Rapid Measurement of Cyclosporine and Sirolimus in Whole Blood by Paper Spray–Tandem Mass Spectrometry

To the Editor:

Paper spray (PS) is a recently described method for the direct mass spectrometry (MS) analysis of blood and other biological samples (1–3). Paper spray is performed by depositing a sample such as whole blood onto a paper substrate contained within a disposable cartridge; extraction and ionization occur directly from the disposable cartridge with an automated MS attachment (Fig. 1). No punching or offline extraction is required. Total analysis time, including sample extraction, is several minutes.

Because of its simplicity, paper spray could lower the barrier for implementation of MS-based assays in clinical laboratories. We previously used PS-MS/MS to monitor tacrolimus in clinical samples and showed good correlation with 2 immunoassays and an external HPLC-MS/MS method (4). Here we extend this approach by demonstrating the simultaneous quantification of cyclosporine and sirolimus by PS-MS/MS. Therapeutic drug monitoring of these drugs is important for organ transplant patient care.

The method used was similar to our previously published method for tacrolimus (4). To prepare 1-g/L stock solutions, stable isotope–labeled internal standards (SIL-Is) [2H₃]cyclosporine (98% purity) and [2H₃]rapamycin (75% purity) (Toronto Research Chemicals) were dissolved in methanol. We prepared a working solution in methanol containing [2H₃]cyclosporine at 1000 ng/mL and [2H₃]rapamycin at 60 ng/mL. An EDTA-anticoagulated blood sample (200 µL) was mixed with 50 µL of the SIL-IS working solution. To accommodate volume-limited samples from pediatric and/or anemic patients, we tested blood aliquots as low as 50 µL with proportionally reduced SIL-IS solution volumes, with equivalent results. The mixture (10 µL) was spotted on paper contained in a disposable cartridge (Prosolia). After allowing the sample to dry, we analyzed the cartridge using an automated paper spray front end interface to a TSQ Vantage. Each drug was detected as a singly charged sodium adduct. We chose sodium over ammonium because sodium adduct ions give more selective fragmentation during MS/MS (larger neutral losses). Two selected reaction monitoring transitions (a qualifier and a qualifier ion) were monitored for each analyte to improve selectivity. The transitions were m/z 1224.9 → 1085 and 1113 for cyclosporine and m/z 936.5 → 345 and 409 for sirolimus.

Representative ion chronograms obtained from a low-concentration calibrator prepared in whole blood, overlaying a representative blank chronogram, are shown in Fig. 1. The limits of detection were 5 and 0.5 ng/mL for cyclosporine and sirolimus, respectively. The lower limit of quantification was determined to be 35 and 2 ng/mL for cyclosporine and sirolimus using the approach described in our tacrolimus article (4).

Interassay imprecision (CV) determined from incurred sample reanalysis was <20% for both analytes. The assay was linear within the analytical measurement range (Fig. 1). Performance was evaluated by analyzing recent College of American Pathologists proficiency samples. Cyclosporine and sirolimus measured at 3 different concentrations all fell within the range reported by the LC-MS/MS peer group. Most of the PS-MS/MS determinations were within 1 SD of the LC-MS/MS mean value. Recovery was further evaluated by analyzing separately prepared spiked blood samples. The determined concentrations were routinely within 15% of the expected value.

We plotted the concentrations determined by PS-MS/MS against Dimension RXL immunoassays (Siemens). Passing–Bablok regression analysis yielded the following correlations: PS-MS/MS = 1.2 × RXL − 21, r² = 0.95 (n = 45), for cyclosporine and PS-MS/MS = 1.1 × RXL − 1, r² = 0.81 (n = 44), for sirolimus.

We evaluated matrix effects by comparing signal intensity for standards prepared in methanol vs whole blood. For each drug, the signal was approximately 3 times lower in blood. The decrease in signal arises from both ion suppression and lower recovery from dried blood (5). Adequate quantification was obtained despite the presence of matrix effects; we used matrix-matched whole blood calibrators and SIL-Is to account for variation arising during extraction and ionization.

We evaluated the effects of hemolysis, lipemia, icterus, and high concentrations of 50 steroids, vitamins, diuretics, and immunosuppressive compounds at both low (0.5 ng/mL sirolimus, 15 ng/mL cyclosporine) and medium (6 ng/mL sirolimus, 100 ng/mL cyclosporine) concentrations. There was no effect at either concentration vs blood samples without these factors.

This study demonstrates that PS-MS/MS is a viable method for immunosuppressive drug measurement. We see several advantages of PS-MS/MS over HPLC-MS/MS for clinical laboratories. First, turnaround times can be shortened because sample prepa-
tion is decreased, and the method is more suited to random access analysis. Second, the personnel time and expertise required are lower compared with HPLC-MS because of the simplification of the method and the removal of the chromatography system. Third, solvent consumption is much lower, and <1 mL per day of solvent waste was generated. Finally, the potential risk of carryover is eliminated, because the entire fluid path that interacts with the sample, including the ionization source, is contained within a disposable cartridge.

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**References**


3. Espy RD, Teunissen SF, Manicke NE, Ren Y, Ouyang Z,
Low measurements of serum thyroglobulin (Tg) when immunometric assays (TgAbs) are used (1). This problem can be overcome by tryptic digestion of patient serum with subsequent measurement of Tg-proteotypic peptides by LC-MS/MS (TgMS) (2–4). However, it remains uncertain how different TgMS assays compare with each other, a crucial question in today’s laboratory testing environment, wherein concern has been raised over patient samples being tested by different methods or in different laboratories over time (5). We therefore performed a pilot study to assess the intermethod concordance between 4 independently developed TgMS methods. The study was approved by the Mayo Clinic institutional review board.

We tested 40 patient serum samples spanning Tg concentrations from <0.1 to 10 ng/mL (assigned by DxI TgIA, Beckman Coulter). We classified the samples as TgAb positive (n = 22) or TgAb negative (n = 18) on the basis of whether they contained TgAb concentrations that exceeded the functional sensitivity of the DxI TgAb assays (1.8 IU/mL). Samples were deidentified and shared between 4 laboratories for TgMS testing: ARUP Laboratories, Laboratory Corporation of America Holdings (LabCorp), Mayo Clinic, and the University of Washington (UWash). Each laboratory digests serum samples directly after different denaturing steps. The peptide targets are also different between the assays: ARUP enriches and monitors the peptide FSPDDSAGASALLR for primary quantification.

Mayo calibrators were also shared between laboratories (Tg-negative/TgAb-negative pooled serum spiked with international reference material BCR-457(6) and run as unknowns in each assay. A different batch of samples (because of sample volume limitations), also spanning Tg concentrations <0.1–10 ng/mL and with similar TgAb status, was assayed across 4 automated TgIA assays: DxI-Tg, Immulite-Tg (Siemens Corp.), Elecsys Tg II (Roche Diagnostics), and Kryptor-Tg (Thermo Fisher).

Comparison of Mayo calibration material between TgMS methods showed excellent correlation but substantial differences in calibration assignment. With Mayo values as a reference, slopes were 0.826, 0.878, and 0.814 for ARUP, LabCorp, and UWash, respectively, and r2 values were 0.999, 0.999, and 0.994. The variation in the observed slopes is likely explained by the different approach to calibration: ARUP and LabCorp use the calibration materials provided with the DxI assay, whereas Mayo uses BCR-457 spiked into Tg-negative serum, and UWash uses human samples assigned by the DxI assay.

Comparison of TgMS methods in the patient samples also showed good correlation (Fig. 1). Defining the mean Tg value (in nanograms per milliliter) across all 4 TgMS assays as the reference, slopes across patient samples were 0.833, 1.027, 1.043, and 1.102 for ARUP, LabCorp, UWash, and Mayo, respectively, and r2 values were 0.961, 0.996, 0.981, and 0.988. When the Mayo assay’s patient values and calibrators were used as the reference instead, slopes were 0.740, 0.920, and 0.942 for ARUP, LabCorp, and UWash, respectively, and r2 values were 0.932, 0.983, and 0.970. The contribution of the calibration bias to the slope could be seen when the values were adjusted for each assay’s calibration bias vs the Mayo calibrators. This yielded slopes of 0.895, 1.05, and 1.16 and identical r2 values.

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1 Nonstandard abbreviations: TgAb, antithyroglobulin autoantibody; Tg, human thyroglobulin; TgIA, Tg measurement by immunometric detection; TgMS, thyroglobulin measurement by LC-MS/MS; LabCorp, Laboratory Corporation of America Holdings; UWash, University of Washington.

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First Steps toward Harmonization of LC-MS/MS Thyroglobulin Assays

To the Editor:

Antithyroglobulin autoantibodies (TgAbs)1 frequently cause falsely

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1 Nonstandard abbreviations: TgAb, antithyroglobulin autoantibody; Tg, human thyroglobulin; TgIA, Tg measurement by immunometric detection; TgMS, thyroglobulin measurement by LC-MS/MS; LabCorp, Laboratory Corporation of America Holdings; UWash, University of Washington.