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Screening for Benzodiazepines in Urine after Hydrolysis of Glucuronide Conjugates

To the Editor:

The EMIT Urine Benzodiazepine Assay (Syva Diagnostics, Palo Alto, CA) is marketed for the detection of benzodiazepine misuse. The recommended limit of reliable detection with 95% confidence has been variously stated as 0.3 or 0.5 mg/L with oxazepam as a reference.

When we obtained several falsely negative results for random urine samples from patients in this hospital who had received 30-mg doses of oxazepam the previous night, we conjectured that most of the drug was excreted as conjugated metabolites, which were not being detected by the test system. In investigating this possibility, we found that the sensitivity of the method could be markedly increased by incubating urine samples with β-glucuronidase (β-D-glucuronide glucuronohydrolase; EC 3.2.1.31) from Helix pomatia ("crude solution"; Sigma Chemical Co., St. Louis, MO).

Urine samples collected over 36 h after a patient had completed a two-day course of oxazepam (30 mg, twice daily) were treated by adding 0.05 mL of crude enzyme (as supplied) to 3.0 mL of urine and incubating at ambient temperature for 2 h. Absorbance was read at 340 nm according to the recommended procedure, after a delay time of 30 s and again 60 s later. The changes in absorbance were as follows:

<table>
<thead>
<tr>
<th>Urine sample</th>
<th>Before hydrolysis</th>
<th>After hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-dose</td>
<td>681</td>
<td>675</td>
</tr>
<tr>
<td>Post-dose</td>
<td>817</td>
<td>1557</td>
</tr>
<tr>
<td>12 h</td>
<td>882</td>
<td>1321</td>
</tr>
<tr>
<td>24 h</td>
<td>707</td>
<td>1252</td>
</tr>
<tr>
<td>36 h</td>
<td>867</td>
<td>1144</td>
</tr>
</tbody>
</table>

Oxazepam calibrator, 0.3 mg/L

The recommendations of the manufacturer direct that, in general, ΔA values should exceed those obtained with the calibrator before a positive result can be reported with 95% confidence. By this criterion, the present results show that patients on a "therapeutic regime" of oxazepam are likely to show negative results, unless the urinary drug conjugates are first hydrolyzed. After hydrolysis, the therapeutic use of oxazepam is detectable for at least 36 h after the last dose.

The EMIT benzodiazepine assay, as marketed, will sometimes give a positive result after a 10-mg dose of diazepam; however, after a 5-mg dose the drug is not detectable in urine (1). After the enzyme treatment step recommended here, a single 5-mg dose of diazepam was detectable in a urine sample collected 24 h after the dose. In some circumstances, a change in the post-hydrolysis ΔA value (relative to the pre-hydrolysis value) may be a more sensitive indicator of benzodiazepine use than comparison with a calibrator of unknown relevance—a possibility we are investigating further.

Reference

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A Syva representative comments:

To the Editor:

Recent studies conducted by Syva on the reactivity of the EMIT® d.a.u. Benzodiazepine Assay to oxazepam glucuronide indicate a low reactivity to this metabolite. With lots M02 and M03 of this assay, greater than 500 μg of oxazepam glucuronide per milliliter would have to be present to be detected. After oxazepam administration, the metabolite composition in urine consists almost entirely of oxazepam glucuronide (1). Nevertheless, the authors cite negative responses for only some of the urine samples from persons given 30-mg doses of oxazepam; this indicates that trace amounts of other metabolites must be contributing to the positive test responses.

With other benzodiazepines, additional metabolic pathways produce demethylated or hydroxylated urinary metabolites, or both. These metabolites are also highly conjugated, but less extensively than oxazepam. For instance, approximately 53% of a dose of diazepam was present in urine as glucuronid conjugates (2). The unconjugated metabolites have higher reactivity in the assay. Therefore, this assay may be relatively less sensitive in detecting the usage of oxazepam, but it is highly sensitive in detecting the usage of many other benzodiazepines without the necessity of hydrolysis. In all cases, the assay is intended to detect abusive use of certain drugs, not to detect very low therapeutic doses of benzodiazepines.

References

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Influence of Uremia on Four Assays for Theophylline: Improved Results with a Monoclonal Antibody in the TDx Procedure

To the Editor:

Recent reports indicate that the fluorescence polarization immunoassay for theophylline in the TDx (Abbott Laboratories, North Chicago, IL) gives higher values for serum from patients with chronic renal failure than does homogeneous enzyme immunoassay (EMIT; Syva Co., Palo Alto, CA) (1, 2) or "high-performance" liquid chromatography (HPLC) (1–3). Here we report confirmation of these observations.