data from a single laboratory.

REFERENCES

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