For amphetamines, positive results could be achieved only with the Abbott ADx assay at a concentration of 1 g/L perazine but with a >2.5-fold lower signal than found in urine of the patient. We assume that the majority of the observed cross-reactivity might result from the urinary excreted perazine metabolites, being much more cross-reactive than that of perazine. Two facts support this assumption: (a) perazine was almost exclusively present in its metabolized form and (b) a complete absorption of 1 g of perazine by the patient would result in urinary concentrations of perazine and its metabolites much below 1 g/L. The Syva Emit-st assay showed no cross-reactivity with perazine or its metabolites.

For opiates, similar high signals were found in urine samples of the patient and a 1 g/L perazine solution by the Abbott ADx. The above-mentioned assumption of an excretion of perazine metabolites at urinary concentrations <1 g/L points to a cross-reactivity of this FPIA with perazine metabolites. In contrast, the Emit-st assay exhibited no cross-reactivity for either perazine as pure compound or for perazine metabolites.

Our findings demonstrate that the lack of cross-reactivity of pure compounds at high concentrations (in this case 100 000 μg/L) tested with immunoassays cannot exclude false-positive results due to their metabolites. Adults receive a daily therapeutic dosage of perazine up to 150 mg per day. Because the ingestion of 1 g of perazine led to a >10-fold higher signal with the ADx amphetamine immunoassay as the cutoff value, a positive result due to a therapeutic application of perazine seems to be possible. This might lead to false-positive results with drugs-of-abuse screening and misinterpretation of preliminary positive results. Further studies are needed to test if such cross-reactivities occur with other neuroleptic drugs.

**References**


Michael Schmolke*
Jürgen Hallbach
Walter G. Guder
Städtisches Krankenhaus Bogenhausen
Englschalkingerstr. 77
81925 München, Germany

* Author for correspondence.

**False-Negative Results with Emit® Amphetamine Confirmation Kit**

To the Editor:

Testing urine specimens for drugs of abuse usually involves an immunoassay as a screening procedure. False-positive results due to sympathomimetic amines occur with some of the amphetamine/metamphetamine kits, most frequently with Emit d.a.u.*, but also with Emit II® immunoassays from Syva Co. (San Jose, CA) [1]. Rescreening of amphetamine/metamphetamine-positive samples after periodate oxidation (Syva confirmation kit) has been designed to eliminate this problem. The confirmation kit may, however, be of limited value. This letter presents 10 cases that were positive for amphetamine by gas chromatography-mass spectrometry (GC-MS), although they were negative according to the Syva confirmation analysis.

Our laboratory performs forensic and drugs-of-abuse analyses in biological samples. Annually, ~20 000 urine specimens are received from prison inmates and analyzed for drugs of abuse. Amphetamine/metamphetamine screening was performed with polyclonal Emit d.a.u. amphetamine/metamphetamine with a 300 μg/L cutoff. The analyses were run on a Monarch 2000 (Instrumentation Lab., Lexington, MA) at 30 °C. Method validation experiments have been carried out to evaluate the normal variability in the assay. Using a calibrator at the cutoff value, accuracy was 97%, and precision in terms of CV was 7.3%. Until recently, all positive results were subjected to the Syva confirmation reaction. Briefly, NaOH and NaIO₄ were added to the specimens, which were then reanalyzed for amphetamines. All procedures were carried out according to Syva’s instructions. Different batches of confirmation kits were used. Positive screening results were analyzed for amphetamine/metamphetamine by GC-MS as described previously [2], with minor modifications. The cutoff value for GC-MS was 135 μg/L (1 μmol/L).

In one case, we received two urine specimens taken 20 min apart from the same prisoner. Both samples were screened for drugs of abuse, and they screened positive for amphetamines. The first sample remained positive after the confirmation reaction, while the second became negative. Both samples were analyzed by GC-MS, and amphetamine was detected in both specimens, in concentrations of 2300 μg/L and 640 μg/L, respectively. The notable difference in the urine amphetamine concentrations between the two samples could at least partly be related to a dilution effect, as the two specimens had somewhat different concentrations of creatinine (22.4 and 14.5 mmol/L), and a pH-effect, as the pH was higher in the second specimen (6.0 and 7.0), leading to reduced urinary excretion of amphetamines.

During the following 3 months, all specimens that screened positive for amphetamines were subjected to GC-MS analysis as well as to the confirmation reaction. Of ~5000 specimens screened, 67 were positive for amphetamine/metamphetamine. After the positive samples were assayed by the confirmation kit, 50 remained positive. When analyzed on GC-MS, amphetamine was detected above the cutoff value in 48 of the 50 specimens confirmed positive, but also in 9 of the 17 negative specimens (Table 1). The concentrations of amphetamine were between 230 and 490 μg/L in these 9 specimens, and >300 μg/L (the corresponding Emit cutoff) in 4 specimens. Altogether, we found false-negative results in 13% (9 of 67) of the confirmation assays.

Our institute is in charge of a national control program for laboratories performing urine analyses for drugs of abuse. Supplemented urine specimens are sent to ~40 other laboratories that are using different types of immunological screening techniques. We added d-1-amphetamine to two different specimens at concentrations of 400 and 440 μg/L,
respectively, on separate occasions during 1995. Six of the laboratories using Emit d.a.u. and Emit confirmation kit found that the first specimen tested amphetamine-positive, but only one laboratory reported a positive result after the confirmation test. Five laboratories obtained positive primary results in the second specimen, but the sample remained positive in only two laboratories when the confirmation assay was used.

We have shown that use of the Syva amphetamine/metamphetamine confirmation assay is associated with false-negative results. The result could not be explained by normal variability in the Emit-assay. Consequently, we no longer use the confirmation assay in our laboratory, and we now analyze all Emit-positive urines by GC-MS. We have done this without increasing the number of chromatographic analyses. In Norway, decongestive sympathomimetic amines are prescription-only drugs, so interferences from these substances are relatively rare. Therefore, omitting the confirmation assay under these circumstances appears cost efficient. False-negative results with the confirmation assay were most often encountered in urine specimens with low concentrations of amphetamine. In countries where sympathomimetic amines are extensively used (e.g., countries where these drugs are sold over the counter), the use of a confirmation assay may be indicated. In some instances (e.g., after khat intake), the detection of sympathomimetic amines is wanted, justifying the use of the Emit d.a.u. immunoassay. Therefore, many aspects must be considered with respect to the immunological screening method, and whether or not a confirmation kit should be used.

<table>
<thead>
<tr>
<th>Amphetamine/metamphetamine confirmation reaction</th>
<th>Amphetamine GC-MS analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed positive by kit (n = 50)</td>
<td>Detected (range 290–52 000 μg/L)</td>
</tr>
<tr>
<td>Negative according to kit (n = 17)</td>
<td>Not detected (range 230–490 μg/L)</td>
</tr>
</tbody>
</table>

References

Henning Mørland*
Anne Smith-Kielland
Natl. Inst. of Forensic Toxicol.
P.O. Box 9934 Ila
N-0132 Oslo, Norway

* Author for correspondence.