From venous blood to VAMS – adaptation of a commercial CE-IVD assay kit for the determination of Tacrolimus, Sirolimus, Everolimus and Cyclosporin A.

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Background: TDM of immunosuppressants is extremely important topic, especially when it comes to sample collection at home coupled with simple and fast sample preparation and analysis. The aims of the study covered check if optimization of ready-to-use kit for determination of Tacrolimus (TAC), Sirolimus (SIR), Everolimus (EVR) and Cyclosporin A (CSA) in venous blood can be successfully applied for VAMS-collected blood and preliminary venous blood to Mitra® (VB/M) correlation study.

Methods: Sample preparation for venous blood was done according to the procedure provided with the kit (Chromsystems kit: 93000). Sample preparation for Mitra®-collected blood included additional sonication, vortexing and combining of internal standards with extraction buffer. Only reagents supplied by the manufacturer were used. All samples were analyzed on QTRAP 5500+ LC-MS/MS system (SCIEX). Data processing, quantitation and basic statistical analysis was done in SciexOS 3.3 software (SCIEX).

Results: The optimized for 10µl and 20µl Mitra® samplers procedure met the validation criteria, including: reproducibility (%CV±15% and accuracy 85-115%), LLOQ (S/N≥10, calibrator 1), linearity (R≥0.995). LOD was not determined, however collected for 10 µl Mitra® sampler data shows that lower that 50 pg/ml concentration for each compound is achieved. The preliminary study of VB/M correlation, included 15 patients per compound where venous blood and 10 µl Mitra® samples were collected at the same time. Obtained VB/M factors were as follows: TAC=0.75, %CV=22.92%, SIR=0.77, %CV=36.10%, CSA=1.15, %CV=19.01%. No patients for EVR were tested during the study.

Conclusions: Optimized for VAMS procedure allowed to achieve validation criteria with the use of kit reagents. Additional steps required for Mitra® sample preparation are simple, robust and automation friendly. The VB/M correlations revealed that the capillary blood concentration of TAC and SIR are lower than in venous blood contrary to concentration of CSA. This data may support the fact that TAC and SIR enters erythrocytes in about 90%, while CSA only in 50% and the capillary blood has higher amount of serum fraction. However, due to relatively small number of patients included in the study it needs further investigation on larger group of patients and deeper statistical analysis.

Key Words: VAMS, immunosuppressants, TDM, CE-IVD, correlation, lc-ms/ms