Lowering cutoffs for initial and confirmation testing for cocaine and marijuana: large-scale study of effects on the rates of drug-positive results

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A large-scale study was conducted to determine whether lowering the initial testing and confirmation testing cutoffs in urine would significantly affect the positive rates for cocaine (COC) and marijuana (THC). Customary cutoffs for COC are 300 µg/L and 150 µg/L for initial testing (screening) and gas chromatography–mass spectrometry (GC-MS; confirmation), respectively; for THC, the usual respective cutoffs are 50 µg/L and 10 µg/L. By applying a screening cutoff of 100 µg/L for COC and lowering the GC-MS cutoff to 50 µg/L, the COC-positive rate increased from 1.2% to 2.1%. For THC, lowering the screening cutoff to 20 µg/L while leaving the GC-MS cutoff at 10 µg/L increased the THC-positive rate from 2.8% to 4.1%. These increases appear noteworthy.

INDEXING TERMS: abused drugs • screening • gas chromatography–mass spectrometry • urine

Five drug classes are monitored by the Substance Abuse and Mental Health Services Administration (SAMHSA), the federally regulated, civilian drug-testing program: cocaine (COC), marijuana (THC), amphetamines, phencyclidine, and opiates. COC and THC are of primary interest because of their relatively high incidence, which is the result almost exclusively of abuse, their medical use being extremely limited. For the first 4 years after the inception of the SAMHSA drug-testing program, the initial testing and confirmation testing cutoffs for THC were 100 µg/L and 15 µg/L, respectively [1]; on September 1, 1994, the initial testing cutoff for cannabinoids was decreased to 50 µg/L [2]. The initial testing and confirmation SAMHSA cutoffs for COC have remained at 300 µg/L and 150 µg/L, respectively. None of the other major civilian drug-testing programs such as the College of American Pathologists or New York State have mandatory reporting cutoffs for the urine drug-testing laboratories enrolled in their programs. The Department of Defense drug-testing program uses respective initial and confirmation test cutoffs of 50 and 15 µg/L for THC and of 150 and 100 µg/L for COC. The present study was undertaken to determine what cutoffs are obtainable with the methodologies now in use for COC and THC and whether applying lower cutoffs would lead to a significant increase in the rates of positive results found.

Materials and Methods

CHEMICALS, REAGENTS, AND SUPPLIES

[3H]11-nor-delta-9-THC-9-carboxylic acid (carboxy-THC-D3; 100 mg/L) was purchased from Research Triangle Institute, Research Triangle Park, NC. [3H]Benzoylecgonine (BZE-D3; 100 mg/L) was purchased from Radian, Austin, TX.

Methanol, methylene chloride, isopropyl alcohol, iso-octane, dimethyl sulfoxide, ethyl acetate, hexane, glacial acetic acid, and methyl iodide were purchased from Fisher Chemical Co., Pittsburgh, PA, all ACS grade. KOH pellets and KH2PO4 were purchased from Mallinckrodt, Paris, KY. 1,1,1,3,3,3-Hexafluoro-2-propanol was purchased from Sigma Chemical Co., St. Louis, MO; pentafluoropropionic anhydride from Supelco, Bellefonte, PA; and tetramethylammonium hydroxide (2 mol/L) from Aldrich Chemical Co., Milwaukee, WI.

Bond-Elut Certify-LRC columns were purchased from Varian, Harbor City, CA. Phosphate buffers and KOH and HCl solutions were prepared as described in the Bond-Elut instruction manual.

For initial testing, Abuscreen OnLine Carboxy-THC 50 calibrator (chiral; carboxy-THC, 50 µg/L) and Abuscreen OnLine calibrator Level 3 (BZE, 300 µg/L) were purchased from Roche Diagnostic Systems, Branchburg, NJ. For GC-MS testing, BZE Calibrator (150 µg/L) and Carboxy-THC Calibrator (15 µg/L) were purchased from Laboratory Corporation of America, Forensic Toxicology, 69 First Ave., Raritan, NJ 08869. Fax 908-526-1822.

1 Nonstandard abbreviations: SAMHSA, Substance Abuse and Mental Health Services Administration; COC, cocaine; THC, tetrahydrocannabinol (marijuana); D, deuterium; BZE, benzoylecgonine; HP, Hewlett-Packard; and SIM, selected ion monitoring.

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ElSohly Labs., Oxford, MS. Appropriate dilutions of the calibrators were made with blank urine.

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Screening tests for COC and THC were done with Abusscreen OnLine reagents; the subsequent light scatter was measured with an Olympus AU5131 (Olympus Corp., Lake Success, NY) to follow the agglutination of microparticles in solution. The degree of inhibition of agglutination is proportional to the amount of analyte in the sample. The COC assay detects BZE, the primary metabolite of COC in human urine; the THC assay detects 11-nor-delta-9-THC-9-carboxylic acid (carboxy-THC).

Reagents were prepared according to the manufacturer’s instructions for using COC and THC on the AU5131. The instrument was calibrated in the AB mode with a single-point calibrator, and the settings used were those recommended by Roche. For the COC study, specimens that produced readings ≥100 vs the 300 µg/L calibrator were tested further by GC-MS. Subsequent experiments determined that the 100 reading is equal to a BZE concentration of ~60 µg/L.

Specimens with results ≥20 vs the OnLine Carboxy-THC 50 calibrator were rescreened with the Cannabinoid 20 Assay from Behring (formerly Syva), San Jose, CA. Reagents and calibrators were reconstituted according to the manufacturer’s instructions for use on the B10 COBAS BIO (Roche Diagnostic Systems); instrument settings were those recommended by Behring. Specimens that produced readings equal to or greater than the 20 µg/L calibrator were considered to be positive and were tested further by GC-MS.

EXTRACTION AND DERIVATIZATION
BZE was extracted from 5.0 mL of urine as described in the Bond-Elut instruction manual but with 150 µg of BZE-D3, internal standard added to each sample. This was followed by derivatization with hexafluoro-2-propanol/pentafluoropropionic anhydride and reconstitution with toluene for analysis by GC-MS.

Carboxy-THC was extracted from 5.0 mL of urine as described in the Bond-Elut manual but with 25 ng of internal standard added to each sample. The extract was then methylated with tetramethy lammonium hydroxide/dimethyl sulfoxide and methyl iodide and then reconstituted with isooctane for analysis by GC-MS.

GC-MS ANALYSIS
All GC-MS analyses were performed on a Hewlett-Packard (HP; Palo Alto, CA) Model 5890 gas chromatograph interfaced with a Model 5970A quadrupole mass spectrometer. Samples were injected with an HP7673 Automated Liquid Sampler. The gas chromatograph was equipped with a 12 m × 0.020 mm (i.d.) HP-1 capillary column (0.33 µm film thickness). The mass spectrometer was operated in selected ion mode (SIM). For BZE, GC column conditions were set to give retention times between 4.5 and 5.5 min. SIM ions used to monitor BZE were m/z 318, 272, and 439. SIM ions used to monitor BZE-D3 were m/z 321 and 275. For carboxy-THC, the GC column variables were set to give retention times between 6.0 and 8.0 min. The SIM ions used to monitor carboxy-THC were m/z 313, 357, and 372; for carboxy-THC-D3, they were m/z 316 and 375.

The limit of detection (mean ± 3SD) was calculated by analyzing 20 blank urines; for BZE, the limit of detection was ~1 µg/L.

Results and Discussion
COCaine
In all, 4911 urines were screened for COC with the COC Online reagent; 59 of these (1.2%) were positive according to the SAMHSA-mandated cutoffs: 300 µg/L for screening, 150 µg/L for confirmation (Table 1). (No donor samples from the SAMHSA-regulated program were used in this study.) Use of a 100 µg/L screening cutoff and a confirmation cutoff of 50 µg/L designated 105 specimens as positive (2.1%). If the confirmation test cutoff was lowered further to 30 µg/L, the number of positives increased to 119 (2.4%), and using the method’s limit of detection (1 µg/L) as the confirmation test cutoff increased the number of positives to 139 (2.8%). Soldin et al.[3] previously had reported similar results in a smaller study done with samples from an adolescent population.

In an additional study, done to determine whether the precision of the COC confirmation method was satisfactory at the 30 µg/L cutoff, several minor modifications were made to the method described in Materials and Methods. The 150 µg/L calibrator was diluted to 30 µg/L with blank urine, and the concentration of internal standard added was 30 µg/L. SAMHSA has set 40% of the cutoff concentration as a target for the lower limit of quantification. To see if this target could be obtained, 11 replicate samples (target concentration = 12 µg/L) were analyzed. The resulting mean and CV were 12.5 µg/L and 5.6%, respectively. Moreover, the chromatography was acceptable, showing no interfering peaks and adequate abundances for each peak.

The increase in the number of positives for COC obtained by first lowering the initial testing and then lowering the confirmation cutoffs was very important.

| Table 1. Effect of screening confirmation cutoffs on cocaine positive rates. |
|-------------------------|-----------------|-----------------|-----------------|
| **COC cutoff**          | **Confirm., µg/L** | **No. of positives** | **% positive**  |
| 300                     | 150             | 59              | 1.2             |
| 100                     | 50              | 105             | 2.1             |
| 100                     | 30              | 119             | 2.4             |
| 100                     | 1a              | 139             | 2.8             |

* Limit of detection.

Total number of urine specimens tested = 4911.

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Using the screening cutoff of 300 μg/L instead of 100 μg/L would have missed 80 COC-positive samples, all of which were subsequently confirmed by GC-MS. In addition, 21 samples with screening readings of ≥100 μg/L were negative by GC-MS—primarily because the routine COC method was calibrated with a 300 μg/L calibrator. Clearly, the discrimination of positives and negatives would be considerably improved if the OnLine reagents and instrument settings were optimized for a cutoff considerably less than the 300 μg/L currently in use. This could be achieved by minor modifications in the reagent or instrument settings, e.g., increasing the sample volume (personal communication, Alan McNally, Roche Diagnostic Systems).

Most importantly, the increases in the positive rate shown in Table 1 were accomplished with only minor changes in the confirmation method. Calibrating the current GC-MS method at 30 μg/L presented no problem. The GC-MS was run with the electron multiplier set at 1600 eV and an injection volume of 1.5 μL. Under these conditions, the control at 40% of cutoff (12 μg/L) produced acceptable chromatography.

No special instrument maintenance was done for this study. The samples were injected in the same manner as the routine batches. Most likely, other forensic toxicology laboratories also are capable of achieving similar results with little or no alterations in their current methodologies. This approach would appear to be a less cumbersome way to increase the positive rate for COC than a recent method that analyzes for ecgonine [4].

Lowering the BZE cutoff increases the concern that positive results might be caused by passive inhalation or occupational exposure. A recent study by Cone et al. [5] obtained a urine concentration of BZE as high as 123 μg/L in one subject exposed to vapor of free base cocaine for 1 h. The maximum urine BZE concentration for aides assisting in that study and who were exposed to sidestream smoke for 4 h was 6 μg/L. More intense exposure to smoke from crack cocaine might lead to higher BZE concentrations in the urine. Whether one could unwillingly expose oneself to this degree is doubtful. Le et al. [6] previously reported BZE concentrations >1000 μg/L in the urine of criminals who were handling or analyzing large amounts of powder cocaine while conducting criminal investigations.

**MARIJUANA**

In all, 6427 urines were screened for THC at a 50 μg/L cutoff with the OnLine reagent. Specimens giving readings ≥20 μg/L were rescreened with the Cannabinoid 20 Emit Assay. Specimens that still gave readings equal to or greater than the 20 μg/L calibrator were tested further by GC-MS. The number of positives reported for the 50 μg/L screening cutoff and the 10 μg/L confirmation cutoff was 179 (2.8%) (Table 2). Using a screening cutoff of 20 μg/L (and keeping the confirmation cutoff at 10 μg/L) increased the number of positives to 262 (4.1%). If, in addition, the confirmation test cutoff was lowered to 5 μg/L, the number of positives increased slightly, to 281 (4.4%). As described in Materials and Methods, calibration at 5 μg/L was accomplished by making only minor changes in the routine method. The chromatography was acceptable, with no interfering peaks, and the peak areas were adequate for quantification. Additional studies done by diluting the low-concentration control demonstrated accurate quantification at 1 μg/L (electron multiplier set at 2000 eV, injection volume = 2 μL).

Lowering the initial test cutoff to 20 μg/L greatly increased the number of positives. The Emit Cannabinoid 20 assay used in this study demonstrated excellent discernment between negatives and positives. Only 1 of 104 specimens that had positive initial test results failed to be confirmed positive at 5 μg/L. Recently, an application of the OnLine reagent at the 20 μg/L cutoff has been published [7]. However, the problem of differentiating between marijuana use and passive inhalation becomes of greater concern when the lower cutoffs are used. Several clinical studies, now ~10 years old, have attempted to evaluate the effects of passive inhalation. Cone et al. [8, 9], Mule et al. [10], and Perez-Reyes et al. [11] all demonstrated that detectable amounts of cannabinoids can be produced in urines of volunteers purposely exposed to substantial amounts of marijuana smoke. Whether an individual could knowingly and unwillingly expose him or herself to such amounts is highly questionable.

An earlier study of Huestis et al. [12] found that lowering the initial screening cutoff to 50 μg/L from 100 μg/L resulted in a large increase in the number of THC positives. Their study was done on urines collected from six volunteers and the percentage increase varied from 23% to 53%, depending on which of the eight immunoassay reagents was used. In the present large-scale study, looking at results from 6427 urines tested in a production laboratory, lowering the initial test cutoff even further to 20 μg/L clearly demonstrates substantial additional increases in the number of positives confirmed by GC-MS.

**CONCLUDING COMMENTS**

Currently, numerous urine specimens containing low concentrations of COC metabolites or THC metabolites are being reported as negative. This supports the findings of Huestis et al. [13], who showed that detection times with the 50 μg/L THC cutoff provide only a 1–2 day

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<th>THC cutoff, μg/L</th>
<th>Screening</th>
<th>Confirm.</th>
<th>No. of positives</th>
<th>% positive</th>
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Total number of urine specimens tested = 6427.
window of detection for acute users. Lowering the cutoffs for both drugs should increase the window of detection and permit a greater percentage of specimens to be confirmed as true positives at concentrations as low as 50 \( \mu g/L \) for BZE and 10 \( \mu g/L \) for carboxy-THC.

Clearly, each laboratory individually must address the issue of increased cost of the additional confirmation required because of using the lower screening cutoff values. Ultimately, these costs will be passed on to the consumers—clients who would benefit from avoiding costs generated by problem employees. This justification for doing drug of abuse testing has been made many times in the past decade. Perhaps it is time to reevaluate the cutoff values used to report these results in light of the technology now available.

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References