Comparison of ELISA method versus MEIA method for daily practice in the therapeutic monitoring of tacrolimus

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Abstract

Background. For the adequate management of transplant patients on tacrolimus therapy, it is important to obtain optimal blood concentrations. The purpose of this study was to determine the most appropriate method for daily practice of tacrolimus determination in whole blood. We compared enzyme-linked immunosorbent assay (ELISA) with microparticle enzyme immunoassay (MEIA), using European controls and blood samples from organ graft recipients treated with tacrolimus. Time, practicability and cost were considered also.

Methods. The assays were performed according to the procedures detailed in the product inserts. In five European controls and 40 blood samples from kidney and liver transplant patients, we determined the blood levels of tacrolimus by both MEIA and ELISA tests.

Results. MEIA gave more reliable results with the European controls ($y = 1.078x + 0.092; r = 0.996$) than ELISA ($y = 0.956x + 1.307; r = 0.946$). For the patient samples, the correlation between the two tests was 0.85 and the extreme range of values was +65% and −56% for ELISA vs MEIA. Although the manufacturer of the ELISA test used claims the best sensitivity and precision, in our experience the MEIA test was quicker and cheaper.

Conclusions. MEIA provides a quick, reliable and easy-to-handle method for routine monitoring of tacrolimus blood levels.

Keywords: ELISA; MEIA; routine monitoring; tacrolimus (FK 506)
Manual-technical method with about eight main steps. The assay was run on a microtitre plate (96 wells) after pre-coating with goat anti-mouse IgG. Standards, controls and blood samples were extracted with a proprietary reagent, and then added to the wells of the microtitre plate, followed by addition of anti-tacrolimus monoclonal antibody. Each sample determination was done in duplicate. After a 20-min incubation at room temperature, tacrolimus horseradish peroxidase conjugate was added and incubated for an additional 60 min. The wells were then washed and chromogen added for a 15-min incubation. This reaction was stopped by addition of sulfuric-acid and the absorbance in each well was read at a dual wavelength of 450/630 nm. Colour development is inversely proportional to the amount of tacrolimus present in the sample. In addition to the blood samples, a six point standard curve, a point of non-specific binding and two internal controls (low and high values) were mandatory [7,8]. Standards, conjugate, controls and digestion reagent were stored at –20°C.

**MEIA technique**

The MEIA test, based on the Abbott IMX® (Abbott Lab., Abbott Park, IL, USA) analyser, is a semi-automated immunoassay with a manual pre-treatment step where the whole blood sample was extracted with a precipitation reagent and centrifuged. The supernatant was poured into the sample well and the IMX Tacro II reagents were added by the probe/electrode assembly to the incubation well in the following sequence: anti-tacrolimus antibody (mouse monoclonal) coated microparticles, and tacrolimus-phosphatase conjugate. Tacrolimus and its conjugate competitively bind to the anti-tacrolimus microparticles forming ‘antibody–antigen’ and ‘antibody–antigen–alkaline phosphatase’ complexes [1].

An aliquot of the reaction mixture containing the sample supernatant, and the ‘antibody–antigen’ and ‘antibody–antigen–alkaline phosphatase’ complexes bound to the microparticles was transferred to the glass fibre matrix. The microparticles bound irreversibly to the glass fibre matrix, which was washed to remove unbound materials. The substrate, 4-methylumbelliferyl phosphate, was added to the matrix and the fluorescent product was measured by MEIA optical assembly [9,10]. Three different quality controls (low, medium, and high) were supplied with the kit.

**Blood samples studied**

The study included 40 blood samples from 40 transplanted patients (27 with kidney transplant and 13 with liver transplant) and 5 European controls (David W. Holt, St George’s Hospital Medical School, London, UK).

Quality control testing across centres provides useful information on overall analytical performance. In June 1995, a European Tacrolimus Quality Assessment Scheme was established. Participating laboratories received three quality control samples per month: one aliquot of a single pool of blood samples from patients on Prograf® therapy and two drug-free blood samples adjusted to two different known concentrations of tacrolimus. The 45 samples (40 patients + 5 controls) stored at −20°C were tested in parallel, in a blind fashion in three consecutive series for both ELISA and MEIA. All were done on the same day, according to the manufacturer’s instructions in the product inserts. Tacrolimus concentration was supposed to be in the therapeutic range.

**Results**

**Internal quality controls**

For both MEIA and ELISA techniques, the tacrolimus concentrations of their own internal controls (low, medium, high) were in the expected range values, but the coefficient of variation (CV) was better for the high controls (Table 1).

**European controls**

We compared the data obtained by both methods using the 5 European controls (0, 3, 7.3, 10, 12 ng/ml) as blood samples (Table 2). The slope of the curves was similar (near 1). The linear correlations obtained were $y = 1.078x + 0.092$ for MEIA with a correlation coefficient of $r = 0.996$ and $y = 0.956x + 1.307$ for ELISA, correlation coefficient $r = 0.946$.

**Blood samples from transplanted patients**

We compared the data obtained with PRO-Trac and MEIA in 40 blood samples from grafted patients. There was no significant difference between the two

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**Table 1. Tacrolimus concentrations (ng/ml) of the internal controls**

<table>
<thead>
<tr>
<th></th>
<th>ELISA Target concentration</th>
<th>MEIA Target concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>CV (%)</td>
</tr>
<tr>
<td>Low</td>
<td>2</td>
<td>2.25</td>
</tr>
<tr>
<td>Medium</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>High</td>
<td>15</td>
<td>17.5</td>
</tr>
</tbody>
</table>

CV, coefficient of variation.
The correlation coefficient between the two methods was fair, $r_s = 0.83$, with the following linear regression equation: $y = 0.85x + 0.94$ (Figure 1). The test of concordance by Bland and Altman method showed that 95% of the values were within the confidence interval ($\pm 2$ SD) (Figure 2).

Different parameters concerning the sensitivity of the two assays are given by the manufacturer and could be useful to the reader (Table 3).

**Table 2. Tacrolimus concentrations (ng/ml) of the European controls**

<table>
<thead>
<tr>
<th>Spiked concentrations</th>
<th>ELISA</th>
<th>MEIA</th>
<th>Inaccuracy (%) ELISA</th>
<th>Inaccuracy (%) MEIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.8</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>4.6</td>
<td>3.8</td>
<td>53</td>
<td>27</td>
</tr>
<tr>
<td>7.3</td>
<td>7</td>
<td>7.5</td>
<td>–4</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>10.4</td>
<td>–10</td>
<td>14</td>
</tr>
<tr>
<td>12</td>
<td>15</td>
<td>13.6</td>
<td>25</td>
<td>13</td>
</tr>
</tbody>
</table>

**Fig. 1.** Linear regression analysis of PRO-Trac II ELISA values vs IMX MEIA values ($n = 40$). The resulting regression equation is $y = 0.85x + 0.94$; $r = 0.83$.

**Fig. 2.** Method comparison between PRO-Trac II ELISA and IMX Tacrolimus II MEIA. The mean value of the two methods is plotted against the difference between the two values (Pro-Trac–IMX).

**Table 3. Characteristics of the assays**

<table>
<thead>
<tr>
<th></th>
<th>ELISA Manual (8 steps)</th>
<th>MEIA Semi-automated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer data</td>
<td>Analytical sensitivity</td>
<td>0.27 ng/ml</td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>78–84%</td>
</tr>
<tr>
<td></td>
<td>Intra-assay precision</td>
<td>Not reported</td>
</tr>
<tr>
<td></td>
<td>Inter-assay precision</td>
<td>&lt;10%*</td>
</tr>
<tr>
<td>Own results</td>
<td>Within day CV</td>
<td>&lt;10%</td>
</tr>
<tr>
<td></td>
<td>Day-to-day CV</td>
<td>&lt;15%</td>
</tr>
<tr>
<td></td>
<td>Number of patients per run</td>
<td>max. 39</td>
</tr>
<tr>
<td></td>
<td>Time for one run</td>
<td>5 h</td>
</tr>
<tr>
<td></td>
<td>Average cost per sample</td>
<td>18.6–60.5 Euros</td>
</tr>
</tbody>
</table>

CV, coefficient of variation.

*For concentrations >3 ng/ml.

**Cost**

When comparing the cost of ELISA and MEIA, even for a small series of samples, MEIA was cheaper. For ELISA, the cost was 18.6 Euros per sample with the kit being used for 1 series (i.e. a maximum of 39 patients). It went up to 60.5 Euros per sample when used for 4 different series (i.e. a maximum of 12 patients).

For MEIA, the cost was 14.4 Euros per sample when the 100-test kit was used for different series, 6 tests for calibration and 3×5 for controls, allowing the exploration of a maximum of 79 patients. It went up to 16.2 Euros per sample when used for 8 different series of about 10 patients (i.e. a maximum of 70 patients). The rent of the IMX analyser was included in that price.

**Discussion**

Therapeutic drug monitoring is generally accepted as a prerequisite for therapy with tacrolimus. Because of the extreme inter- and intra-patient variability and lack of correlation between drug dosage and drug blood levels, a consistent and reliable therapeutic drug monitoring method is needed.
For ELISA, extrapolations of 4PL curve fits were known to be susceptible to unpredictable behaviour. Between zero and the lowest standard, the sample value obtained by a 4PL curve fit is an algebraic formula, not a realistic assessment \( w_1 x \). Those 4PL fits were also used by the IMX analyser.

With European controls, the slope of the curves was greater than 1 for MEIA (1.078), compared with ELISA (0.956), and the correlation coefficient was better for MEIA \( (r = 0.996 \text{ vs } r = 0.946) \). Therefore, it appeared that the results obtained with MEIA were closer to the real values than those obtained with ELISA.

When testing the patient samples, the two techniques gave quite similar results. The resulting correlation coefficient of the linear regression analysis was 0.83 and there was no significant difference between the two assays (paired \( t \)-test; \( t = 0.479; P = \text{NS} \)). The concordance test was acceptable with more than 95% of the values in the confidence interval, even if the extreme range of values was \( +65\% \) and \( -56\% \) for ELISA vs MEIA (data not shown).

The number of samples tested by the two methods in parallel was relatively small and we should be cautious in interpreting the results.

In terms of time spent and practicability, MEIA was a faster technique than ELISA and could be adapted to all types of analyses, namely large and small series. With this technique, blood samples could be kept for a maximum of 14 days at 4°C or frozen at \(-20°C\); the standards and reagents could be used until the expiration date when kept at \( 4°C \). For ELISA, blood samples have been reported to be stable at room temperature for up to 14 days, or frozen at \(-20°C\) for 6 months with a maximum of three freeze/thaw cycles. Standards, controls, PRO-Trac II conjugate, and digestion reagent should be stored at \(-20°C\) and up to three freeze/thaw cycles are allowed. Microtitre plate, antibody, wash concentrate, conjugate diluent, stop solution and chromogen should be stored at \( 4°C \) [8]. The cost of MEIA was somewhat lower than that of ELISA for large series of samples. For smaller series, the cost saving was striking with MEIA, namely divided by a factor of 4. This calculation did not include technician costs, which would give an additional advantage to MEIA.

References

10. MacFarlane G, Scheller D, Ersfeld D. A simplified whole blood enzyme-linked immunosorbent assay (PROTrac II) for tacrolimus (FK 506) using proteolytic extraction in place of organic solvents. Ther Drug Monit 1996; 18: 698–705

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