# Simultaneous LC-MS/MS quantification of creatinine, iohexol and five immunosuppressants in renal transplant recipients using volumetric dried blood spot sampling: analytical validation

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**Background**
Renal function and immunosuppressant exposure are considered key diagnostic markers in the clinical management of renal transplant recipients. These markers are typically determined during outpatient clinic visits. The dried blood spot (DBS) technique has brought about options for remote renal function and immunosuppressant monitoring. Here, we report on the development and analytical validation of a liquid chromatography tandem-mass spectrometry (LC-MS/MS) assay for simultaneous quantification of tacrolimus, everolimus, sirolimus, cyclosporine, mycophenolic acid, iohexol and creatinine in DBS.

**Methods**
Method development was based on previously validated assays for quantification of tacrolimus, everolimus, sirolimus, cyclosporine, mycophenolic acid, iohexol and creatinine in DBS using LC-MS/MS. The analytical validation for the DBS samples was carried out on a Thermo Quantiva LC-MS/MS system, according to the European Medicines Agency guidelines on bioanalytical method validation, including selectivity, carry-over, linearity, accuracy, precision, recovery, matrix effect, stability and cross-validation versus EDTA venous whole blood samples.

**Results**
The assay was validated successfully for all analytes. Lower limits of quantification were established for tacrolimus (1.2 µg/L), sirolimus (2.6 µg/L), everolimus (1.6 µg/L), cyclosporine (10.6 µg/L), mycophenolic acid (0.3 mg/L), iohexol (7.5 mg/L) and creatinine (3 mg/L). Analytical cross-validation with 20 manually spotted DBS samples versus their corresponding EDTA whole blood samples indicated adequate method agreement for tacrolimus, everolimus and cyclosporine, with regression slopes of 0.93, 0.99 and 1.04 and bias of -0.32 µg/L (-1.17%), +0.33 µg/L (+3.58%) and -2.63 µg/L (-1.86%), respectively. The cross-validation regression slope and bias were 0.97 and -1.99 mg/L (-10.2%) for creatinine, showing moderate accuracy. The performance of the assay for creatinine was, however, adequately accurate for its purpose, namely to detect divergent renal function fluctuations on the individual patient level. For mycophenolic acid, the results closely resembled that of our previously validated, routinely used LC-MS/MS DBS assay. Analytical cross-validation of the assay for iohexol and sirolimus is currently being conducted.

**Conclusions**
A promising multi-analyte LC-MS/MS assay to enable simultaneous remote renal function evaluation and immunosuppressant monitoring using DBS sampling was developed. Clinical validation with paired DBS and EDTA whole blood samples from renal transplant recipients is warranted before considering this method for routine clinical care.