**Evaluation of electrochemiluminescence immunoassays for immunosuppressive drugs on the Roche cobas e411 analyzer**

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**Supplementary File 1: Supplementary Methods**

*ESI-LC-MS/MS method*

The ESI-LC-MS/MS MRM method for CSA, TAC, and SRL were analyzed on a 4000 QTrap mass spectrometer (SCIEX) at the Hospital for Sick Children (Toronto, ON, Canada). Samples were pretreated by mixing 40 µL of sample with 100 µL of sample pretreatment reagent consisting of 0.04M zinc sulfate, and internal standards 100.0 µg/L cyclosporine D and 10.0 µg/L ascomycin in methanol. Samples were vortexed and centrifuged for 5 minutes at 15,000 x g to obtain the supernatant for analysis. The analyte is separated by liquid chromatography (Nexera X2 Shimadzu) with a reverse phase C18 column (Phenomenex, 4 x 3.0 mm at 45oC) and gradient elution from 100% B to 50% B (Buffer A: 2 mM ammonia acetate and 0.1% formic acid in water and Buffer B: 2 mM ammonia acetate and 0.1% formic acid in methanol) at a flowrate of 650 µL/min and electrospray ionization into the mass spectrometer. The following precursor/product ion pairs in positive ion mode were used 1220.8/1203.8 *m/z* for CSA, 821.5/768.5 *m/z* for TAC, and 931.6/864.5 *m/z* for SRL. CSA and TAC were calibrated with a 6-point calibration curve using Emit 2000 CSA or TAC specific calibrators (Syva Company, Siemens Healthcare). SRL was calibrated with a 6-point calibration curve using 6Plus1 Multilevel immunosuppressant calibrators (Chromsystems). Internal QC were evaluated with Bio-Rad Lyphochek Whole Blood ISD Controls levels 1, 3, and 4.