Comparison of the Sensitivity and Specificity of Six Immunoassays for the Detection of Amphetamines in Urine

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Abstract
We analyzed 225 urine samples with FPIA (Abbott Amphetamine/Methamphetamine II on ADx and AxSYM), EMIT (Emit II Plus Monoclonal Amphetamine/Metamphetamine assay and EMIT II Plus Amphetamines assay, EMIT N), and KIMS (standard protocol and MDMA protocol, KIMS and KIMS X, respectively) immunoassays and compared their sensitivity and specificity. All assays were calibrated and used semi-quantitatively. All samples that screened positive by any amphetamine screening method and 15% of the negative samples were confirmed by liquid chromatography-tandem mass spectrometry (LC-MS-MS). A sample was considered positive for amphetamines if amphetamine, methamphetamine, methylenedioxymethylamphetamine, methylenedioxyethylamphetamine, or methylenedioxyamphetamine was present at 250 ng/mL. Ninety (40%) of the samples were positive by LC-MS-MS. The areas under the receiver operating characteristic curve varied between 0.972 (KIMS X) and 1.000 (ADx). The optimal cut-off concentrations varied between 271 ng/mL (EMIT N) and 723 ng/mL (AxSYM). The sensitivity was 100% for ADx and between 93 and 95% for the other assays. The specificity varied between 88% (KIMS) and 100% (EMIT N). Use of a 500 ng/mL screening cut-off would have resulted in identical or very similar results for ADx and KIMS X and large increases in the false positives for AxSYM and EMIT and the false negatives for EMIT N and KIMS.

Introduction
According to the United Nations Office on Drugs and Crime, amphetamine-type substances (ATS) are the second-most abused drugs, after cannabis. In the period 2001–2003, about 30 million people used amphetamines, primarily methamphetamine and amphetamine, and 8 million used ecstasy. Methamphetamine and amphetamine together account for some 80%, and ecstasy for some 20% of global ATS production. At least two thirds of the ‘amphetamines’ manufacture is accounted for by methamphetamine. Methamphetamine use is encountered most often in the North America, Australia, and Asia, whereas amphetamine use is encountered in Europe. Ecstasy use is prevalent in Europe, and its use is increasing in North America and Asia. During the last decade, the highest increases in illicit drug use, after cannabis, were for the ATS (mainly ecstasy).

While in the late 1980s and early 1990s laboratories producing methylenedioxyamphetamine (MDA), and to a lesser extent, methylenedioxymethylamphetamine (MDMA) and other ecstasy-type substances, still played a role, almost all clandestine laboratories seized in 2000 and subsequent years produced methylenedioxymethylamphetamine (MDMA, ecstasy). Over the 2000–2002 period, 56% of all ecstasy laboratories dismantled were in Europe (of which 98% were in Western Europe), 27% were in North America, 13% were in Asia (mainly East and Southeast Asia), and 4% were in other parts of the world (1).

In April 2004, the Substance Abuse and Mental Health Services Administration (SAMHSA) published its proposed revisions to mandatory guidelines for federal workplace drug-testing programs (2). The initial test cut-off concentration for amphetamines (methamphetamine is the target analyte) and MDMA is 500 ng/mL. The confirmatory test cut-off concentration for amphetamine, methamphetamine, MDMA, MDA, and MDEA is 250 ng/mL. In the case of methamphetamine, the specimen must also contain amphetamine at a concentration greater than or equal to 100 ng/mL.

In drug-of-abuse screening, the ideal amphetamine immunoassay should detect amphetamine, methamphetamine, and the different illicit amphetamine analogues (e.g., MDMA, MDEA, MDA) without false-positive results from anorectics, other stimulants or other drugs. For clinical toxicology, however, it has been recommended that tests directed toward a broad spectrum of sympathomimetic amines as a class be used (3).

In point-of-care screening, most panel tests will now include an amphetamine assay combined with a methamphetamine assay to detect methamphetamine and MDMA (4), in laboratory screening, combined amphetamine/methamphetamine assays that should detect the different amphetamines are more often used.

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There have been several evaluations of single kits (5–11), studies with spiked samples (12–14), and studies after drug administration to volunteers (15), but relatively few comparisons of the different amphetamine immunoassays have been performed (16–22), often on a limited number of samples. A review on the cross-reactivity of amphetamine assays has been published (23).

Many interferences have been described with the (meth)amphetamine screening immunoassays: benzathine (24,25), cyclamate (26), isomethypentene (27), labetalol (28), mebeverine (29), mexiletine (30), and ranitidine (31,32). Several studies have examined the effect of use of Vicks nasal inhaler, which contains l-methamphetamine (9,33,34). In addition, many medicinal drugs are precursors of methamphetamine and amphetamine, which can make the result interpretation difficult (35,36).

We compared the sensitivity and specificity of 6 commercial urine amphetamine immunoassays for the analysis of the urine samples that were sent to our laboratory during a 2.5-month period.

Methods

Samples

We tested 225 consecutive urine samples that were sent to our laboratory for screening or confirmation of amphetamines. One hundred and fifty samples (67%) were referred by other laboratories for screening and/or confirmation of positive immunoassay results, and 75 (33%) came from our hospital. Samples obtained from other laboratories were sent by mail or by a courier of the lab, usually within 24 h. Samples were kept refrigerated between the analyses. No preservative was used. All analyses were performed within a 4-month period.

Table I. Target Molecule of the Calibrators, Concentrations, and Recommended Cut-Off Concentrations

<table>
<thead>
<tr>
<th>Analyte in Calibrator</th>
<th>Concentrations (ng/mL)</th>
<th>Recommended Cut-off (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADx d-Amphetamine</td>
<td>0, 150, 300, 1000, 3000, 8000</td>
<td>300</td>
</tr>
<tr>
<td>AxSYM d-Amphetamine</td>
<td>0, 150, 300, 1000, 3000, 8000</td>
<td>1000</td>
</tr>
<tr>
<td>EMIT d-Methamphetamine</td>
<td>0, 500, 1000, 2000</td>
<td>1000</td>
</tr>
<tr>
<td>EMIT N d-Methamphetamine</td>
<td>0, 300, 500, 1000</td>
<td>300, 500 or 1000</td>
</tr>
<tr>
<td>KIMS d-Amphetamine</td>
<td>0, 500, 1000, 2000</td>
<td>1000</td>
</tr>
<tr>
<td>KIMS X d-Amphetamine</td>
<td>0, 500, 1000, 2000</td>
<td>500</td>
</tr>
</tbody>
</table>

Table II. Distribution of the Urine Concentrations (ng/mL) of the Different Amphetamines in the Samples

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Number Positive</th>
<th>Number ≥ 250 ng/mL</th>
<th>Lowest Concentration</th>
<th>Median Concentration</th>
<th>Highest Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>75</td>
<td>73</td>
<td>71</td>
<td>2560</td>
<td>155,000</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>30</td>
<td>1</td>
<td>15</td>
<td>33</td>
<td>305</td>
</tr>
<tr>
<td>MDMA</td>
<td>30</td>
<td>26</td>
<td>46</td>
<td>5975</td>
<td>108,000</td>
</tr>
<tr>
<td>MDA</td>
<td>31</td>
<td>21</td>
<td>15</td>
<td>516</td>
<td>12,400</td>
</tr>
<tr>
<td>MDEA</td>
<td>7</td>
<td>4</td>
<td>27</td>
<td>1530</td>
<td>24,800</td>
</tr>
</tbody>
</table>
MBDB, 4-MTA, and PMA. The precision of the assay is < 4% within-run and < 6% between-day.

A sample was considered positive for amphetamines if amphetamine, methamphetamine, MDMA, MDEA, MDA, MBDB, 4-MTA, and/or PMA were present at ≥ 250 ng/mL.

Statistical analysis
The results were analyzed using Microsoft Excel (Microsoft Corporation, Redmond, WA). MedCalc (Mariakerke, Belgium) software was used for statistical analysis and in particular to draw receiver operating characteristic (ROC) curves and calculate cut-offs.

Results
No particular problems were encountered with the screening assays, except that 16 samples (7.1%) gave an "exception" error on AxSYM ("intensity too high"). They were diluted 1:3 and rerun; the obtained result was multiplied by 3 and used for the further evaluation. This percentage is probably an overestimation, because some samples were referred to us for this reason. In the samples from our hospital, the percentage was 5.3%.

Ninety (40%) of the samples were positive by LC–MS–MS at the 250 ng/mL cut-off. The numbers of positive samples, lowest, median, and highest concentration (in ng/mL) are given in Table II. MBDB, 4-MTA, and PMA were not found in any sample. Figure 1 gives an overview of the distribution of the concentrations of the different amphetamines found in the samples.

Sixty samples contained only amphetamine, 15 samples contained MDMA and/or MDA and MDEA, and 15 samples contained both amphetamine and MDMA. Methamphetamine was found only in very low concentrations in samples that contained high amphetamine or MDMA concentrations.

The performance characteristics of the different assays (sensitivity, specificity, area under the ROC curve) are given in Table III. The areas under the ROC curve varied between 0.972 (KIMS X) and 1.000 (ADx). The optimal cut-off concentrations, as calculated by the MedCalc software, varied between 271 ng/mL (EMIT N) and 723 ng/mL (AxSYM). The sensitivity was between 93 and 95% for all assays, except ADx (100%). The specificity varied between 88% (KIMS) and 100% (EMIT N). The number of false-negative samples varied between 0 (ADx) and 6 (EMIT N), and the number of false-positive samples varied between 0 (EMIT N) and 16 (KIMS).

Use of a 500 ng/mL screening cut-off would have resulted in identical or very similar results for ADx and KIMS X. For AxSYM and EMIT it would slightly reduce the number of false negatives, but dramatically increase the number of false positives (from 1 to 14 for AxSYM and from 9 to 20 for EMIT). For EMIT N, the use of a 500 ng/mL cut-off would triple the number of false negatives without increasing the number of false positives. For KIMS, use of a 500 ng/mL cut-off would double the number of false negatives while slightly reducing the number of false positives.

Discussion
Forty percent of our samples were positive for amphetamines, which can be explained by the fact that the majority of the samples were referred to us by other laboratories for confirmation of positive results.

We did not attempt to look for the substances that could cause the false-positive results as
Table IV. Results of Published Evaluations of Amphetamine Assays*

<table>
<thead>
<tr>
<th>Assay</th>
<th>N</th>
<th>% Confirmed</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDX</td>
<td>70</td>
<td>82.9</td>
<td>96.6</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>AxSYM</td>
<td>146</td>
<td>26.0</td>
<td>100</td>
<td>100</td>
<td>18</td>
</tr>
<tr>
<td>CEDIA</td>
<td>146</td>
<td>26.0</td>
<td>100</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>CEDIA Amphetamines/ecstasy</td>
<td>256</td>
<td>64.5</td>
<td>99.4</td>
<td>97.8</td>
<td>11</td>
</tr>
<tr>
<td>DRI AMP</td>
<td>27,435</td>
<td>0.08</td>
<td>78.5</td>
<td>96.1</td>
<td>21</td>
</tr>
<tr>
<td>DRI XTC</td>
<td>27,435</td>
<td>0.08</td>
<td>75.9</td>
<td>100</td>
<td>21</td>
</tr>
<tr>
<td>DRI amphetamine</td>
<td>146</td>
<td>26.0</td>
<td>100</td>
<td>100</td>
<td>18</td>
</tr>
<tr>
<td>EMIT</td>
<td>2964</td>
<td>3.7</td>
<td>100</td>
<td>96.1</td>
<td>16</td>
</tr>
<tr>
<td>EMIT dau monoclonal</td>
<td>533</td>
<td>8.4</td>
<td>48.9</td>
<td>95.3</td>
<td>38</td>
</tr>
<tr>
<td>EMIT dau monoclonal</td>
<td>503</td>
<td>25.6</td>
<td>100</td>
<td>99.2</td>
<td>9</td>
</tr>
<tr>
<td>EMIT II</td>
<td>70</td>
<td>82.9</td>
<td>84.2</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>EMIT II</td>
<td>1007</td>
<td>0.2</td>
<td>100</td>
<td>98.1</td>
<td>7</td>
</tr>
<tr>
<td>EMIT II plus monoclonal</td>
<td>146</td>
<td>26.0</td>
<td>81.6</td>
<td>100</td>
<td>18</td>
</tr>
<tr>
<td>Roche Online</td>
<td>2964</td>
<td>3.7</td>
<td>100</td>
<td>99.1</td>
<td>16</td>
</tr>
<tr>
<td>Abuscreen Online</td>
<td>27,460</td>
<td>0.08</td>
<td>81.5</td>
<td>99.1</td>
<td>21</td>
</tr>
<tr>
<td>Roche Online</td>
<td>146</td>
<td>26.0</td>
<td>94.7</td>
<td>100</td>
<td>18</td>
</tr>
<tr>
<td>Roche Hitachi AMSPS</td>
<td>70</td>
<td>82.9</td>
<td>94.3</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>Roche HS AMP/MDMA Hitachi</td>
<td>70</td>
<td>82.9</td>
<td>100</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>Roche Integra AMSPS</td>
<td>70</td>
<td>82.9</td>
<td>84.4</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>Roche Integra AMPSX cut-off 1000</td>
<td>70</td>
<td>82.9</td>
<td>86.2</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>Roche Integra AMPSX cut-off 1000</td>
<td>70</td>
<td>82.9</td>
<td>94.8</td>
<td></td>
<td>22</td>
</tr>
</tbody>
</table>

* In most cases, the prevalence, sensitivity, and specificity were not given in the original publications and had to be calculated based on the data mentioned in the text.
GC–MS, which corresponds to a positive predictive value of 11.3%.

Finally, the CEDIA assay was not evaluated in this study. Hsu et al. (17) concluded that CEDIA Amp/MDMA appeared to be the most effective screening assay for both MDMA and amphetamine/methamphetamine.

A potential weakness of our study was the fact that the analysis was not performed on fresh samples, but on samples that were stored refrigerated and not frozen, and that some degradation products could have been formed and cause false positives in the immunoassays. However, the package insert of the EMIT and KIMS test documents a very low cross-reactivity for tyramine and phenethylamine and more recent experiments (data not shown) with fresh samples yielded similar results for KIMS.

The optimal cut-offs, calculated by analysis of the receiver operating characteristic curves, varied between 271 and 723 ng/mL. We have shown that better results (fewer false positives or false negatives) can be obtained if the cut-off is optimized and not set at 500 ng/mL. We recommend that, in situations where the laboratory can choose its screening cut-off, it chooses the most appropriate cut-off level, based on its own validation or literature data.

Conclusions

We compared six screening immunoassays for the detection of amphetamine or MDMA in 225 patient urine samples. The best results were seen for the FPIA assay on the ADx analyzer. FPIA on AxSYM gave a few more false positives and negatives at a cut-off of 723 ng/mL. The EMIT II amphetamines assay gave no false positives, but 6 false negatives at a cut-off of 271 ng/mL. The other assays (EMIT, KIMS, KIMS X) showed more overlap between the negative and positive samples with 5 false negatives and 9–16 false positives. At a 500 ng/mL cut-off, ADx also gave the best results, followed by AxSYM (many FP), EMIT N (many FN), both KIMS methods (FN and FP), and EMIT (many FP). In situations where the cut-off is not mandated, we recommend use of the optimal cut-off based on literature or in-house data.

Acknowledgments

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References

20. P.R. Stout, K.L. Klette, and C.K. Horn. Evaluation of ephedrine,
pseudoephedrine and phenylpropanolamine concentrations in human urine samples and a comparison of the specificity of DRI Amphetamines and Abuscreen® online (KIMS) amphetamines screening immunoassays. _J. Forensic Sci._ **49**:160–164 (2004).


