out simultaneous measurements of other activities with the ultramicromethod, because 30 μL of leukolysate is generally obtained. In addition, the greater economy of reagents is not negligible, especially with respect to the oligo-dA, which is very expensive.

References


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**Fluorescence Polarization Immunoassay for Ethosuximide Evaluated and Compared with Two Other Immunoassay Techniques**

Clinton F. Stewart1,2 and Michael B. Bottorff1

We evaluated a new fluorescence polarization immunoassay (FPIA) for ethosuximide in the Abbott TDx® and compared results with those by two other ethosuximide immunoassays, emit® (Syva Co.) and ace® (DuPont). The FPIA assay produced within- and between-day CVs of <5% at the low, medium, and high ranges of the standard curve. For the ethosuximide FPIA assay the standard curve was stable during the 47 days of the study. By all three methods, we analyzed 100 serum and plasma samples from patients who were receiving ethosuximide. The coefficient of determination (r²) for TDx versus emit was 0.973 (slope, 0.96; intercept, −0.80); for TDx vs ace it was 0.985 (slope, 1.00; intercept, −2.44); both relationships were statistically significant (p <0.05). Values for patient’s specimens were significantly lower by the TDx than by the ace or emit methods (p <0.05).

**Additional Keyphrases:** anticonvulsant drugs · enzyme immunoassay compared

Ethosuximide is a succinimide drug useful in the treatment of absence (petit mal) seizures (1). Because of wide interpatient variability in pharmacokinetic parameters, monitoring the ethosuximide concentration in serum has been recommended, to optimize drug dosage and therapeutic response (2, 3). Based on clinical studies, a therapeutic range of 40 to 100 mg of ethosuximide per liter of serum has been suggested (4).

Advances in immunoassay technology have produced rapid, automated methods for measurement of ethosuximide in serum. We evaluated the comparative performance of a new fluorescence polarization immunoassay (FPIA) in the Abbott TDx® with two other commercially available immunoassays, ace® and emit®.

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Materials and Methods

Reagents and instrumentation. Reagents, calibrators, and controls for the EMT assay were obtained from Syva Co., Palo Alto, CA. Reagents for the acu (DuPont Co., Wilmington, DE) assay were obtained from DuPont. As suggested in the acu product literature, EMT calibrators and controls were also used for the acu ethosuximide assay. The Syva LP 6000 system was used for those samples assayed by EMT; the acu III instrument was used for the acu analyses. The FPIA reagents, controls, and calibrators used were in TDX kits supplied by Abbott Diagnostics Division, Dallas, TX. Six-point standard curves were constructed for all three methods with use of the described calibrators in concentrations of 0, 10, 25, 50, 100, and 150 mg/L.

Precision and accuracy. After calibrating the TDX system on day 1, we assayed 20 replicates each of the low- (35 mg/L), medium- (70 mg/L), and high-concentration (120 mg/L) controls to evaluate for within-day precision. To evaluate between-day precision, we assayed five replicates each of the TDX low, medium, and high controls in 10 runs made during two weeks. To evaluate accuracy, we quantified the TDX calibrators in quadruplicate by both the EMT and acu methods. In addition, we assayed the EMT calibrators (used for both the EMT and acu methods) in quadruplicate by the TDX assay. Stability of the standard curve for the TDX assay was assessed by assaying the six TDX calibrators in duplicate on days 1, 13, and 47 (see Table 4).

Clinical specimens. Serum and plasma were sampled from patients who were receiving ethosuximide for seizure control and stored at −70 °C until analysis. A total of 100 specimens were analyzed by the three methods. The EMT and TDX assays were performed in duplicate; the acu was a single analysis.

Statistical analysis of data. Precision was estimated by methods outlined in the NCCLS EP5 precision evaluation document (5). Correlation analysis was performed on values determined for serum by the TDX, EMT, and acu techniques. Median values for ethosuximide concentrations determined by the three methods were compared by Friedman's two-way analysis of variance with Nemenyi's multiple comparison test (6). Statistical analyses were performed with the BMDP (7) and SAS (8) statistical packages as implemented on a Data General MV 8000 computer. The significance level was pre-chosen as alpha = 0.05.

Results and Discussion

Table 1 gives the results of the precision studies. Use of the TDX method for ethosuximide in serum resulted in CVs of <5% for the three control concentrations for the low, medium, and high ranges of the standard curve.

Table 2 summarizes our assessment of the accuracy of the TDX standard curves. The CV for the assay of the TDX 10 mg/L calibrator by the acu method was 17%; all other TDX calibrator determinations had CVs <10%. Because the value of this calibrator is well below the reported therapeutic range for ethosuximide and fewer than 2% of the samples assayed in this study were in this range, no apparent effect on our results was observed. The results of the assay of EMT calibrators by the TDX are presented in Table 3.

The results of the standard curve stability test are shown in Figure 1. The millipolarization units vs concentration plots for days 1, 13, and 47 were practically superimposable, indicating standard curve stability over the duration of the study period (see Table 4).

Correlation of the serum concentrations for ethosuximide by the TDX method vs those by EMT and acu are depicted as scattergrams in Figure 2. The results indicate a high degree of association between the TDX and the two comparative methods, with coefficients of determination ($r^2$) of 0.973 (TDX vs EMT) and 0.985 (TDX vs acu). Both of the relationships were statistically significant ($p < 0.05$). Least-squares

Table 2. TDX Calibrators Assayed by EMT and acu

<table>
<thead>
<tr>
<th>Target concn, mg/L</th>
<th>0</th>
<th>10</th>
<th>25</th>
<th>50</th>
<th>100</th>
<th>150</th>
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<tbody>
<tr>
<td>EMT (n = 4)</td>
<td></td>
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<tr>
<td>Mean</td>
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<td>52.50</td>
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<tr>
<td>SD</td>
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<td>2.40</td>
<td>3.90</td>
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<tr>
<td>CV, %</td>
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<td>8.00</td>
<td>2.50</td>
<td>2.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>acu (n = 4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>9.20</td>
<td>26.80</td>
<td>53.90</td>
<td>105.30</td>
<td>158.20</td>
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<tr>
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<td>1.40</td>
<td>6.40</td>
<td>12.10</td>
<td></td>
</tr>
<tr>
<td>CV, %</td>
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<td>3.40</td>
<td>2.50</td>
<td>6.10</td>
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Table 3. EMT Calibrators Assayed by TDX (n = 4)

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<th>Target concn, mg/L</th>
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<th>25</th>
<th>50</th>
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<td>SD</td>
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<tr>
<td>CV, %</td>
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<td>2.70</td>
<td>2.50</td>
<td>1.90</td>
<td>2.70</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Plot of FPIA standard curve stability

The six points represent ethosuximide concentrations of 0, 10, 25, 50, 100, and 150 mg/L.
linear regression analysis was performed and the results are described in the legend of Figure 2. Since both variables are subject to error, this approach could cause underestimation of the "true" slope (9). However, the large $r^2$ observed in our comparison would minimize this effect.

The results of the pairwise comparisons between the TDx and the aca or EMIT methods indicate a statistically significant difference between the median concentration determined by TDx and the other two methods. The median (and range) TDx value for the 100 ethosuximide samples was 49.57 (8.1-192.22) mg/L, which was significantly lower than the values for the aca (52.05, 3.90-183.60 mg/L), and the EMIT (52.25, 11.00-198.00 mg/L) techniques ($p < 0.05$). The median values assayed by the EMIT and aca methods were not statistically different from each other.

The lower values for the TDx assay may be attributed to differences in antibody specificity. In humans, as much as 20% of the drug is excreted unchanged in the urine, the remainder (80%) presumably being metabolized (9-12). The major metabolites are the hydroxethyl derivative, the keto derivative, and succinamic acid (13). Because the aca ethosuximide assay is an adaption of the EMIT ethosuximide assay, it is not surprising that they yielded similar results. On the other hand, if the TDx assay were more specific (i.e., less subject to metabolite interference), it would yield lower concentrations. In addition to the differences in antibody specificity, differences in EMIT and TDx calibrators could also account for the observed differences in concentrations. Although the TDx method was determined to give results statistically different from those of the other two methods, we doubt, given the magnitude of the difference and the therapeutic range of 40-100 mg/L, that this difference would be clinically significant.

Our results indicate that the TDx FPIA method for ethosuximide in serum is as accurate and precise as the other methods currently available, EMIT and aca. The standard curve for the TDx assay remained stable for the 47 days of the study period. Finally, for the range of concentrations measured in this study, any of the immunoassays studied would be clinically acceptable methods for therapeutic drug monitoring of ethosuximide therapy.

References