

Comparison of Tacrolimus Quantification using the Waters MassTrak LC-MS/MS assay with the Abbott Architect Immunoassay

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Long Abstract:

Background: The use of immunosuppressant drugs following organ transplantation is essential in order to reduce the risk of organ rejection. The most commonly used immunosuppressants in solid organ transplantation are sirolimus, cyclosporine and tacrolimus. Tacrolimus is a calcineurin inhibitor and, due to its narrow therapeutic window, requires periodic therapeutic drug monitoring. The first line assay used to monitor tacrolimus concentrations is an immunoassay which utilizes monoclonal antibodies raised against tacrolimus. However, tacrolimus is metabolized by CYP3A subfamily of enzymes giving rise to many other metabolites which are structurally similar to the parent drug and are known to cross react with the assay antibody. **Methods:** We verified performance claims of the Waters MassTrak assay and performed a patient comparison relative to the Abbott tacrolimus immunoassay on the Architect platform. We performed linearity studies by using pooled blood from patient samples measured in quadruplicates with 9 concentrations: 0.95, 4.9, 8.8, 12.7, 16.7, 20.6, 24.5, 28.5, and 32.4 ng/mL. Precision studies were performed by using three concentrations of blood samples; one low (0.5 ng/mL), medium (15 ng/mL) and high pool (30 ng/mL) concentrations. The lower limit of quantification of the assay was also verified. A total of 40 patient blood specimens were run on both the MassTrak and the architect immunoassay over 20 days. In order to cover the dynamic range of the assay, we choose to divide the samples into four groups: 10 samples from each of the four ranges (0-6 ng/mL; > 6-13 ng/mL; > 13-21 ng/mL and > 21 ng/mL). **Results:** Our linearity studies revealed an excellent linear correlation from 1 – 32.4 ng/mL with an $R^2 = 0.997$. Precision studies at three different concentrations span over the measured analytical range

were as follows (mean \pm CV): 0.6 ng/mL \pm 20%, 16.0 ng/mL \pm 5.4%, 31.2 ng/mL \pm 5.8%. Matrix effect studies were conducted to determine whether blood could suppress ionization of tacrolimus; comparison of signal intensity between pretreated blood samples and solvent samples revealed a difference of less than 10% for both tacrolimus and the internal standard (ascomycin). Our patient comparison data between the Mastrak LC-MS/MS and Abbott immunoassay showed excellent correlation with an $R^2 = 0.97$ (Figure 1). However, the MassTrak method produced concentrations for tacrolimus that were consistently lower than the immunoassay with a negative bias ranging from 0 to 37% (Figure 2) ($p < 0.0001$; via two-tailed paired t-test test). Calibrator samples that were run on Abbott immunoassay were also run on the MassTrak LC-MS/MS; similarly, the LC-MS method produced a constant negative bias ranging from 0 to 16% ($p < 0.0001$; via two-tailed paired t-test test).

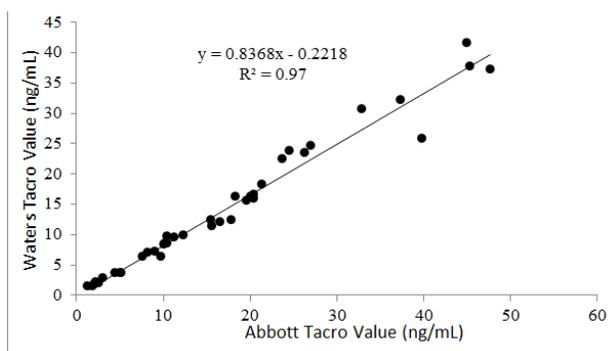


Figure 1: Correlation analysis between Abbot and MassTrak Waters method.

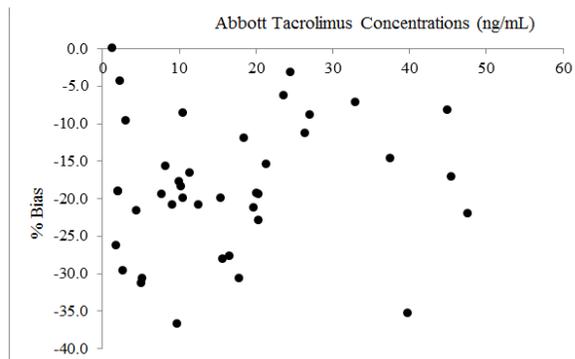


Figure 2: Bland-Altman plot showing comparison of the Abbott assay vs. Water's MassTrak assay.

Conclusion: Overall, the MassTrak LC-MS/MS assay was linear over the clinically relevant range of 1 to 32.4 ng/mL. Tacrolimus measurements in patient blood samples correlated well with the immunoassay ($R^2 = 0.97$), however, the immunoassay values were consistently higher than those measured using the MassTrak method. The LC-MS method was not affected by matrix effects. The higher measured concentrations in the immunoassay method are likely due to cross reactivity with tacrolimus metabolites by the antibody used in this assays. This is supported, in part, by the fact that a larger negative bias was observed between the LC-MS/MS method vs. Abbott immunoassay when patient samples were analyzed as opposed to the Abbott calibrator specimens, i.e., in the absence of drug metabolites.

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