3-GP oxidase (EC 1.1.3.21; Boehringer Mannheim) per milliliter of reaction mixture. Within 20 min, [U-14C]3-GP is quantitatively converted to [U-14C]DHAP, which remains stable for at least 1 h.

During this preincubation, which routinely lasts 20 min, 175 μL of the reaction mixture is pipetted into the incubation vials. Incubation is started by adding 25 μL of fibroblast suspension (2 mg of protein per milliliter, suspended in, per liter, 50 mM of NaCl, 5 mmol of NEM, 1 g of taurocholate, and 5 mmol of MOPS, pH 7.5) and transferring the stopped vials to a shaking water bath at 37 °C. After 20 min the reaction is stopped by adding 750 μL of chloroform/methanol (1/2, by vol) and the samples are processed as described by Schutgens et al. (3).

3-GP oxidase catalyzes the irreversible oxidation of 3-GP to DHAP. No cofactors are needed other than molecular oxygen, which is reduced to H2O2. H2O2 is converted to H2O by catalase.

The oxidation of 3-GP is rapid and quantitative, even at low concentrations. Moreover, DHAP can be generated in the reaction mixture used for the subsequent assay of DHAPAT. The medium we use includes NEM, which inhibits microsomal DHAPAT and makes the assay specific for the peroxisomal activity (2), and taurocholate, which causes complete disruption of the fibroblasts. Neither reagent affects the generation of DHAP. Catalse and 3-GP oxidase do not interfere with the DHAPAT assay and need not be inactivated after the preincubation.

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Metabolites of Chlorpromazine and Brompheniramine May Cause False-Positive Urine Amphetamine Results with Monoclonal EMIT® d.a.u. Immunoassay

To the Editor:

The new monoclonal EMIT® d.a.u.™ Amphetamine/Methamphetamine Immunoassay (Syva Co., Palo Alto, CA) specific for the detection of amphetamine was recently introduced (1). Interference from phenylisopropylamine derivatives (e.g., phenylpropanolamine, ephedrine, pseudoephedrine, and phenylephrine) with the polyclonal EMIT d.a.u. and with other immunoassays used for drug screening is well known (1). The monoclonal immunoassay, however, is reportedly highly stereospecific for amphetamines and does not cross-react with phenylpropanolamine derivatives (1).

We (2) have used the polyclonal EMIT d.a.u. immunoassay for drug screening in blood and urine for several years. We reanalyze urine samples positive for amphetamine, using the EMIT immunoassay confirmatory test (EMIT d.a.u. Amphetamine Confirmation Kit; Syva) and adding sodium hydroxide and periodic acid to the urine sample to exclude phenylpropanolamine and other common derivatives. We further confirm positive amphetamine results from urine and blood with specific chromatographic methods (3).

We recently evaluated the monoclonal EMIT d.a.u. immunoassay. We demonstrated interference with metabolites from chlorpromazine and possible interference from metabolites of the antihistamine brompheniramine. Product information from Syva indicated that therapeutic doses of chlorpromazine might produce positive results. Possible cross-reactivity caused by chlorpromazine metabolites was mentioned (1) but was not verified by specific chromatographic methods or substantiated with information about drug use. We therefore report two cases of false-positive amphetamine results obtained after screening with the monoclonal EMIT d.a.u. assay.

Case 1: Three urine samples collected at different times from a patient being treated in a psychiatric department were screened for drugs in a local hospital laboratory. All samples were positive for amphetamine, but the patient denied taking this drug. Because the doctor relied on the laboratory results, he lost confidence in his patient, who was then discharged from treatment. The monoclonal EMIT d.a.u. assay was used for screening without confirmatory analyses, and the staff had no information on possible interference with other drugs or metabolites. The samples were sent to our institute for confirmatory analyses and were screened with monoclonal and polyclonal EMIT d.a.u. assays, followed by gas-chromatographic (GC) and thin-layer-chromatographic (TLC) analyses (3). Alkalized urine extracts were also analyzed for antidepressive and neuroleptic drugs (GC; SP-2250 column). Amphetamine was not detected by the polyclonal immunoassay, GC, or TLC but was detected by the monoclonal immunoassay (Table 1). Metabolites of chlorpromazine were detected by GC and TLC. Only minor amounts of chlorpromazine were detected. The GC pattern was similar to patterns for samples from other patients taking chlorpromazine. The doctor said that the patient had been taking chlorpromazine medication for a long time.

To test for interference, we added chlorpromazine to drug-free urine samples to give concentrations of 100, 50, and 15 mg/L. All samples were negative by the monoclonal immunoassay, indicating that metabolites might be causing interference.

Further evaluation of the monoclonal EMIT assay showed an additional 10 false-positive results for amphetamines in urine, all from different patients taking chlorpromazine. Amphetamine was not detected in any of these samples by the polyclonal EMIT d.a.u. or by chromatographic methods.

Case 2: A urine sample from an employee with former drug problems was positive for amphetamine when screened in a local hospital laboratory by the monoclonal EMIT d.a.u. immunoassay. The employee denied use of amphetamine, but was temporarily dismissed from his job. During the sampling period, he had been treated with a β-receptor blocker and with a drug containing phenylpropanolamine and brompheniramine. The sam-

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Table 1. Urine Amphetamine Results by EMIT d.a.u. Monoclonal and Polyclonal Immunoassays and by Chromatographic Confirmatory Methods

<table>
<thead>
<tr>
<th>Drug use</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 2 after 4 wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>EEMIT monoclonal</td>
<td>Chlorpromazine</td>
<td>β-Receptor blocker, phenylprop-</td>
<td>β-Receptor blocker</td>
</tr>
<tr>
<td>EEMIT polyclonal</td>
<td>Positive</td>
<td>anolamine, and brompheniramine</td>
<td></td>
</tr>
<tr>
<td>Periodic acid confirmation</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>GC and TLC confirmation</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Possible interfering compounds</td>
<td>Metabolites of chlorpromazine</td>
<td>Negative</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Metabolites of brompheniramine</td>
<td>Negative</td>
<td>—</td>
</tr>
</tbody>
</table>

A sample was sent to our laboratory for confirmatory analyses and was screened with monoclonal and polyclonal EMIT immunoassays, followed by GC and TLC. The sample tested positive in the polyclonal immunoassay (because of phenylpropanolamine) but negative after the periodic acid confirmatory test. Amphetamine was not detected by GC or TLC, but possible brompheniramine metabolites were detected by GC. The concentration of phenylpropanolamine in urine was determined by GC (3) to be 34 mg/L.

The employee was retested after four weeks when he was using only the β-receptor blocker. This time both monoclonal and polyclonal immunoassay results were negative. Drug-free urine samples with brompheniramine added to give concentrations of 100, 50, and 15 mg/L were negative when screened by the monoclonal assay. Reference samples of brompheniramine metabolites were not available.

The monoclonal assay package insert states that a phenylpropanolamine concentration of 75 mg/L gives a negative response. Concentrations ≤150 mg/L also give a negative response (1) as do urine samples containing 35 and 75 mg of phenylpropanolamine per liter, tested in our laboratory. The concentration of phenylpropanolamine in our patient’s sample was considerably lower and should not have caused a positive response. No cross-reactivity was observed when only the β-receptor blocker was being used. Although we did not test brompheniramine alone (no drug available in Norway contains only brompheniramine), our results suggest that metabolites of brompheniramine may interfere with the monoclonal immunoassay.

We conclude that the EMIT d.a.u. monoclonal immunoassay of amphetamine may cross-react with drug metabolites. The manufacturer has tested an extensive list of compounds at various concentrations for cross-reactivity but has not tested drug metabolites, which may also cause interference. Interference with ranitidine was recently shown, but no indication of cross-reactivity with ranitidine metabolites was given (4). It is important to be aware of possible interferences with drug metabolites when introducing a new immunoassay for drug abuse testing. False-positive results from unspecific immunoassay screenings lead to unnecessary, time-consuming, expensive confirmation analyses and can cause serious problems for the person tested. Because of a lack of reference substances, the problem is difficult to evaluate. Recently, Kelly (Syva Co.) strongly recommended (5) that all positive EMIT results be confirmed by specific alternative methods. Unfortunately, many laboratories report positive results from immunoassays without using specific confirmatory tests, and information about therapeutic drug use and interpretation of positive results is not always available.

References

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