References

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Impact of Revised NIDA Guidelines for Methamphetamine Testing in Urine

To the Editor:
In 1988, The National Institute on Drug Abuse (NIDA) established guidelines for federal workplace drug-testing programs (1). However, because of reports of false-positive results (2), NIDA altered their reporting criteria for amphetamines in terms of confirmation by gas chromatography/mass spectrometry (GC/MS). Effective December 19, 1990, a positive methamphetamine was to be reported only if, by GC/MS, the concentration of methamphetamine exceeded 500 μg/L and the concentration of the major metabolite, amphetamine, exceeded 200 μg/L. These changes were necessary because of false-positive reports for methamphetamine when carboxyhexafluorobutyryl chloride (CB) was used as the derivatizing agent. We previously determined that such false-positive results could be produced by the conversion of the CB-ephedrine derivative to the CB-methamphetamine derivative when high injection temperatures were used (3). The modification in the reporting criteria eliminated the possibility of a false-positive result due to the presence of other sympathomimetic amines because these drugs do not metabolize to amphetamine (4).

We examined 83 urine specimens that were positive for methamphetamine by either the EMIT assay (Syva Co., Palo Alto, CA) adapted to the Hitachi 717 analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN) or the Triage Panel (BioSite, San Diego, CA) (5) to determine how many samples fulfilled the revised criteria for reporting positive results. The cutoff limits for both assays were 1000 μg/L. We assayed all samples by full-scan GC/ion-trap MS with the ITS40 GC/MS (Finnigan, San Jose, CA), using a confirmation procedure involving derivatization with heptafluorobutyric anhydride (6). The limits of detection, quantification, and linearity of the assay used in our laboratory are 50, 100, and 2000 μg/L, respectively. The heptafluorobutyryl derivatives of other sympathomimetic amines do not produce false-positive methamphetamine results when full-scan mass spectral detection is used (7).

We found that, of the 83 urine specimens, 12 were positive for amphetamine alone, and 52 met the revised criteria for reporting positive methamphetamine. The remaining 19 urine specimen had methamphetamine concentrations >500 μg/L but amphetamine concentrations <200 μg/L. The results for these 19 urine specimens are listed in Table 1. All would have met the original NIDA criteria for positive methamphetamine results, but must be reported as negative under current guidelines. As with most drugs, the rate and extent of hepatic metabolism of methamphetamine depend on many factors, including the amount consumed, interindividual variations, preexisting disease, and genetic factors. Furthermore, the renal excretion of methamphetamine depends on the urine pH. Beckett and Rowland (8) reported that, in acid urine, as much as 76% of a dose of methamphetamine (plus 7% as amphetamine) is excreted in 24 h. In alkaline urine, <0.1% of a dose of methamphetamine is ordinarily excreted as amphetamine in 24 h. In our samples, we do not know why no amphetamine was detected in sample 10, given the very high methamphetamine concentration that was detected. Lebish et al. (9), examining urine of seven chronic methamphetamine abusers, found urinary excretion rates of amphetamine to methamphetamine ranging from 0.003 to 0.35. Perhaps the time of collection in relation to administration is also a factor in the metabolism and excretion of methamphetamine.

We support the intent of the revised regulations for reporting methamphetamine, which was designed to eliminate the possibility of reporting false-positive results. The National Laboratory Certification Program has put increasing emphasis on amphetamine accuracy by including other sympathomimetic amines such as ephedrine, pseudoephedrine, and phenylpropanolamine in recent proficiency survey samples. However, we encourage laboratories to conduct their own evaluations so the problem of false-positive results can be eliminated. We hope that NIDA will be able to return to the original criteria.

Table 1. Urine Samples Positive for Methamphetamine and Negative for Amphetamine

<table>
<thead>
<tr>
<th>Urine no.</th>
<th>Concentration, μg/L</th>
<th>Meth.</th>
<th>Amph.</th>
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<tr>
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<td>2169</td>
<td>164</td>
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</tr>
<tr>
<td>2</td>
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</table>
for reporting methamphetamine-positive results without the criteria for detecting its metabolite, so that all individuals with indisputable evidence of methamphetamine abuse, such as our sample 10, can be reported as being positive.

References

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Inaccuracy of Dispensing Sample and Reagent Volume by the Cobas-Bio Analyzer

To the Editor:

In their Technical Brief, Sonntag and Weidemann (1) demonstrated the influence of the diluent volume on the enzyme activity measured by a Cobas-Bio centrifugal analyzer (Hoffmann-La Roche, Basel, Switzerland). We support their findings and present additional data related to this problem.

During a comparison of enzymatic methods performed with a Cobas-Bio and those performed with other instruments (ACP 5040, SMAC, and Eppendorf enzyme automate) we found that measured activity was as much as 10% less in the experiments done with the Cobas-Bio. Several causes for the discrepancies were considered, such as photometric irregularities, temperature abnormalities, improper function of the pipetting unit, or the mathematics program available in the analyzer. After testing all the other possibilities, the cause for the discrepancies appeared to be the pipetting unit, on which we then focused our attention. We checked its accuracy by three methods: weight (mass), absorbance, and radioactivity.

In the weight experiment, the Cobas-Bio pipetted distilled water into its cuvettes (20 μL of sample and 5 μL of diluent). The theoretical weight of the water, confirmed by manual pipetting, was 900 mg (26 × 25 μL + 250 μL), as corrected for the measured water temperature. Five observations gave an average weight that was too low by 2.7%.

In the first absorbance experiment, the Cobas-Bio pipetted into its cuvettes a series of samples with increasing concentrations (20.0, 50.0, 100.0, 150.0, 200.0, and 250.0 mg/L) of potassium dichromate (National Institute of Standards and Technology, Gaithersburg, MD; Standard Reference Material 935) and measured the absorbances. We checked the absorbance with a Pye Unicam (Cambridge, UK) spectrophotometer. The pipetting schedule was as follows: sample, 25 μL of potassium dichromate; diluent, 72 μL of distilled water; reagent, 150 μL of perchloric acid, 0.001 mol/L. The absorbances measured by Cobas-Bio, and for comparable manual pipetted volumes by the Pye Unicam, showed lower values for the former instrument but were not significantly different (n = 5; P < 0.055).

In the second absorbance experiment, an aqueous solution of NADH (final concentration 23.4 μmol/L) was pipetted by the Cobas-Bio into its cuvettes and measured at 334 nm, according to the following schedule: 5, 10, and 20 μL of sample plus a fixed diluent volume (10 μL), adjusted to a total volume of 250 μL with 0.1 mol/L NaOH. The temperature in the measuring cells was kept at 25 °C. The absorbances (average of five observations) were compared with those obtained with an Eppendorf (Hamburg, Germany) enzyme automate (volumes pipetted by the machine) and a Zeiss (Jena, Germany) spectrophotometer (pipetted manually). The results by each instrument were 10.6%, 11.0%, and 7.1% too low, respectively. Cobas-Bio results for each sample volume were 10.6%, 11.0%, and 7.1% lower than the results by the Eppendorf and Zeiss. (The Eppendorf and Zeiss give equal results.)

In the radioactivity experiment, a concentrated solution of 125I was mixed with serum from an apparently healthy volunteer. First, we made the Cobas-Bio pipet different volumes of sample and diluent into its cuvettes; no reagent was dispensed. The cuvettes were separated from the cuvette ring, and the radioactivity of the solutions pipetted into the cuvettes was counted individually (α). The radioactivity of the remaining 125I solution in the sample cup was counted as well (γ). Subtraction of the sum (α + γ) from the total original radioactivity indicated the extra volume (α′) aspirated by the sample unit at a nominal sample volume (S). The percentage of extra aspirated volume was calculated as follows: [(S + α′)/S] × 100% (Table 1). After aspiration, the sample unit dispenses a part of its contents (α) into the cuvette. Depending on its diluent volume, this may be either greater than, equal to, or less than S. These results, calculated as (S/α′) × 100%, are also shown in Table 1. Repeating this experiment five times for all combinations of sample and diluent volumes yielded an average CV for the sample volumes pipetted by the Cobas-Bio of 1.18%. Manual pipetting of 30 μL of the 125I solution showed a CV of 0.65% (n = 10). Finally, we checked the accuracy of the dispensed reagent volumes. The Cobas-Bio was made to pipet only 125I solution, without any sample or diluent liquid. We then counted the radioactivity in the 125I solutions in the separated cuvettes individually. The data (n = 5 per reagent volume) are presented in the lower part of Table 1 as a percentage of the nominal reagent volume. The average of the CVs of the reagent