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Analytic Performance of Immunoassays for Drugs of Abuse Below Established Cutoff Values

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Background: **The analytic performance and accuracy of drug detection below Substance Abuse and Mental Health Services Administration (SAMHSA) cutoffs is not well known. In some patient populations, clinically significant concentrations of abused drugs in urine may not be detected when current SAMHSA cutoffs are used. Our objectives were to define the precision profiles of three immunoassay systems for drugs of abuse and to evaluate the accuracy of testing at concentrations at which the CV was <20%.**

Methods: **Drug-free urine was supplemented with analytes to assess the precision in three commercial drugs-of-abuse immunoassay systems below the SAMHSA-dictated cutoffs for amphetamines, opiates, benzoylecgonine, phencyclidine, and cannabinoids. Consecutive urine samples with signals associated with a CV <20% by Emit® immunoassay and below SAMHSA cutoffs were then subjected to confirmatory analysis.**

Results: **The CV of all immunoassay systems tested remained <20% to drug concentrations well below SAMHSA cutoffs. The accuracy of urine drug-screening results between the SAMHSA-specified cutoffs and the precision-based cutoffs was less than accuracy for specimens above the SAMHSA cutoffs, but the use of the precision-based cutoff produced a 15.6% increase in the number of screen-positive specimens and a 7.8% increase in the detection of specimens that yielded positive results on confirmatory testing.**

Conclusion: **The precision of three commercial immunoassay systems for drugs-of-abuse screening is adequate to detect drugs below SAMHSA cutoffs. Knowledge of the positive predictive values of screening immunoassays at lower cutoff concentrations could enable efficient use of confirmatory testing resources and improved detection of illicit drug use.**

Workplace and clinical drug screening typically begins with a rapid, low-cost screening test. Because of their low cost and ability to be automated for high throughput, various immunoassay techniques have become the mainstay for urine screening tests. Depending on the medical and legal consequences of a positive test, confirmatory analysis may be applied to specimens when screening tests indicate the presence of drugs above predetermined cutoff concentrations. According to College of American Pathologists survey data from 2003 *(1)*, nearly 3000 laboratories performed immunoassay-based drug screening, but only 50 were certified to perform federal workplace drug testing *(2)*. Despite the small percentage of laboratories certified to perform testing under the Substance Abuse and Mental Health Services Administration (SAMHSA),[7] the federally mandated threshold concentrations differentiating "positive" from "negative" specimens are widely used by laboratories that perform drug testing for other reasons.

Thresholds or cutoffs in the federal workplace drug testing program were established in the mid-1980s *(3)*. An objective of the program is to identify drug use without producing false-positive results. The original screening immunoassay cutoff concentrations were 300 μg/L for

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[7] Nonstandard abbreviations: SAMHSA, Substance Abuse and Mental Health Services Administration; PCP, phencyclidine; THC, 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid; BEG, benzoylecgonine; PPV, positive predictive value; EIA, enzyme immunoassay; FPIA, fluorescence polarization immunoassay; GC/MS, gas chromatography–mass spectrometry; and TLC, thin-layer chromatography.

"opiate metabolites", 100 μg/L for "marijuana metabolites", 300 μg/L for "cocaine metabolites", 1000 μg/L for amphetamines, and 25 μg/L for phencyclidine (PCP). The latter three threshold values have remained unchanged since their inception, but the cutoff concentrations for opiates and marijuana have been modified. The marijuana metabolite cutoff was lowered from 100 μg/L to 50 μg/L in 1994, reflecting, ". . . advances in technology of immunoassay" *(4)*. Huestis et al. *(5)* reported that this change produced a 23–53% increase in the identification of specimens yielding positive results on confirmatory testing depending on the immunoassay used for screening. The opiate cutoff was increased in 1997 from 300 to 2000 μg/L to avoid false-positive screening results attributable to poppy seed ingestion *(6)*. Thus, mixed criteria are used to adjust SAMHSA-specified cutoffs for screening immunoassays. The intent of adjusting the cutoff for marijuana metabolites was to increase the frequency of identifying specimens that yielded positive results on confirmatory testing. In raising the opiate cutoff, the intent was to reduce the rate of analytically true-positive results that did not reflect drug abuse.

With few exceptions, the consequences of using federally mandated cutoffs in clinical settings have not been evaluated. By lowering the screening cutoff for 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC) to 10 μg/L and for benzoylecgonine (BEG) to 100 μg/L, Wingert *(7)* reported increases in the yield of specimens testing positive on confirmation of 1.3% and 0.9%, respectively. Soldin et al. *(8)* reported a >100% increase in cocaine-positive specimens when they lowered the cutoff from 300 μg/L to 80 μg/L in a pediatric population. The issue is particularly important in a pediatric population because neonates are not able to concentrate urine to the same extent as adults. Urine osmolality ranges from 15 to 585 mosmol/kg in the neonatal period and does not approach adult values until 2 years of age *(9)*. This fact may have contributed to the findings of Hattab et al. *(10)* that 50% of cocaine-exposed newborns had urinary cocaine metabolite concentrations below the SAMHSA cutoff.

The current study uniquely aims to establish the performance of multiple immunoassays for drugs of abuse at the limits of analytic sensitivity in a diverse clinical population. Our specific objectives were to (*a*) assess the imprecision of immunoassay systems in the detection of abused drugs below SAMHSA cutoff concentrations and establish precision-based cutoffs; (*b*) determine the incidence of urine drug-screening results between the SAMHSA cutoff concentrations and the precision-based cutoffs; and (*c*) compare the predictive value of a positive (PPV) immunoassay screening test determined with currently specified cutoff concentrations to that obtained with precision-based cutoffs. The results of our study provide data relevant to developing customized drug-of-abuse detection schemes in clinical settings.

Materials and Methods

IMMUNOASSAY PRECISION

For the precision study, identical samples were analyzed at three sites by use of Emit® reagents on a Hitachi 717 (Barnes Jewish Hospital, St. Louis, MO), Beckman enzyme immunoassay (EIA) reagents on a Synchron® CX-9 (Children's Mercy Hospital, Kansas City, MO), or fluorescence polarization immunoassay (FPIA) technology on an Abbott AxSYM® (Children's Hospital of Wisconsin, Milwaukee, WI) according to the manufacturer's instructions in each case. The Hitachi 717 and the Synchron CX-9 were calibrated by use of a single calibration point, whereas the AxSYM was calibrated by use of a six-point curve. As part of routine clinical practice at these sites, SAMHSA cutoff values were used on all three platforms with the following exceptions: the Beckman EIA cannabinoid assay was calibrated to a 20 μg/L cutoff, the Emit cocaine metabolite assay was calibrated to a 150 μg/L cutoff, and all three opiate immunoassays used a 300 μg/L cutoff.

Precision profiles were generated by use of drug-free urines supplemented with 1250 μg/L d-methamphetamine, 70 μg/L THC, 25 μg/L PCP, 180 μg/L BEG (all from Alltech), and 380 μg/L morphine (Cerilliant Corp.). The supplemented urines were diluted to 80%, 60%, 40%, and 20% of the original analyte concentrations. Freshly thawed aliquots of each urine pool were measured twice a day for 10 days on the three instruments specified above. Instrument signal was converted to analyte units by nonlinear regression *(11)*, and total imprecision was calculated in terms of analyte concentration, then expressed as a CV. An imprecision-based cutoff was defined as the lowest concentration of analyte with a CV <20% or, in the case when the CV never exceeded 20%, the lowest concentration tested.

ACQUISITION OF URINE SPECIMENS YIELDING IMMUNOASSAY SIGNALS ABOVE BLANK BUT BELOW SAMHSA-SPECIFIED CUTOFFS

All specimens submitted to the Drug Analysis Laboratory at Barnes-Jewish Hospital for drug screening and subsequent confirmation were eligible. The Barnes-Jewish Drug Analysis Laboratory performs ~10 000 annual drug screens and serves as the primary drug-testing venue for a medical campus serving 1500 inpatient beds, including St. Louis Children's Hospital. The primary source of drug screens is the emergency department (67%), followed by labor and delivery (19%), St. Louis Children's Hospital (9%), and chemical dependency wards (5%). During an 8-week period from November 2002 to January 2003, urine specimens with Emit signals below the currently used cutoffs but above the signals associated with a CV of 20% were collected until 20 consecutive samples were accrued for each of the five immunoassays. These specimens are referred to as "subcutoff-positive" specimens and were stored at −20 °C until selected for confirmatory testing.

CONFIRMATORY TESTING

All confirmatory tests except gas chromatography–mass spectrometry (GC/MS) were performed in the Barnes Hospital Drug Analysis Laboratory. GC/MS analyses were performed by the Christian Hospital Northeast (St. Louis, MO) Toxicology Laboratory. Samples were thawed and then maintained at 4 °C during transport and storage before processing for confirmatory analyses. All analyses were completed within 24–48 h after the samples were thawed.

Specimens with non-zero immunoassay signals but below the 1000 μg/L cutoff for (meth)amphetamine were subjected to thin-layer chromatography (TLC) analysis for sympathomimetic amines (Toxilab®; Ansys Diagnostics). If TLC analysis was negative, the sample was subjected to GC/MS analysis capable of detecting 250 μg/L amphetamine or methamphetamine. The presence of opiates was confirmed by two simultaneous procedures: (*a*) TLC analysis of unhydrolyzed urine, after solid-phase extraction, by a method capable of detecting various natural, synthetic, and semisynthetic opiates at concentrations exceeding 200 μg/L *(12)*; and (*b*) specific GC/MS analysis for morphine and codeine, after β-glucuronidase treatment, by a method with a detection limit of 50 μg/L. The presence of BEG and PCP was confirmed by GC/MS analysis with detection limits of 50 and 10 μg/L, respectively. THC specimens with immunoassay signals above SAMHSA cutoff values were not routinely confirmed because past experience indicated almost perfect concordance between screening immunoassay results and confirmation procedures (J. Koenig and A. Saunders, unpublished results). THC specimens with immunoassay signals below the SAMHSA cutoff but above the 20% CV cutoff were confirmed, after potassium hydroxide treatment, by GC/MS analysis capable of detecting 5 μg/L THC. Confirmatory testing for PCP, BEG, and THC was considered positive only when these substances were detected by GC/MS. Confirmatory tests for (meth)amphetamine were considered positive when amphetamine, methamphetamine, ephedrine, pseudoephedrine, phenylpropanolamine, methylenedioxymethamphetamine, or methylenedioxyamphetamine was detected by a single confirmatory technique. Confirmatory tests for opiates were considered positive when morphine, codeine, 6-acetylmorphine, norcodeine, dihydrocodeine, hydrocodone, hydromorphone, oxycodone, or oxymorphone was detected by a single confirmatory technique.

Results

Precision profiles for the three drugs-of-abuse immunoassay systems studied are shown in Fig. 1. In general, each immunoassay system exhibited a CV <20% at analyte concentrations below the SAMHSA cutoffs. At the lowest concentration of morphine tested (75 μg/L), all three opiate immunoassays displayed imprecision <20%. The CVs in the Emit amphetamine, BEG, and THC assays

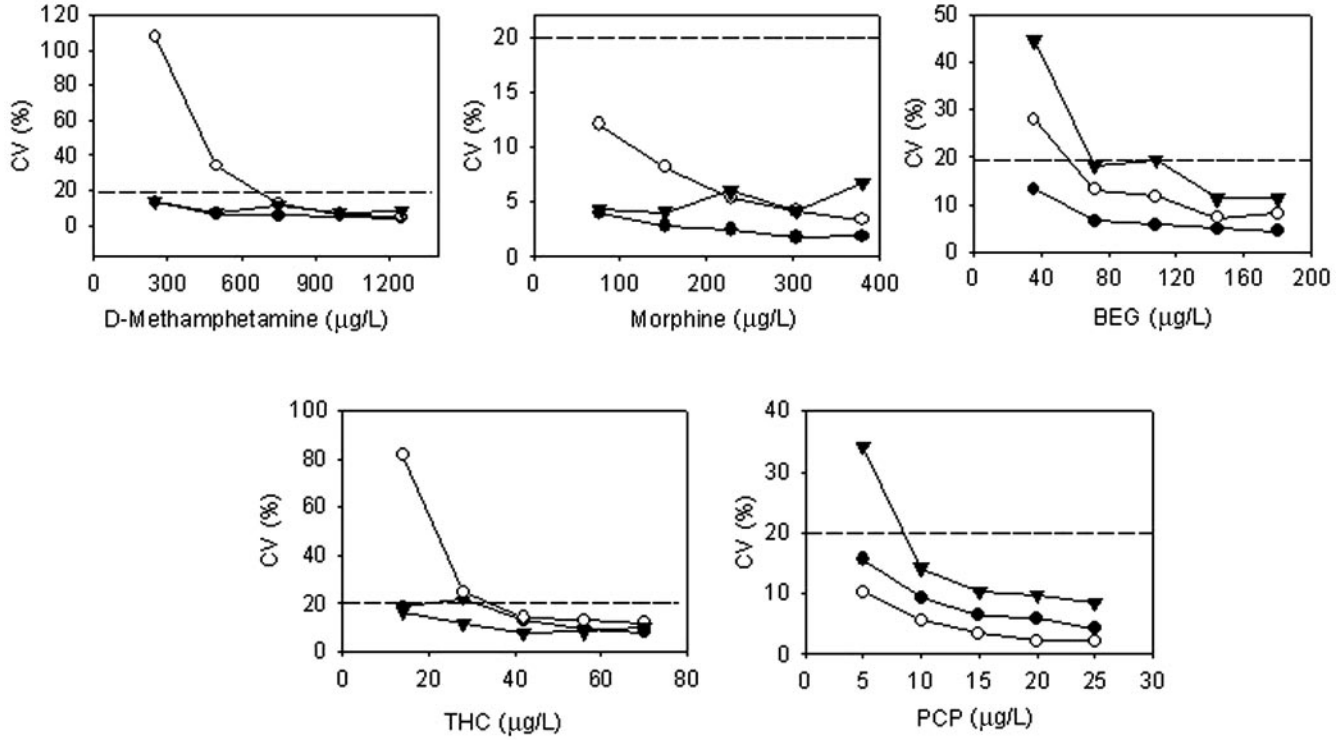

Fig. 1. Precision profiles of immunoassays for drugs of abuse at concentrations below SAMHSA cutoffs.

Drug-free urine was supplemented with the indicated concentrations of each compound and analyzed by Beckman EIA (●), Syva Emit (○), or Abbott FPIA (▼). Imprecision was calculated in terms of analyte concentration for each immunoassay and expressed as CV (*y axis*). A CV of 20% (*dashed line* in each *panel*), was used to derive the precision-based cutoff values.

exceeded 20% only at the lowest drug concentrations tested. The CV in the Abbott FPIA assays exceeded 20% only at the lowest concentrations of PCP and BEG tested. The CV in the Beckman EIA assays never exceeded 20% at the drug concentrations tested. On the basis of these result, we propose assay-specific cutoffs equal to the point at which the CV exceeded 20% or, when the CV never exceeded 20%, equal to the lowest drug concentration tested (Table 1).

Having defined the limits of precision in the three immunoassay systems, we evaluated the accuracy of drug detection by Emit in subcutoff-positive specimens. Emit-"negative" urine specimens with signals at or above those corresponding to 700, 76, 60, 35, and 5 μg/L for methamphetamine, morphine, BEG, THC, and PCP, respectively, were subjected to confirmatory analysis. Consecutive specimens were collected until ~20 were identified that fulfilled the above criteria. The results of these analyses are shown in Table 2. We examined the results of 1100–1300 drug screens to obtain specimens for amphetamines, THC, opiate, and PCP analysis. Examination of 825 screens yielded 21 specimens for BEG confirmation. On a percentage basis, the increase in the number of screen-positive specimens was largest for amphetamines (157%) followed by PCP (45%), BEG (14%), opiates (10%), and THC (9%). Overall, 102 new screen-positive samples were subjected to confirmation.

The confirmatory rates for these subcutoff specimens were lower than for Emit-positive specimens. At one extreme, only 6% of subcutoff PCP specimens contained detectable PCP by GC/MS analysis. At the other extreme, 90% of subcutoff-positive THC specimens contained THC by GC/MS analysis. Confirmatory rates for BEG, amphetamines, and opiates were 57%, 41%, and 27%, respectively. Of the 102 new screen-positive specimens examined, 46 yielded positive results on confirmatory testing. This represents a 7.8% increase over results obtained by use of conventional Emit cutoffs. When combined with Emit-positive results, the overall effects on the PPVs of screening for THC, BEG, and opiates were modest (<10%), whereas the PPVs for amphetamine and PCP screening were reduced by 23% and 25%, respectively.

Finally, we investigated the possibility that the decreased confirmatory rate of subcutoff screen-positive specimens was attributable to insufficiently sensitive confirmatory assays. We compared the Emit signal strength of the subcutoff-positive specimens with their outcomes in confirmatory testing (positive or negative). As shown in Fig. 2, there was no correlation between immunoassay signal and confirmatory outcome in any of the screening assays tested. Both positive and negative specimens were evenly distributed across the range of signal strengths indicated. Therefore, the immunoassay signal in subcutoff specimens likely resulted from nonspecific interfering compounds in urine or cross-reacting metabolites not detected by GC/MS.

Discussion

The issue of immunoassay cutoffs in drugs-of-abuse screening receives considerable attention in the context of federal workplace drug testing programs. The attention paid to the suitability of these cutoffs in everyday

Table 1.

Proposed cutoffs for drugs-of-abuse immunoassays based on imprecision (CV).

Assay	Amphetamines, μg/L	THC, μg/L	BEG, μg/L	Opiates, μg/L	PCP, μg/L
Syva Emit	700[a]	35[a]	60[a]	76[b]	5[b]
Beckman EIA	250[b]	14[b]	36[b]	76[b]	5[b]
Abbott FPIA	250[a]	14[b]	72[a]	76[b]	10[a]
SAMHSA cutoff	1000	50	300	2000	25

[a] Cutoff derived from point at which total CV exceeds 20%.
[b] CV <20% at all concentrations tested; cutoff derived from lowest concentration tested.

Table 2.

Effect of lowered cutoffs on confirmatory PPVs.

		Established (SAMHSA)			Precision-based		
Assay	Screens, n	Screen+, n	Confirmed+, n	PPV, %	Screen+, n	Confirmed+, n	PPV, %
Amphetamines	1134	14	11	79	36	20	56
THC	1133	222	222[a]	100[a]	242	240	99
BEG	825	155	148	95	176	160	91
Opiates	1296	225	180	80	247	186	75
PCP	1299	38	31	82	55	32	58
Total		654	592	91	756	638	84

[a] Confirmation rates were derived from previous experience. Only 20 subcutoff-positive specimens were subjected to GC/MS analysis.

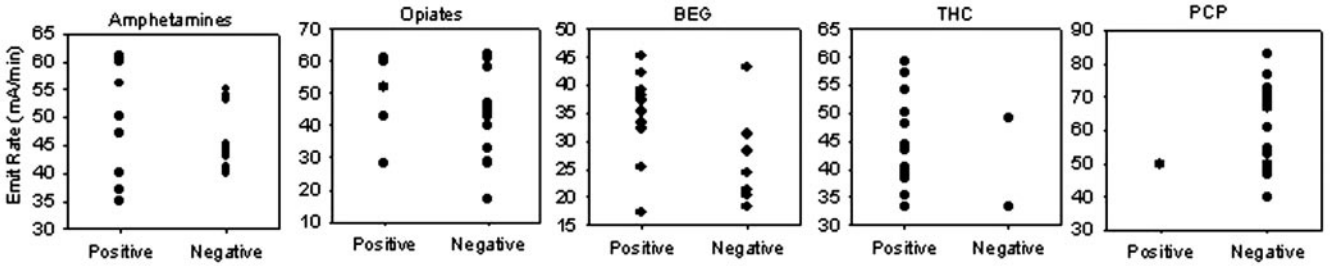

Fig. 2. Lack of correlation between immunoassay results and confirmatory outcomes for urine specimens with rates above blank but below currently used Emit cutoffs.

Rates from the original Emit immunoassay are plotted vs the confirmatory outcome of each specimen by TLC or GC/MS. *mA*, milliabsorbance units.

clinical use, however, has been sparse. The scattered reports that have addressed this issue have uniformly concluded that large numbers of urine specimens labeled negative will yield positive results on confirmatory analysis *(5, 7, 8, 10)*). The use of SAMHSA-specified cutoffs might be necessary to avoid false-positive results for workplace drug testing, but medical drug testing is performed for different reasons that might be better suited by optimizing the sensitivity and specificity of screening immunoassays. In pediatric populations, dilute urines have great potential to cause false-negative screens, which could lead to discharge of a child to an environment in which caregivers are actively using illicit drugs.

In the present study we have taken a first step toward optimizing the sensitivity and specificity of immunoassays for detecting drugs of abuse. We defined new cutoffs below existing SAMHSA cutoffs based on the precision in three different immunoassay systems. We then identified urine specimens with immunoassay signals below SAMHSA-specified cutoffs but above precision-based cutoffs and re-examined these for the presence of illicit drugs by confirmatory testing procedures. Approximately one-half of specimens thus identified yielded positive results on confirmatory testing. Our results indicate that lower cutoffs would significantly increase the number of specimens yielding positive results on confirmatory analysis but, not surprisingly, at the expense of a significant increase in specimens that are negative for the presence of drugs of abuse by confirmatory analysis.

The increased numbers of false-positive specimens represent increased expenditures for confirmatory analysis. Extrapolating to an annual volume of 10 000 drug screens, an additional 900 screen-positive specimens would be identified. The cost associated with the confirmatory analyses on these specimens may be offset by foregoing confirmatory testing when it is unnecessary. For example, the Barnes-Jewish Drug Analysis Laboratory has discontinued routine confirmation of THC screens >50 μg/L because of near-perfect concordance between screening and confirmatory results. This change in practice would more than offset the added expense of discovering a significant number of new true-positive specimens that would otherwise have gone undetected.

The conclusions of our study are limited in several ways. We confirmed subcutoff screen-positive specimens only from the Emit immunoassay system, and our findings might not be applicable to other immunoassay systems. The degree of cross-reactivity for metabolites of illicit drugs and other substances is likely different for each immunoassay system. Huestis et al. *(13)*, for example, reported considerable interassay variability in the length of time that THC was detected after cessation of marijuana use, suggesting substantial differences in the detection of cross-reacting cannabinoid metabolites. The population being screened will also profoundly affect results because of the influence of the prevalence of drug use on PPV. For example, our study of the Beckman drug-screening assays was performed in the setting of a chemical dependency program, which led to higher PPVs than would be experienced in screening the general population, where the prevalence of drug use would be lower *(14)*. Finally, it is likely that the performance characteristics of immunoassays for drugs of abuse below the SAMHSA cutoffs are not closely scrutinized by manufacturers because these properties are not relevant to current laboratory practices. Lot-to-lot fluctuations in performance may therefore be severe, and our conclusions with the single lot of reagents used during the time of this study might not be applicable to other lots.

Our study lays the groundwork for an alternative, performance-based approach to establishing cutoff values for drug-screening immunoassays. With further studies matching specific assays to specific populations, the decision to confirm an immunoassay result may be based on a signal-specific PPV rather than a predetermined cutoff. This approach could increase the frequency of identifying specimens that will yield positive results on confirmatory testing and could be used to tailor the selection of cutoff values in specific testing settings that might differ in their objectives.

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