proteins. We show here that storage at −70 °C is another option.

Urinary concentrations of albumin were remarkably stable in this study, which seems somewhat in contrast to our previous work (5) and to the findings of other investigators (1–4, 6–10). We feel that this discrepancy can be explained by the fact that most studies involved the measurements of albumin in the microalbuminuric range, whereas in the present study most urine samples contained high concentrations of albumin. Therefore, our conclusion can be applied only to urine samples that contain relatively large amounts of albumin. In our study, IgG was more sensitive to storage effects than albumin, and we would expect that losses of IgG might be even more pronounced in urine samples with low protein concentrations. Recent data presented by Hoogenberg et al. (24) seem to confirm this idea. The use of BSA may be warranted when urinary protein concentrations are low (except for albumin measurement because of cross-reactivity).

We conclude that if urinary proteins can be measured within 4 weeks after sampling, storage at 4 °C appears to give the best results. For longer time periods, a storage temperature of 2–4 °C should be chosen. Storage at −20 °C (without appropriate preservatives) is not recommended.

We are grateful for the help of all the technicians who were involved in the production and processing of the results of this study.

References


Evaluation of the New AxSYM Cyclosporine Assay: Comparison with TDx Monoclonal Whole Blood and Emit Cyclosporine Assays, Pierre E. Wallemacq and Kathy Alexandre (Laboratory of Therapeutic Drug Monitoring, Department of Clinical Chemistry, University Hospital St Luc, University of Louvain, 10 Hippocrate Avenue, B-1200 Brussels, Belgium, and 2 Abbott Diagnostics Division, Rue du Bosquet 2, B-1348 Ottignies-Louvain-la-Neuve, Belgium; * author for correspondence: fax 32 2 764 90 44, e-mail wallemacq@lbcm.ucl.ac.be)

Cyclosporine (CsA) is a cyclic undecapeptide that has potent immunosuppressive activity, but its narrow therapeutic range and variable pharmacokinetics in humans make monitoring of CsA mandatory. The generally accepted whole-blood therapeutic ranges of CsA (with specific assay) are 100–200 \(\mu g/L\) for renal transplant patients and 150–250 \(\mu g/L\) for cardiac, hepatic, and pancreatic transplant patients during the maintenance phase (2, 3), whereas slightly higher ranges are recommended during the induction phase. The CsA metabolites arising from oxidative pathways (4) cross-react in immunoassays (5, 6), leading to results higher than those by HPLC. Trough concentrations of metabolite AM1, the major metabolite in human blood (1, 4), can exceed those of the parent drug (1). For metabolites AM1, AM9, and AM4N, immunosuppressive activity is <10% of that observed for CsA (7, 8), and at least in rats, various CsA metabolites are not nephrotoxic (9). Thus CsA itself,
rather than its metabolites, is the major pharmacologically active substance (10), and specific analytical methods are recommended (3,11,12). HPLC is specific but time-consuming and labor-intensive; it also displays the highest between-center CV (1). Three major immunoassays based on different CsA monoclonal antibodies were developed by Abbott, Inc Star, and Syva Laboratories: a fluorescence polarization immunoassay (FPIA; for the TDx analyzer), a Cyclo-Trac RIA, and an Emit, respectively (13,14). All of these immunoassays yield slightly higher concentrations than does HPLC, with FPIA concentrations high by 20–30% (15). The results can be summarized as HPLC < Emit < RIA < TDx.

Very recently, a new monoclonal CsA FPIA assay was introduced by Abbott Laboratories for the AxSYM analyzer; it was aimed at reducing metabolite cross-reactivity further and, therefore, at correlating better with HPLC. We first evaluated the analytical performances of this new assay by comparing it with the TDx whole blood monoclonal assay and the Emit assay on Cobas Mira plus. The clinical performance of this AxSYM assay was further evaluated by comparing a total of 150 clinical samples analyzed with the three methods on the same day. In addition, the three methods were evaluated regarding their cross-reactivity with three CsA metabolites. Finally, to evaluate the role of the type of organ transplanted in the correlation AxSYM/TDx, these methods were compared using 608 renal transplant and 112 hepatic transplant samples.

The AxSYM assay requires 184 μL of sample, and 59 samples/h can be analyzed after a rapid organic extraction (150 μL of whole blood + 50 μL of solubilization reagent + 300 μL of precipitation reagent). The blood samples after pretreatment were vortex-mixed individually for 10 s and centrifuged for 5 min according to the manufacturer’s instructions. The pretreatment volumes are thus similar for the TDx CsA monoclonal whole blood method (150 μL of whole blood + 50 μL of solubilisation reagent + 300 μL of precipitation reagent, which is a zinc sulfate solution in methanol and ethylene glycol). For the Emit method, a pretreatment with methanol and ethylene glycol was used. The analytical performance of each method is summarized in Table 1. The within-run imprecision (CV) was determined for the AxSYM and the comparative methods (TDx and Emit). Each of the three controls (low, medium, and high) was analyzed in replicates of 20 during 3 consecutive days. In addition, to assess the between-day CV, each of the three controls was analyzed in replicates of two during 10 different days. The results are shown in Table 1. To estimate the detection limit, 10 samples of calibrator A (0 μg/L) were analyzed with the three methods on 4 different days; the mean ± 2 SD was 7.4, 4.2, and 1.5 μg/L, respectively, for AxSYM, TDx, and Emit (Table 1). To assess dilution linearity, the AxSYM calibrator B (800 μg/L) was serially diluted with the AxSYM calibrator A (0 μg/L) to obtain target concentrations of 800, 400, 200, 100, 50, and 25 μg/L; the mean results were 800, 399, 196, 94, 45, and 26 μg/L. To determine whether hepatic or renal impairment interfered, we selected seven samples with bilirubin values of 242–797 μmol/L and two samples with creatinine of 496 and 520 μmol/L. These samples were CsA-free. After addition of known amounts of CsA (300 μg/L), we compared the CsA concentrations with results in two nondiseased blood specimens (bilirubin, 9 μmol/L; creatinine, 88 μmol/L). The mean CsA concentration in the control samples was 292 μg/L, whereas CsA concentrations were 285–317 μg/L in the cholestatic samples and 299–306 μg/L in the samples from patients with renal impairment. Neither bilirubin nor creatinine correlated with the measured CsA concentration.

We assayed 150 samples (54 from kidney, 35 from hepatic, 29 from heart, and 32 from bone marrow transplantation patients treated with CsA) simultaneously with the three techniques (AxSYM as y-axis and TDx or Emit as x-axis). Deming regression analyses performed with these data (all transplant types together) for AxSYM-TDx yielded the correlation coefficient r = 0.981 and the equation y = (0.78 ± 0.012)x – (3.9 ± 0.216); Syx = 11.35; and for AxSYM-Emit, it yielded the correlation coefficient r = 0.974 and the equation y = (0.86 ± 0.015)x + (19.7 ± 2.11); Syx = 13.1.

AxSYM results were lower than those from TDx by ~20% and were much closer to the Emit values (Fig. 1). A significant decrease in the blood concentrations was observed vs TDx but not vs Emit.

To evaluate the cross-reactivity of CsA metabolites in the three assays, we added 400–1700 μg/L of metabolites AM1, AM9, and AM4N (provided by Novartis Pharmaceuticals, Basel, Switzerland) to the 100 μg/L calibrator in all three methods. All measurements were made in duplicate on the same day. The cross-reactivities for AxSYM

<table>
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<tr>
<th>Table 1. Analytical performance of the new AxSYM cyclosporine assay compared with the TDx and Emit.</th>
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<tr>
<td><strong>Within-run CV,a %</strong></td>
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<td>Low</td>
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<td><strong>Between-day CV,b %</strong></td>
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<td><strong>Detection limitc</strong></td>
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a CV for each of the three controls in replicates of 20 during 3 consecutive days.
b CV on measurements of each of the three controls (means of duplicates) during 10 different days.
c Mean ± 2 SD of the measurements of calibrator A (0 μg/L) in replicates of 10 on 4 different days.

*NS, not significant; the measured value is within the within-run imprecision range calculated for the measured value.*
were significantly lower than the values obtained with the TDx and closer to those obtained by Emit (Table 1).

In addition, we compared the results obtained for 608 renal and 112 hepatic transplants using the AxSYM with those obtained using the TDx; the least-squares equations were $y = (0.81 \pm 0.006)x - (2.6 \pm 1.03); r = 0.980; S_{yx} = 12.2$ for renal transplants; and $y = (0.71 \pm 0.02)x - (3.2 \pm 2.1); r = 0.975; S_{yx} = 13.3$ for hepatic transplants. The slope we observed for the hepatic transplants (0.71) was significantly smaller than those obtained for the total population of transplants (slope = 0.78) and the kidney transplant population (slope = 0.81), which was consistent with the better specificity of the AxSYM method for the CsA parent compound.

The lower results with the AxSYM assay may appear surprising because the AxSYM and TDx assays use the same monoclonal antibody. The increased specificity may arise from the pretreatment and extraction steps. The AxSYM pretreatment solution contains more methanol and less ethylene glycol than that of the TDx. Moreover, the pipetting sequences and incubation times are modified. The FPIA technology is based entirely on the binding constants of the antibody for the analyte. The binding of an antibody to its ligand is affected by several factors, including the concentrations of the protein and ligand, temperature, pH, buffering conditions, and others. In addition, binding is dependent on the length of incubation required to reach equilibrium. The AxSYM method appeared to be reproducible and stable. No calibration was required during the study (>7 weeks), whereas it was necessary to recalibrate the Emit method weekly. The analytical performance (within- and between-run imprecision and detection limit) of the TDx was slightly better. However, it should be stressed that the quality-control samples used in the AxSYM had considerably lower mean values than those used in the TDx. Contrary to the CsA assay on TDx, no special probe cleaning step was required on AxSYM. The concentrations of the AxSYM CsA calibrators were 0, 40, 100, 200, 400, and 800 µg/L, whereas they were 0, 100, 250, 500, 1000, and 1500 for the TDx. Thus, the AxSYM calibrators included more concentrations in the range most frequently encountered in samples.

The major advantage of this new method is better specificity for the parent drug. This improved specificity is demonstrated by the values obtained for clinical specimen compared with the TDx and confirmed by metabolite cross-reactivity, which is ~50% lower than the cross-reactivity observed for TDx. The higher specificity of the new AxSYM method should yield CsA blood concentrations much closer to HPLC data, reflecting more accurately the concentration of the immunosuppressive parent compound. Furthermore, measurement of CsA on a new generation analyzer such as the AxSYM should substantially reduce the laboratory technologist time required for the assay through true random access, shorter time to first result, and the ability of the AxSYM to be bidirectionally connected to a laboratory information system.

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References


