
Modified TDx® Assay for Cyclosporine and Metabolites, for Use with Whole-Blood Samples

Wolfgang Vogt and Irene Welsch

A recently introduced fluorescence polarization immunoassay (FPIA) for determination of cyclosporine A in serum and plasma is discussed with regard to its use for whole-blood samples, with and without hemolysis before the assay. The performance characteristics of the modified method are highly satisfactory (within-run CVs 2.06 to 5.50% and 1.99 to 3.39%, respectively; long-term between-run CVs under routine conditions 5.73 to 8.95%). The limit of detection is 30 μg/L. Results agree well with those obtained with the RIAs compared, but the modified FPIA is more convenient and faster.

Additional Keyphrases: fluorescence polarization immunoassay • transplantation • immunosuppression • therapeutic drug monitoring

Cyclosporine A (CyA) is widely used as an immunosuppressant to combat tissue rejection after organ transplants. Its clinical usefulness in the treatment of patients with autoimmune diseases is also under investigation. Given the poor dose-response relationship and the considerable toxicity of the agent, therapeutic drug monitoring of CyA concentrations is indispensable. Radioimmunoassays (RIA) and high-performance liquid chromatography (HPLC) methods have to date been the only commercially available techniques for this purpose. These methods are cumbersome and time consuming, though the 125I assay is comparatively easy to perform. A fluorescence polarization immunoassay (FPIA) has recently been described (1) and now has also become available commercially. However, this method is designed for use on plasma specimens only.

Given the clearly temperature-related distribution of CyA

Institut für Klinische Chemie und Laboratoriumsmedizin, Deutsches Herz-Zentrum München des Freistaates Bayern, Lothstraße 11, 8000 München 2, F.R.G.
Received October 9, 1987; accepted March 30, 1988.

among the various blood components, whole blood is obviously the preferred material for analysis (2, 3). We therefore sought to adapt this new FPIA to allow tests on whole-blood samples. For comparison we used a tritiated RIA and an 125I-labeled RIA. Because we receive both unprocessed whole-blood samples and deep-frozen hemolysates for testing, we studied the usefulness of the modified FPIA for both these materials.

Materials and Methods

Instrumentation

The FPIA was performed in a TDx Analyzer (Abbott, Irving, TX). Radioactivity was measured with a 1261 Multi-gamma γ-scintillation counter and a 1219 Betarack β-scintillation counter (KLB-Wallac, Turku, Finland). Dilutions were made with a Microlab 1000 dilutor (Hamilton, Bonaduz, Switzerland).

To prepare hemolysates, we subjected blood cells to -20 °C for at least 1 h or used a Soniprep 150 ultrasonic disintegrator (MSE Scientific Instruments, Crawley, Sussex, U.K.), three 20-s bursts.

Reagents

We used test kits from Sandoz Ltd., Basle, Switzerland (Cyclosporin RIA-Kit); INC-DRG-Instruments, Marburg, F.R.G. (CYCLO-Trac); and Abbott Laboratories, Chicago, IL (TDx Cyclosporine and Metabolites Assay).

Specimens

Blood anticoagulated with EDTA (1 mg of K2EDTA per milliliter of whole blood) was used for all assays. All patients were being treated with CyA after heart transplants. EDTA-treated blood from healthy blood donors was used to prepare the calibration standards and to dilute samples with high CyA concentrations for checking linearity.
Specimen Preparation

**Fluorescence polarization immunoassay. Hemolysate technique (method A).** We deep-froze (−20 °C) 500 μL of EDTA-treated blood for at least 1 h to hemolyze the blood cells. The samples were thoroughly mixed. After thawing the sample in a water bath at 37 °C for 15 min, then cooling it to room temperature, we added 50 μL of TDx-buffer solution to 50 μL of hemolysate in a 1.5-mL Eppendorf plastic vial provided with a cover. After vortex-mixing, we pipetted 300 μL of the precipitation solution (ammonium acetate in water/propan-2-ol, 98.5/1.5 by vol), sealed the vials, and vortex-mixed again for 20 s. The remaining steps of the procedure were those in the manufacturer’s test instructions for serum/plasma.

**Unprocessed whole blood (method B).** Instead of the hemolysate, we used 50 μL of EDTA-treated blood directly, diluted this with 50 μL of TDx-buffer solution, and precipitated with 300 μL of the ammonium acetate solution as described above. The remaining steps were as in method A.

**Radioimmunoassays.** Erythrocytes were destroyed exclusively by ultrasonic disintegration for the 125I assay, but also partly by deep-freezing for determination with the tritiated assay. All tests were performed according to the manufacturer’s instructions. Tritium counting was corrected for quench.

**Standards**

CyA in pure form was kindly made available by Sandoz Ltd. We dissolved 4 mg in aqueous ethanol (70 mL of ethanol diluted to 100 mL with distilled water).

**Control Materials**

Hemolysate controls with high (target CyA concentration range 686–1030 μg/L) and low (121–181 μg/L) concentrations were supplied by Bio-Rad ECS, Anaheim, CA.

**Calibration**

Calibrators of the following concentrations were prepared from hemolysate of healthy blood donors: 0, 50, 100, 200, 500, and 1000 μg/L. The TDx analyzer was calibrated according to the operating instructions of the manufacturer for plasma samples.

**Results and Discussion**

**Sample Preparation**

Use of methods A and B resulted in a clear, practically hemoglobin-free (hemoglobin concentration about 20 mg/dL) supernate, which could be analyzed without additional processing by FPIA (Table 1). No statistically significant difference could be detected by Wilcoxon’s signed-rank test for matched pairs.

**Accuracy**

Accuracy was checked by measuring CyA concentrations of commercially available hemolysate controls. The accuracy data given by the manufacturer refer to the tritiated assay. Deviation from the mean was +7 μg/L (+1.1%) and −11 μg/L (−7.3%); all values were within the target range.

**Within-Run Imprecision**

**Method A:** Imprecision was studied by assaying 10 replicates of hemolysate specimens with low, medium, and high CyA concentration. Mean (and SD) values were 156.4 ± 8.6 μg/L, 497.6 ± 13.2 μg/L, and 896.4 ± 18.5 μg/L; the respective coefficients of variation (CVs) were 5.50%, 2.65%, and 2.06%.

**Method B:** The mean (and SD) results obtained with method B for patients’ whole-blood samples with concentrations similar to those of the specimens used for determining the precision of method A were 152.3 ± 5.16 μg/L, 588.5 ± 8.59 μg/L, and 936.1 ± 18.6 μg/L; the corresponding CV values were 3.39%, 1.51%, and 1.99% for 10 replicates each. These results are somewhat better than those observed with the same reagents but with plasma samples: 79.2 ± 3.53 μg/L (CV 4.45%), 251 ± 11.07 μg/L (CV 4.41%), and 681.7 ± 21.05 μg/L (CV 3.09%).

**Between-Run Imprecision**

**Method A:** Between-assay imprecision (10 runs) was studied by measuring the same controls (hemolysates) as those used for determining intra-assay variation. The mean (and SD) results were 149.2 ± 13.2 μg/L, 510.2 ± 15.8 μg/L, and 903.6 ± 32.6 μg/L. The respective CVs were 8.85%, 3.61%, and 3.68%.

**Long-term imprecision under routine conditions (different lots, 10 different technicians, high workload) was 135.7 ± 12.16 μg/L and 812.4 ± 46.6 μg/L; the corresponding CVs were 9.5% and 5.73% for 96 runs.**

**Method B:** Between-run precision of method B was not determined, because it was not known whether prolonged, refrigerated storage of unprocessed whole-blood controls might give rise to matrix alterations, thus affecting the CyA measurement. However, results for whole-blood samples were very similar to those for plasma samples (78.2 ± 6.58 μg/L, CV 8.42%; 243.4 ± 5.68 μg/L, CV 2.33%; 709.9 ± 32.55 μg/L, CV 4.59%).

**Detection Limit**

The lowest concentration significantly distinguishable from zero was 30 μg/L under the conditions described. We determined this as the concentration on a calibration curve that would be obtained for the fluorescence polarization reading plus 3 SD for a sample without CyA.

**Linearity**

A patient’s sample with a high concentration of CyA (927 μg/L) was variously diluted with a CyA-free hemolysate and assayed. A strong linear correlation between the amounts measured and expected was found (r = 0.9992, see Table 2).

**Comparison of Results by FPIA and RIA**

Blood samples of 70 patients were assayed by the tritiated RIA and 64 specimens were analyzed by the 125I-labeled

---

Table 1. Comparison of Results of CyA Determinations by Methods A and B for Samples from 10 Patients

<table>
<thead>
<tr>
<th>CyA, μg/L</th>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td>399</td>
<td>406</td>
<td></td>
</tr>
<tr>
<td>462</td>
<td>447</td>
<td></td>
</tr>
<tr>
<td>640</td>
<td>640</td>
<td></td>
</tr>
<tr>
<td>603</td>
<td>581</td>
<td></td>
</tr>
<tr>
<td>477</td>
<td>467</td>
<td></td>
</tr>
<tr>
<td>605</td>
<td>658</td>
<td></td>
</tr>
<tr>
<td>417</td>
<td>409</td>
<td></td>
</tr>
<tr>
<td>516</td>
<td>516</td>
<td></td>
</tr>
<tr>
<td>548</td>
<td>547</td>
<td></td>
</tr>
<tr>
<td>573</td>
<td>566</td>
<td></td>
</tr>
<tr>
<td>393</td>
<td>392</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1. Comparison of CyA concentrations measured by FPIA (ordinate) and (left) tritiated RIA (abscissa) or (right) $^{125}$I RIA (abscissa).

The origins of the coordinate sets correspond to the arithmetic mean of both methods, the unit of the scale values is the standard deviation (SD)

Table 2. Results for a Patient's Hemolysate (a) Diluted with CyA-free Hemolysate (b) ($r = 0.9992$)

<table>
<thead>
<tr>
<th>Dilution ($a + b$)</th>
<th>Expected</th>
<th>Found</th>
<th>($C_{found}/C_{exp}$) x 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted</td>
<td>927</td>
<td>927</td>
<td>100</td>
</tr>
<tr>
<td>1 + 0.5</td>
<td>618</td>
<td>591</td>
<td>96</td>
</tr>
<tr>
<td>1 + 1</td>
<td>464</td>
<td>475</td>
<td>102</td>
</tr>
<tr>
<td>1 + 2</td>
<td>309</td>
<td>316</td>
<td>102</td>
</tr>
<tr>
<td>1 + 5</td>
<td>155</td>
<td>168</td>
<td>108</td>
</tr>
<tr>
<td>1 + 7</td>
<td>116</td>
<td>126</td>
<td>109</td>
</tr>
<tr>
<td>1 + 10</td>
<td>84.3</td>
<td>87</td>
<td>103</td>
</tr>
</tbody>
</table>

RIA; all samples were also assayed by the FPIA. Correlation was estimated by main component analysis (Table 3). The TDx results tended to be somewhat higher (10%) than those obtained with the RIAs. Figure 1 shows that there is an acceptable correlation between these methods.

Workload

The mechanization of the FPIA procedure offers a definite reduction in workload over the RIAs. In addition, the time until the final results are available is only 50 min for 10 patients' samples for the FPIA, whereas the $^{125}$I-labeled RIA takes 5 h and the tritiated RIA as long as 6 h to complete.

References