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Short Communication

Diagnostic efficiency of different amphetamine screening tests – the search for an optimal cutoff

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Abstract

Increased use of designer drugs (amphetamines and amphetamine-like substances) raises the need for fast screening tests in urine in clinical settings, workplace and drug rehabilitation. Immunological assays currently used are subject to unwanted crossreactivities, partly depending on the cutoff concentrations used. The values recommended in Europe and the USA are 500 and 1000 ng/ml, respectively. In Switzerland, the recommended concentration of 300 ng/ml results in a high rate of false-positive urine samples and expensive, time-consuming confirmation testing. Using the Abbott Axsym analyzer, we found numerous false positives from patients in rehabilitation centers due to concomitant medication. Therefore, the diagnostic sensitivity and specificity of the Abbott test at different cutoff concentrations and the sensitivity of the Roche Cobas Integra, Beckman Synchron and Biosite Triage point-of-care test were examined. HPLC Bio-Rad Remedi was chosen as the method of higher hierarchical order. The specificity of the Axsym analyzer (300 ng/ml) was 86%. At 500 ng/ml or 1000 ng/ml the specificity was increased to 99 or 100%, respectively, while the sensitivity only decreased from 97 to 91 or 81%, respectively. In summary, the cutoff concentration for amphetamine screening tests should not be below 500 ng/ml to avoid a high rate of false-positive results.

Keywords: amphetamine; Axsym; cutoff; HPLC; screening test.

The abuse of amphetamine, methamphetamine and other amphetamine-like substances (so-called designer drugs) has been increasing in Europe due to increased sales and demand at popular rave parties. Therefore, the rapid and inexpensive detection of amphetamines and amphetamine-like substances in urine samples is important for physicians in emergency rooms as well as for workplace testing and in addiction rehabilitation clinics. In a clinical routine laboratory, immunological techniques are the only methods that can be used for the rapid detection of amphetamine in urine samples. The cutoff values of initial screening tests are currently under re-evaluation in the United States. A lower cutoff concentration of 500 ng/ml for screening of amphetamines in urine has been proposed by the Substance Abuse and Mental Health Services Administration (SAMHSA) (1), while the cutoff value of 1000 ng/ml is currently used for workplace drug testing (2). A cutoff concentration of 500 ng/ml is recommended by the European Workplace Drug Testing Society (EWDTS) (3). In this regard, the Swiss working group for drugs of abuse testing (AGSA) is recommending 300 ng/ml and 1000 ng/ml for addiction rehabilitation centers and workplace testing, respectively, in its own guidelines (4).

The immunoassays used for initial drugs of abuse testing have to detect a wide variety of different molecules (e.g., the amphetamine derivatives methylenedioxymethamphetamine MDMA and methylenedioxyamphetamine MDA) and are, therefore, subject to unwanted crossreactivities (e.g., with different antidepressant and neuroleptic drugs). Patients in addiction rehabilitation centers often have prescriptions for many different psychoactive drugs. Therefore, numerous false-positive results are expected. As a consequence, the high rate of positive urine samples leads to expensive, time-consuming confirmation testing, which is not desirable and can cause unnecessary confusion for patients and physicians.

In our laboratory many of the urine samples requiring an amphetamine screening test are obtained from addiction rehabilitation centers. Interestingly, we found a surprisingly high number of positive tests for amphetamine using the Axsym analyzer (Abbott, Illinois, USA) that could not be confirmed by HPLC and that were classified as false-positive results. The cutoff value used was 300 ng/ml as recommended by the Swiss AGSA. To clarify this issue, that very little information can be found in the literature on sensitivity and specificity, especially with respect to the different cutoff values and methods (5–10), which stands in striking contrast to the frequency of amphetamine tests performed. We therefore decided to study the diagnostic sensitivity and specificity of the fluorescence polarization immunoassay (FPIA) screening test amphetamine/methamphetamine II (Axsym, Abbott) at different cutoff levels (300, 500, 1000

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Table 1 Diagnostic specificity of the Axsym FPIA test at different cutoff levels and diagnostic sensitivity of the Axsym FPIA at different cutoff levels, the Beckman Synchron, the Roche Abuscreen online and the Triage tests.

Sensitivity, %			
Cutoff level, ng/ml	300	500	1000
FPIA, Abbott Axsym	96.9	90.8	81.5
Synchron, Beckman	–	–	86.2
Abuscreen online, Roche	–	93.9	–
Triage, Biosite	–	–	69.2
Specificity, %			
Cutoff level, ng/ml	300	500	1000
FPIA, Abbott Axsym	86	99	100

Sensitivity was calculated using the following equation: Screening test positives/HPLC positives [true positives/(true positives + false negatives)]. Specificity was determined as follows: Screening test negatives/HPLC negatives [true negatives/(true negatives + false positives).

ng/ml), as well as the diagnostic sensitivity of the FPIA test in comparison with the Abuscreen online test (Cobas Integra, Roche Diagnostics, Basel, Switzerland; cutoff value 500 ng/ml), the Synchron system amphetamines test (Beckman-Coulter, Fullerton, USA; cutoff value 1000 ng/ml) and the Triage point-of-care test (Biosite, San Diego, USA; cutoff concentration 1000 ng/ml). Detection of amphetamine and amphetamine-like substances by HPLC on a Remedi HS system (BioRad, Munich, Germany) was chosen as a method of higher hierarchical order. The Remedi HS system has been demonstrated to identify amphetamines, methamphetamines and their derivatives with a high degree of reliability (11, 12), allowing its use as a confirmation method that is faster and cheaper than gas chromatography-mass spectrometry. The cutoff concentrations for amphetamine and amphetamine-like substances on the Remedi HS system are 150 ng/ml for amphetamine and methamphetamine, and 100 ng/ml for MDA and MDMA, respectively. Ten drug-free samples from healthy volunteers, 65 samples positive for amphetamine as determined by HPLC from confirmed users of amphetamine and amphetamine-like drugs, and from clinical routine, as well as 365 HPLC-negative urine samples from routine testing were compared with all screening methods. A higher number of positive samples would have been preferable. However, the initially determined analytical performance values calculated with even fewer positive urine samples did not significantly change upon inclusion of more samples.

All drug-free samples from the healthy volunteers tested negative with all methods. All HPLC-positive urine samples were analyzed with the Axsym test using different cutoff values as well as with the Abuscreen online, Synchron and Triage point-of-care assay. Consequently, the sensitivity was calculated for all immunoassay tests (Table 1). Sensitivity was defined as screening test positives divided by all HPLC-positive samples. Three hundred and sixty five HPLC-negative urine samples were analyzed with the Abbott Axsym test using different cutoff concentrations and the diagnostic specificity was calculated (Table 1). None of the false-positive tests found with the Axsym FPIA test could be reproduced with any other immunoassay system tested. Of the urine samples tested false positive with the Axsym assay at cutoff values of 300 or 500 μg/l, the following 11 non-amphetamine-like substances were found by HPLC analysis: methadone, mianserine, bupivacaine, domperidone, venlafaxine, monoacetylmorphine (metabolite of heroin), fluoxetine, olanzapine, atenolol, paroxetine and quinine. Of these compounds only fluoxetine is known to cross-react with the Axsym FPIA test. Therefore, drugs not detected by HPLC or endogenous compounds may cause the false-positive immunochemical findings as suggested by Schutz et al. (13).

The positive urine samples, which have been confirmed by HPLC, contained amphetamine, methamphetamine and the amphetamine-like substances MDA, MDMA, phenylpropanolamine and ephedrine either alone or in different combinations.

Further, as shown in Table 1, increasing the cutoff concentration of the FPIA Axsym test from 300 to 500 ng/ml led to a substantial increase in specificity (+13%) and a smaller loss in sensitivity (−6%). Increasing the cutoff value to 1000 ng/ml only slightly increased the specificity (+1%), however, led to a substantial decrease in sensitivity (−9%).

Most of the positive results using the FPIA Axsym test at a cutoff concentration of 1000 ng/ml had multiples of cutoff of more than 2 (69%) or even more than 8 (45%). Consequently, these strongly positive results would not suggest a decrease in the cutoff value, resulting in even higher multiples of the cutoff. The multiple of cutoff values of the positive Roche Abuscreen tests are likewise high (>2 in 60%), despite the fact that the cutoff concentration of the Abuscreen test is already set at 500 ng/ml. In contrast, only 19% of the positive samples have a multiple of the cutoff >2 with the Beckman Synchron test. Unfortunately, the results of this test are expressed in optical densities and not in concentrations. Therefore, the determination of sensitivity and specificity using different cutoff values is not possible.

In conclusion, the recommended cutoff concentration for amphetamine screening tests in Switzerland (300 ng/ml) is set far too low and only leads to a high rate of false-positive results using the Axsym analyzer or presumably any other screening test set at such a low cutoff concentration. A cutoff value of 500 ng/ml as recommended by the EWDTS is a reasonable compromise between good sensitivity and an acceptable specificity. However, as currently no data are available this applies to the Axsym FPIA test only. Data for the diagnostic efficiency of the numerous other tests that are on the market should be established and taken into account before lowering the actual cutoff value of 1000 ng/ml recommended in the United States (1). Finally, we found the diagnostic sensitivity of the point-of-care device to be substantially lower than the sensitivity of automated immunological tests. This is in line with the higher threshold concentrations (factor 3–6) for different amphetamine derivatives of the

Triage test compared to the FPIA test as described by Felscher et al. (11). For this, whenever possible, automated quantitative immunoassays should be used.

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