INDIRECT COMPARISON OF THE MASSTRAK REAGENT KIT FOR LC/MS/MS ANALYSIS OF WHOLE BLOOD TACROLIMUS WITH THREE ESTABLISHED IMMUNOASSAYS

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ABSTRACT

Introduction: In routine clinical laboratories it is not practical to maintain multiple assays for the same analyte for logistic and financial reasons, making it difficult to perform long-term method comparison studies when new analytical tests are introduced. We have used external quality assurance (EQA) returns as an indirect means to compare the MassTrak™ LC/MS/MS whole blood tacrolimus assay with established immunoassays. For some of the assays, there are a large number of test centres contributing data so that the method mean provides a reliable measure of the accuracy of that technique. Furthermore, the same sample set may be used for all the comparisons and long-term comparative assay performance inferences can be made.
Methods: Banked tacrolimus EQA samples (n=41) from the Tacrolimus International Proficiency Testing (IPT) Scheme (Analytical Services International, London, UK) were analysed in singlicate using the MassTrak LC/MS/MS assay as described in the user manual. The MassTrak results were compared to the Method Means for the same sample sets analysed by the three immunoassay groups, as published by the IPT Scheme. Linear regression analysis was performed using Microsoft Excel.
Results: A summary of the results from the comparison of the MassTrak determinations against the iummunoassay method means is shown in Table 1.

Assay	Dade EMIT (N=90)		DiaSorin (N=3)		Abbott (N=200)	
Samples	Slope	R^2	Slope	R^2	Slope	R^2
All (n=41)	0.95	0.995	0.99	0.96	1.09	0.96
Spike (n=26)	0.94	0.997	0.97	0.98	1.06	0.97
Patient Pools (n=15)	1.01	0.991	0.69	0.24	1.22	0.72

Table 1: *Summary of linear regression parameters for the comparison of MassTrak data against IPT method means for n samples from N laboratories.*

Conclusions: The indirect comparison of MassTrak and immunoassay results obtained by the study of IPT returns is a valid approach since good correlations are obtained for the spiked samples with all three immunoassays. A comparison made in this way may provide a more accurate estimate than a comparison made using immunoassay data from a single laboratory as any laboratory bias is minimised, and outliers are eliminated. The comparisons for patient pool samples show a larger discrepancy than the spiked samples, and this is believed to be attributed to the presence of the tacrolimus metabolites.

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, EP5A2[2], 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 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 laboratories that subscribe to the scheme. 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 statistical analysis. The statistical analysis is broken down into groups according to the analytical technique. The table below 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

* *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 (PASS) and those outside the window are rejected (FAIL). 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).

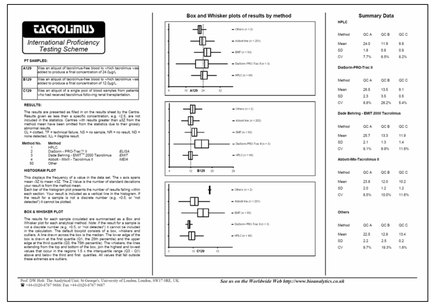

Figure 1: *Example report from the Tacrolimus IPT scheme showing statistical analysis of the results returned for each method type. (www.bioanalytics.co.uk)*

A Test Protocol was developed in which IPT samples are used for testing the accuracy of the MassTrak kit by comparing MassTrak results to the HPLC (= MS & MS/MS) method means. Thus data had previously been collected (Houston) for the analysis of a series of 41 IPT samples.

Using the IPT reports described above, it is also possible to obtain the mean values for three different 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. A statistical comparison (e.g. linear regression analysis) between MassTrak and immunoassay results can therefore be made.

Immunoassays have been reported to suffer from interference, particularly from metabolites that will be present in the pooled patient IPT samples but will not be present 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. However, this is an artificial situation and to demonstrate the relationship between MassTrak results and immunoassay results for patient samples, it is important to exclude spiked samples from the data analysis and focus on the patient pool samples. This inevitably reduces the range of the comparison to a relatively narrow band around the target therapeutic range.

RESULTS

Figures 2, 3 & 4 show comparisons of the existing MassTrak IPT results to the method mean results for the Dade Behring EMIT 2000 MEIA, DiaSorin Pro-Trac II ELISA and the Abbott Tacrolimus IMx immunoassays respectively. In each case, comparisons for all samples, spiked samples only and patient pools only are presented. The linear regression parameters for each of the comparisons are summarised in Table 2.

Assay	Dade EMIT (N=90)		DiaSorin (N=3)		Abbott (N=200)	
Samples	Slope	R^2	Slope	R^2	Slope	R^2
All (n=41)	0.95	0.995	0.99	0.96	1.09	0.96
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Table 2: *Summary of linear regression parameters for the comparison of MassTrak data from Houston against IPT method means for n samples from N laboratories.*

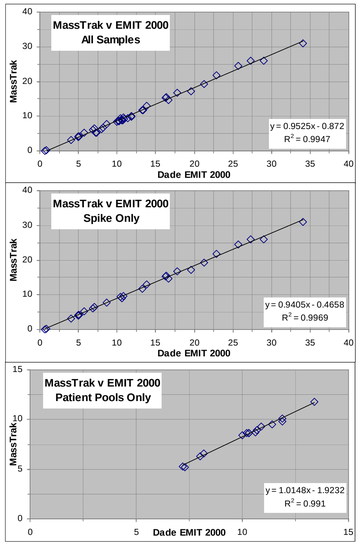

Figure 2: *Linear regression analysis of Houston MassTrak results for Tacrolimus IPT samples against Dade Behring EMIT 2000 MEIA results for the same samples.*

The MassTrak results are comparable to the results for all three immunoassays when looking at the spiked samples.

When only the patient pools are considered the EMIT assay compares favourably with the MassTrak method, whilst the Abbott IMx is poor. This agrees with the documented relatively high degree of cross-reactivity of metabolites in the IMx assay.

The comparison data for the DiaSorin ELISA are the worst of the three assays, but these results are based on ELISA data returned from only 3 laboratories compared to 90 and 200 laboratories for the EMIT and IMx data, respectively. Although there were few laboratories using the method, the number of samples tested is adequate to demonstrate a good correlation between MassTrak and DiaSorin ELISA for the spiked samples (Table 1 and Figure 3). The lack of correlation with the more specific method suggests that the immunoassay also suffers from considerable cross-reactivity with the metabolites.

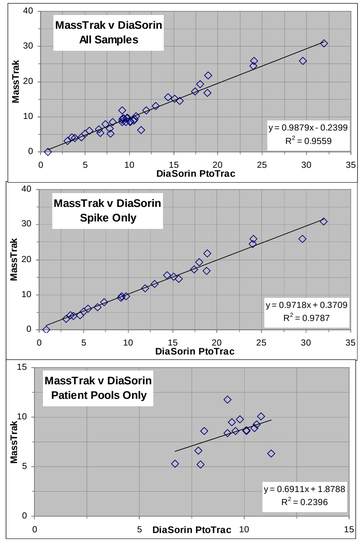

Figure 3: *Linear regression analysis of Houston MassTrak results for Tacrolimus IPT samples against DiaSorin Pro-Trac ELISA results for the same samples.*

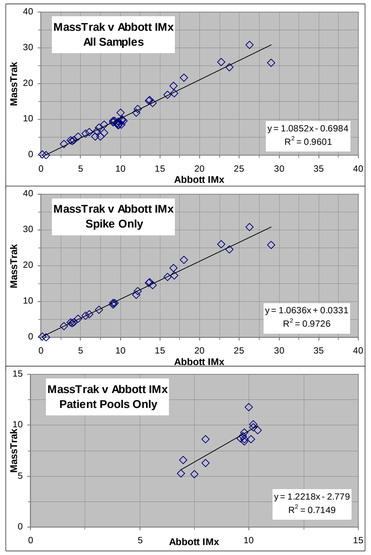

Figure 4: *Linear regression analysis of Houston MassTrak results for Tacrolimus IPT samples against Abbott IMx results for the same samples.*

CONCLUSION

The indirect comparison of MassTrak and immunoassay results obtained by the study of IPT returns appears to be valid since good correlations are obtained for the spiked samples for all three immunoassays. It could be argued that a comparison made in this way provides a more accurate estimate than a comparison made using immunoassay data from a single laboratory as any biases are minimised and outliers are eliminated.

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