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Evaluation of Immunoassay Methods for the Screening of Cocaine Metabolites in Urine

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ABSTRACT: Immunoassay kits for urine cocaine (and metabolite) screening, obtained from two commercial sources, were examined for correlation of their results, expressed in terms of equivalent benzoylecgonine concentration, with the gas chromatography/mass spectrometry (GC/MS) concentration of benzoylecgonine. The correlation coefficients obtained, based on 62 (out of a total sample population of 3295) highly relevant samples, were 0.467 and 0.766 for Abuscreen® (ARIA) and TDx® (TDX), respectively. The preliminary screen cutoff values, which correspond to 150 ng/mL benzoylecgonine (as determined by GC/MS), were calculated based on the resulting regression equations and found to be 380 and 190 ng/mL for ARIA and TDX, respectively. With these cutoff values, ARIA generates 5 false negatives and 16 unconfirmed presumptive positives, while TDX results in 3 false negatives and 6 unconfirmed presumptive positives.

KEYWORDS: toxicology, immunoassay, cocaine, urine

In response to the drug abuse problem in today's society, many preliminary and confirmatory methods have been developed. Generally, the tests begin with an immunoassay screening procedure [*1–3*], such as the enzyme multiplied immunoassay (EMIT®), Abuscreen® (ARIA), or TDx® (TDX), to exclude the large number of negative specimens from those that require further testing. Since screening assays of this type are vulnerable to interferences, all specimens producing positive results are confirmed by a separate, more specific method, such as gas chromatography/mass spectrometry (GC/MS) [*3,4*].

The purpose of this study was to compare two methods, commonly used for preliminary

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drug screening, for the establishment of a cutoff value corresponding to the concentration of a specific drug or metabolite as determined by GC/MS. The use of an appropriate cutoff value for a screening procedure will minimize generating *false negatives* (samples that are determined negative in the preliminary screening step, but contain the drug/metabolite at a concentration higher than the confirmation cutoff level) and *unconfirmed presumptive positives* (samples that are determined positive in the preliminary screening step, but determined negative in the confirmatory procedure). Specifically, urine samples were assayed for cocaine metabolites with the ARIA system marketed by Roche Diagnostics (Montclair, New Jersey) and with the TDX system from Diagnostics Division, Abbott Laboratories (North Chicago, Illinois). Since samples with drug concentrations near the positive/negative cutoff levels are of particular concern, only those generating ARIA data within the ±20% of the count per minute obtained for the cutoff standard (300 ng/mL benzoylecgonine) were selected for the correlation study. The results were then compared with those obtained using GC/MS methods.

Materials and Methods

Immunoassay

Both the ARIA and TDX methods are based on the competitive binding of labeled antigen and free unlabeled antigen (analyte) to antibody in proportion to their concentrations in the reaction mixture. Reagents and procedures [*5,6*] provided by Roche Diagnostic Systems and Abbott Laboratories were followed for these tests.

Gas Chromatography/Mass Spectrometry

All specimens selected for this study were ultimately extracted and derivatized for analysis by GC/MS. Standard operating procedures [*7*] of the U.S. Navy's Drug Screening Laboratories were adopted for the extraction/derivatization of benzoylecgonine from urine samples and for the GC/MS analysis. Solid-phase extraction was performed using the Dupont Prep I automated sample processor (Wilmington, Delaware). Benzoylecgonine was extracted with the Dupont Prep Type W extraction cartridge. The cocaine metabolite was then alkylated with tetramethylammonium hydroxide/trimethyl phosphate/dimethyl sulfoxide (TMAH/TMPAH/DMSO) and iodopropane. Deuterated benzoylecgonine was used as the internal standard.

A Hewlett-Packard (HP) 5970B mass selective detector (MSD) coupled to an HP 5890A gas chromatograph was used for analysis. A 15-m [0.251-mm inside diameter (ID)] J & W DB-5 (0.25-μm film thickness) capillary column (Folsom, California) was connected to the MSD through a direct capillary interface. The injection port was a capillary split injector with a silanized glass insert. The carrier gas, helium, was at a flow rate of approximately 1.0 mL/min with a split ratio of 10:1. The MSD was used in the electron impact selected ion monitoring mode. The following ions were monitored: benzoylecgonine, *m/z* 210, 272, and 331; deuterated benzoylecgonine, *m/z* 213 and 334. The first ion listed for each compound was used for quantification.

Sample Selection

Samples from patients, which presumably included a normal distribution of urinary metabolites derived from cocaine use, were used in this study. Out of a total sample population of 3295, 62 specimens generated ARIA data that were within ±20% of the counts per minute obtained for the cutoff standard (300 ng/mL benzoylecgonine). These samples were then tested by TDX and GC/MS and used for correlation studies. Ben-

zoylecgonine controls in 300-ng/mL amounts, as obtained from the suppliers, were used to establish the cutoff values for both TDX and ARIA. This cutoff value was selected based on the recommendation provided by the "Guidelines for Federal Drug Testing Programs" [3].

Results and Discussion

These two initial test procedures were evaluated in two different ways. First, results from each of the two screening methods were correlated with the results from GC/MS for a consistency comparison. The second evaluation was a determination of appropriate screening cutoff values that correspond to a specific metabolite concentration as determined by GC/MS. Since results obtained by GC/MS are of ultimate value for legal purposes, a good correlation between results obtained by a preliminary screening and GC/MS methods would facilitate the selection of an appropriate cutoff value for the screening method to determine whether a specific specimen should be submitted for further GC/MS analysis.

Correlation of Abuscreen and TDX with GC/MS

The numerical results obtained in this study are listed in Table 1. These results are plotted in Figs. 1 and 2 with correlation coefficients. Results obtained with ARIA in counts per minute were converted to nanograms per millilitre using a standard curve [5] before the correlation analysis was attempted. The GC/MS versus ARIA plot (Fig. 1) resulted in a correlation coefficient of 0.467, which may be attributed to the cross-reactivities of the immunoassay to compounds closely related structurally [5]. Both cocaine and ecgonine cross-react with the antibody by more than 50% when present in amounts capable of giving results equivalent to 300 and 1000 ng/mL benzoylecgonine, respectively [5]. This correlation coefficient also indicates that the relative concentrations of cross-reactive compounds in these specimens were not constant. It has been concluded that the relative concentrations of various cocaine metabolites that will cross-react with the antibody in ARIA vary with the time elapsed after drug use [8]. The GC/MS versus TDX plot (Fig. 2) demonstrates a correlation coefficient of 0.766, because of the more specific nature [6] of the antibody used for the TDX assay.

Preferred Method

As a preliminary screening procedure for GC/MS determination of a specific compound, the dependability of an immunoassay is primarily determined by the specificity of the particular antibody, that is, the antibody's ability to distinguish the specific structural characteristics of the target compound from those compounds possessing similar structures. Since the antibodies used in TDX and ARIA are not absolutely specific and are responsive to various cocaine metabolites present in urine, it is expected that the apparent concentrations (as expressed in equivalent nanograms per millilitre of benzoylecgonine) obtained by these assays will be higher than those obtained by GC/MS. Thus, a higher cutoff value would have to be adopted for the screening procedure. This cutoff value can be appropriately selected as long as the screening procedure provides a reliable correlation with the GC/MS result. It should be noted that a good screening/confirmation correlation may still exist if the metabolites of high cross-reactivity present in the samples maintain constant ratios with the target compounds in the urine specimen. The correlation plots shown in Figs. 1 and 2 demonstrate that TDX may be more effective in predicting the concentration of benzoylecgonine.

TABLE 1—*Numerical results (in nanograms per millilitre)[a] from Abuscreen, TDX, and GC/MS analysis.*

Sample No.	Abuscreen	TDX	GC/MS	Sample No.	Abuscreen	TDX	GC/MS
1101	146	36	62	1187	280	156	130
1239	538	268	292	1316	445	225	0
1334	506	280	301	1362	415	85	69
1828	608	142	92	30017	329	7	40
30065	212	34	48	32064	484	270	296
32082	610	272	70	32095	265	133	101
32256	265	60	47	33023	395	326	216
33061	300	247	198	34089	438	177	66
34095	470	255	100	37053	265	123	165
38032	562	276	292	38051	384	155	131
38088	273	90	41	38108	317	174	128
40016	433	358	296	40098	405	304	318
40135	588	304	267	41098	382	245	0
41102	590	249	187	42026	472	123	26
42072	441	256	194	43002	283	171	129
43084	227	174	147	44029	300	111	148
45102	327	122	70	46027	218	48	56
46045	511	172	349	46049	410	215	257
47042	269	95	100	47050	185	99	66
49037	288	68	139	49042	381	193	121
49097	518	356	316	50018	239	161	223
50026	420	182	258	50094	239	101	134
50135	298	111	128	51029	470	267	393
51060	445	148	146	51118	333	115	71
53021	600	187	76	53022	485	297	289
53045	392	110	73	55048	320	188	94
55054	535	355	268	55126	419	161	123
57087	234	202	148	57123	246	148	96
58009	361	250	183	58052	497	460	443
61003	208	199	216	61032	616	494	428
61080	510	370	320	64071	429	176	146

[a]The GC/MS concentration is expressed in nanograms of benzoylecgonine per millilitre, while the Abuscreen and TDX concentrations are expressed in equivalent units of measure, based on the calibration curves generated at the time of analysis.

Selection of Appropriate Screening Cutoff Concentration Levels

Since only the results obtained by GC/MS are specific enough for forensic science purposes, the cutoff concentration adopted by a preliminary screening method is selected at a level that will correspond with the cutoff concentration of the target drug/metabolite as determined by GC/MS. The use of an inappropriately low cutoff value will result in too many confirmation testings, producing negative results, and the overall analytical procedure would not be economical. On the other hand, selecting a cutoff value for an immunoassay to avoid producing any unconfirmed presumptive positive is not desirable either. If a cutoff value that will achieve this goal is used, it will have to be set at a relatively high level, thereby reporting negative (false negative) for many samples that are scientifically above the GC/MS cutoff level. The choice of a cutoff level is thus a compromise between how many false negatives one can tolerate and how many unconfirmed presumptive positives one is willing to prove negative by the costly GC/MS test.

Using 300 and 150 ng/mL of benzoylecgonine as the ARIA and the GC/MS cutoff values, ARIA generated 3 false negatives and 21 unconfirmed presumptive positives (Table 2). Using these same cutoff values for TDX and GC/MS assays, TDX produced 16 false negative and no unconfirmed presumptive positive. It appears that the 300-ng/

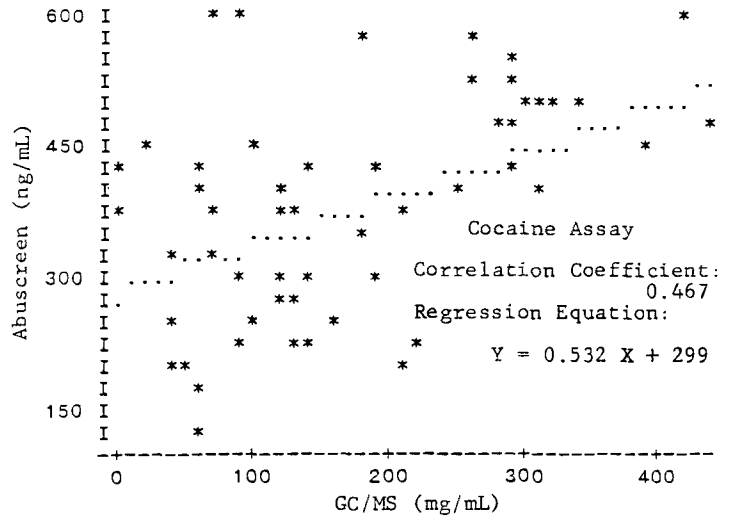

FIG. 1—*Correlation of GC/MS benzoylecgonine concentration versus Abuscreen cocaine metabolites concentration (expressed in terms of benzoylecgonine).*

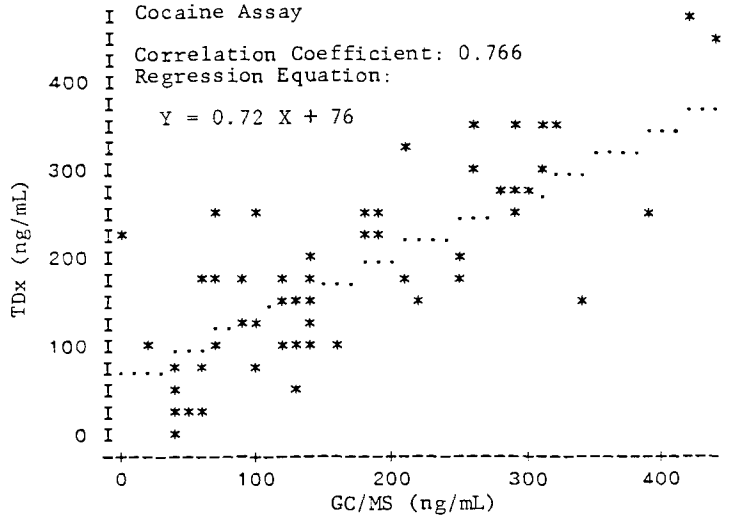

FIG. 2—*Correlation of GC/MS benzoylecgonine concentration versus TDX cocaine metabolites concentration (expressed in terms of benzoylecgonine).*

mL cutoff value was too low for ARIA, but too high for TDX for the selected 150-ng/mL GC/MS cutoff concentration of benzoylecgonine. It is interesting to explore the possibility of calculating an appropriate cutoff value for an initial test method based on the resulting regression equation. Thus, the calculated TDX cutoff value which corresponds to 150 ng/mL benzoylecgonine as determined by GC/MS is $0.766 \times 150 + 76 = 191$ ng/mL, while the corresponding value for ARIA is $0.532 \times 150 + 299 = 379$ ng/mL. Using 380 and 190 ng/mL as the cutoff values for ARIA and TDX, respectively, ARIA generates 5 false negatives and 16 unconfirmed presumptive positives, while TDX

results in 3 false negatives and 6 unconfirmed presumptive positives (Table 2). The difference in the number of "false" results observed in these two initial tests reflects the correlation coefficients that were established between these initial tests and the GC/MS results.

Conclusions

Based on the correlations (Figs. 1 and 2) established between the initial screening and GC/MS methods, it is apparent that TDX is more effective in predicting benzoylecgonine concentrations. Based on the use of 150 ng/mL of benzoylecgonine as the GC/MS cutoff values, an examination on the numbers of unconfirmed presumptive positives and false negatives suggests that 380 (ARIA) and 190 (TDX) ng/mL are more appropriate cutoff values for a cocaine initial assay.

It should be noted that the 62 samples used in this study were selected from 3295 routine specimens. The concentrations of drug metabolites in these selected samples are all near the cutoff values, while others are true negatives or contain either insignificant or high drug/metabolite concentrations that generate comparable results among the TDX, ARIA, and GC/MS methods.

Both ARIA and TDX have become established procedures in the practice of forensic toxicology. Each screening method has its own advantages and disadvantages, and either may be more economical under different sets of testing circumstances. Each testing laboratory will have to consider these circumstances and choose the method that will provide the most efficient screening for the particular drug group under consideration.

TABLE 2—*Possible cutoff values.*

Test Name	Cutoff Concentration[a]		False Negative	False Presumptive Positive
	GC/MS	Initial Test		
ARIA	150	300	37053, 50018, 61003	1316, 1362, 1828, 30017, 32082, 34089, 34095, 38051, 38108, 41098, 42026, 44029, 45102, 49042, 51060, 51118, 53021, 53045, 55048, 55126, 64071
TDX	150	300	1239, 1334, 32064, 33061, 37053, 38032, 41102, 42072, 46045, 46049, 50018, 50026, 51029, 53022, 58009, 61003	
ARIA	150	380	33061, 37053, 50018, 58009, 61003	1316, 1362, 1828, 32082, 34089, 34095, 38051, 41098, 42026, 49042, 51060, 51118, 53021, 53045, 55126, 64071
TDX	150	190	46045, 50026, 61003	1316, 32082, 34095, 41098, 49042, 57087

[a]The GC/MS concentration is expressed in nanograms of benzoylecgonine per millilitre, while the Abuscreen and TDX concentrations are expressed in equivalent units of measure, based on the calibration curves generated at the time of analysis.

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