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Validity Testing of Commercial Urine Cocaine Metabolite Assays: II. Sensitivity, Specificity, Accuracy, and Confirmation by Gas Chromatography/Mass Spectrometry

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ABSTRACT: A comprehensive validity assessment study was performed on eight commercial urine assays for detection of cocaine use. Sensitivity, specificity, and accuracy of each assay were evaluated by analyzing, in random order and under blind conditions, specimens spiked with known drug concentrations and clinical specimens obtained from human subjects after intravenous cocaine use. Commercial assay results were compared with gas chromatography/mass spectrometry (GC/MS) assay of the same specimens for benzoylecgonine. All of the assays examined were determined to have utility in screening for cocaine use, with the exception of the KDI Quik Test®, which was not a reliable test for detection of cocaine use. Major differences in sensitivity, specificity, and confirmation rate by GC/MS were noted among the assays, differences which should be taken into consideration when implementing a urine screening test for cocaine use or interpreting test results involving use of these assays.

KEYWORDS: toxicology, cocaine, benzoylecgonine, urine, chemical analysis, urine testing, validity, GC/MS assay, TDx, Toxi-Lab, Coat-A-Count, Double Antibody, KDI Quik Test, Abuscreen, EMIT dau, EMIT st

Urine testing of personnel for drugs of abuse is now well established in the military services and in industry. Recent estimates of Fortune 500 companies indicate that 30 to 50% practice pre-employment screening [*1,2*]. Many governmental agencies also are implementing drug-testing programs [*1*]. The reliability of urine testing has been questioned in an early study conducted in 1981 of laboratories engaged in testing for methadone centers [*3*]. Clearly, the technology and practices involved in drug testing have improved since this study [*4*]. Recent technical guidelines published in the *Federal Register* [*5*] for certification of laboratories engaged in urine testing for Federal agencies indicate a requirement of $\leq 10\%$ false negative rate (that is, $\geq 90\%$ of all specified drugs must be detected) and a 0% false positive rate (that is, no incorrect drug identifications are acceptable) on proficiency test specimens. It is probable that in the future these standards will also apply to or be adopted by most laboratories providing drug-testing services to the private sector as well.

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A substantial number of commercial tests can be implemented by drug-testing laboratories in meeting the certification requirements outlined in the guidelines for urine testing of Federal employees [5]. For example, there are at least six commercial immunoassay tests presently available for detection of cocaine use. Validity studies on these and other tests being used for drug screening are needed for assessment of the effects of pharmacologic variables (for example, dose, route of administration, inter-subject variability in metabolism, and excretion rates) on test outcome. Impartial evaluations of the sensitivity, specificity, accuracy, and potential for confirmation by gas chromatography/mass spectrometry (GC/MS) also are needed. This type of commercial test assessment provides the health professional with guideline data for selection of reliable testing methods and also for use in interpretation of test results.

In an earlier study (in this issue), we reported the effects of selected pharmacologic variables upon the performance of eight commercial urine tests for detection of cocaine use [6]. The present study documents the performance of these tests by the additional validity criteria of sensitivity, specificity, accuracy, and confirmation rate by GC/MS. Clinical specimens obtained from five human subjects after intravenous administration of cocaine together with control urines containing known amounts of cocaine metabolites and derivatives were analyzed under blind conditions in randomized order for the presence of cocaine metabolite. Together, these two reports comprise a complete validity-assessment study of most existing commercial urine tests for detection of cocaine use and present comparisons of results with those obtained by a reference GC/MS method.

Materials and Methods

Subjects, Dosing, and Specimen Collection

Five healthy male volunteers with a history of intravenous cocaine abuse participated in the study. The subjects were cocaine-free at the time of the study as indicated by urinalysis of their pre-drug specimens by EMIT® dau assay (300-ng/mL cutoff) for cocaine metabolite. Each subject received a single intravenous dose of 20 mg of *l*-cocaine hydrochloride on the test day. One subject received a second dose of 40 mg of *l*-cocaine hydrochloride on the second day. Detailed subject characteristics, specimen collection, and handling procedures have been described earlier [6]. Following collection and freezing, freshly thawed clinical specimens and standardized urines containing known amounts of cocaine, metabolites, cocaine isomers and derivatives, and other drugs were coded and organized in random order for analysis.

Drugs

Cocaine hydrochloride (*l*-isomer) was purchased from Mallinckrodt Inc., St. Louis, MO. *d*-Cocaine, *l*-pseudococaine, *d*-pseudococaine, *l*-benzoylecgonine, *l*-ecgonine methyl ester, *l*-ecgonine, *l*-benzoylnorecgonine, *l*-pseudoecgonine methyl ester, *d*-pseudoecgonine methyl ester, and *l*-norcocaine were provided by the Research Technology Branch, National Institute on Drug Abuse.

Assays

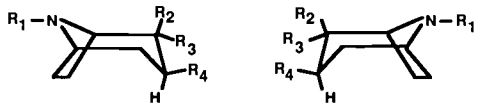
All assays were performed on freshly thawed, identical sets of clinical specimens. The specimens were assayed in random order under blind conditions. Results were decoded only after completion of the assay. Specimen sets were assayed with the following commercial drug testing kits: TDx® Cocaine Metabolite assay (Abbott Laboratories, Irving, TX); Toxi-Lab® Drug Detection Systems (Analytical Systems, Division of Marion Laboratories, Inc.,

Irvine, CA); Coat-A-Count® Cocaine Metabolite radioimmunoassay and Cocaine Metabolite Double Antibody radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA); KDI Quik Test™ Drug Screen (Keystone Diagnostics, Inc., Columbia, MD); Abuscreen® RIA for Cocaine Metabolite (Roche Diagnostics Systems, Nutley, NJ); and EMIT® dau™ Cocaine Metabolite Assay and EMIT® st™ Urine Cocaine Metabolite Assay (Syva Co., Palo Alto, CA). The TDx, Coat-A-Count, Double Antibody, and Abuscreen assays were evaluated in the quantitative mode by means of a benzoylecgonine standard curve; the Toxi-Lab, EMIT dau, and EMIT st assays were evaluated in the qualitative mode employing a 300-ng/mL benzoylecgonine calibrator when appropriate. Manufacturer's procedures were followed for all assays with the exception of a modification of the Toxi-Lab system. Standard Toxi-Lab A procedures were employed for detection, but only Stage IV dip in Dragendorff reagent was used for visualization of cocaine. Benzoylecgonine was detected by the Toxi-Lab Benzoylecgonine Special Procedure. All quantitative assay data are reported as nanogram/millilitre equivalents of benzoylecgonine.

The measurement of benzoylecgonine in urine by GC/MS was performed according to the procedure for confirmation of benzoylecgonine employed by the Navy Drug Screening Lab, Norfolk, VA, as described earlier [7]. The presence of benzoylecgonine was determined on the basis of three criteria: comparison of the relative retention times of specimens to standardized urine containing benzoylecgonine; occurrence of appropriate selected ions; and comparison of ion ratios of processed standards with those obtained for the clinical specimens. A specimen was assayed for benzoylecgonine only if all three criteria were within established ranges. Quantitation was performed by means of internal standardization with deuterated standard. Under the conditions employed, the sensitivity for benzoylecgonine was 10 ng/mL. The lower limit for quantitation of benzoylecgonine by this method was 20 ng/mL.

Determination of Specificity

The cross-reactivities of cocaine isomers and a variety of metabolites and derivatives (Fig. 1) were determined for seven commercial cocaine metabolite assays and the GC/MS assay for benzoylecgonine (Table 1) by measuring apparent benzoylecgonine concentrations of



L-ISOMER D-ISOMER

R_1	R_2	R_3	R_4	COMPOUND
CH_3	$COOCH_3$	H	$OCOC_6H_5$	COCAINE
CH_3	COOH	H	$OCOC_6H_5$	BENZOYLECGONINE
CH_3	$COOCH_3$	H	OH	ECGONINE METHYL ESTER
CH_3	COOH	H	OH	ECGONINE
H	$COOCH_3$	H	$OCOC_6H_5$	NORCOCAINE
H	COOH	H	$OCOC_6H_5$	BENZOYLNORECGONINE
CH_3	H	$COOCH_3$	$OCOC_6H_5$	PSEUDOCOCAINE
CH_3	H	$COOCH_3$	OH	PSEUDOECGONINE METHYL ESTER

FIG. 1—*Structure of optical isomers of cocaine, cocaine metabolites, and cocaine derivatives tested for cross-reactivity in commercial assays.*

TABLE 1—*Cross-reactivity of cocaine, cocaine metabolites, isomers, and derivatives in urine tests for detection of cocaine use.*

Assay	Cross-Reactivity,[a] %										
	l-BE	*l*-COC	*l*-EME	*l*-ECG	*l*-BNE	*l*-NCOC	*d*-COC	*d*-PCOC	*l*-PCOC	*l*-PEME	*d*-PEME
GC/MS	109.0[b]	7.1[c]	0	0	1.4	0	6.6[c]	0	0	0	0
TDx	95.7[b]	1.2	0.1	1.1	1.2	0.1	0.2	0.1	0.1	0.1	0.2
Toxi-Lab (BE)	pos[d]	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
Toxi-Lab (COC)	neg	pos[e]	neg	neg	neg	neg	pos	neg	neg	neg	neg
Coat-A-Count	104.0[b]	7259.0[f]	1.3	5.6	1.9	63.5[f]	7.4	1.0	0.1	0.3	0.3
Double Antibody	95.0[b]	5498.0[f]	5.5	9.9	3.4	83.8[g]	19.3	0.5	0	0.7	0.1
Abuscreen	108.0[b]	215.0[b]	0.6	0.8	1.3	5.1	0.4	0.5	0.1	0.1	0.1
EMIT dau	pos[b]	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
EMIT st	pos[b]	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

[a]Percent cross-reactivity is defined as: (apparent benzoylecgonine concentration/concentration of added standard) × 100. Cross-reactivity was determined at 5000 ng/mL concentration of standard in control urine unless otherwise noted. BE = benzoylecgonine; COC = cocaine; EME = ecgonine methyl ester; ECG = ecgonine; BNE = benzoylnorecgonine; NCOC = norcocaine; PCOC = pseudococaine; PEME = pseudoecgonine methyl ester.

[b]Cross-reactivity was determined at 300-ng/mL concentration in control urine.

[c]Cross-reactivity was likely due to trace hydrolysis of cocaine to benzoylecgonine.

[d]Tested positive at 1000-ng/mL concentration in control urine.

[e]Tested positive at 150-ng/mL concentration in control urine.

[f]Cross-reactivity was determined at 50-ng/mL concentration in control urine.

[g]Cross-reactivity was determined at 500-ng/mL concentration in control urine.

analytes in varying concentrations in control urine. The cross-reactivities of these compounds in the Toxi-Lab assay for cocaine also were determined in the same manner. For those assays evaluated in the quantitative mode, percent cross-reactivity was defined as: (apparent benzoylecgonine concentration/concentration of the added standard) × 100. For the assays evaluated in the qualitative mode, cross-reactivity was recorded as positive (identified as benzoylecgonine) or negative based on detection at the specified concentration. In the Toxi-Lab assay for cocaine, cross-reactivity was recorded as positive or negative for detection of cocaine. Generally, the cross-reactivities of the analytes were determined at 5000-ng/mL concentration in control urine unless the apparent benzoylecgonine concentration measured in excess of the highest standard on the standard curve. If this occurred, the concentration of the analyte was reduced to a level which appeared on the standard curve constructed for that assay.

Results

Assessment of Sensitivity and Accuracy of Commercial Assays with Spiked Specimens

Assay responses by TDx, Coat-A-Count, Double Antibody, and Abuscreen were linear across a wide range of standard benzoylecgonine concentrations (50 to 5000 ng/mL) added to control urine (Fig. 2). At the lowest concentration tested (50 ng/mL), quantitative measures ranged from a mean of 45.5 ng/mL for the Double Antibody assay to 66.6 ng/mL by the Coat-A-Count assay (Table 2). The precision of the measures at this concentration, as measured by the coefficient of variation (CV), varied among the four commercial assays from 3.9% for Coat-A-Count to 13.2% for Abuscreen. Also, the magnitude of responses by the four assays with the 50-ng/mL standard was well removed from equivalent responses with drug-free control urine indicating that sensitivity was < 50 ng/mL for benzoylecgonine. The mean GC/MS determination for benzoylecgonine with the 50-ng/mL standard was 73.7 ng/mL with a CV of 33%. Accuracy and precision were generally high for GC/MS and the four commercial assays in measuring benzoylecgonine concentrations ranging from 150 to 2500 ng/mL. At the highest concentration tested, 5000 ng/mL, accuracy and precision were high for TDx and Abuscreen, but were somewhat variable for the other assays (Table 2).

Detection by EMIT dau and EMIT st (300-ng/mL cutoff) of benzoylecgonine added to control urine was 67% accurate (two of three were positive) at 300-ng/mL concentration and was 100% accurate at all higher concentrations. Below 300-ng/mL concentration, no positives were recorded.

Toxi-Lab detection of benzoylecgonine was 100% accurate at a standard concentration of 1000 ng/mL and higher and was consistently negative below 1000 ng/mL. Toxi-Lab detection of standard cocaine in control urine was 100% accurate at concentrations ≥ 150 ng/mL.

KDI Quik Test detection of standard benzoylecgonine in control urine was not accurate due to color interference from control urine. This high background interference resulted in production of a high rate of false positives in all specimens.

Specificity of Commercial Assays for Benzoylecgonine

Cross-reactivity of the cocaine metabolites, *l*-ecgonine methyl ester, *l*-ecgonine, and *l*-benzoylnorecgonine generally was $< 10\%$ of that of benzoylecgonine in all quantitative assays and was not detectable in the qualitative assays (Table 1). Cross-reactivity was extremely low ($< 1\%$) for the geometric isomer of *l*-cocaine, *l*-pseudococaine, and for the geometric isomer of *l*-ecgonine methyl ester, *l*-pseudoecgonine methyl ester, as well as their respective optical isomers, *d*-pseudococaine and *d*-pseudoecgonine methyl ester. Cross-reactivity also was low ($< 10\%$) or was not detectable for *l*-cocaine, *d*-cocaine, and *l*-norcocaine in all assays except

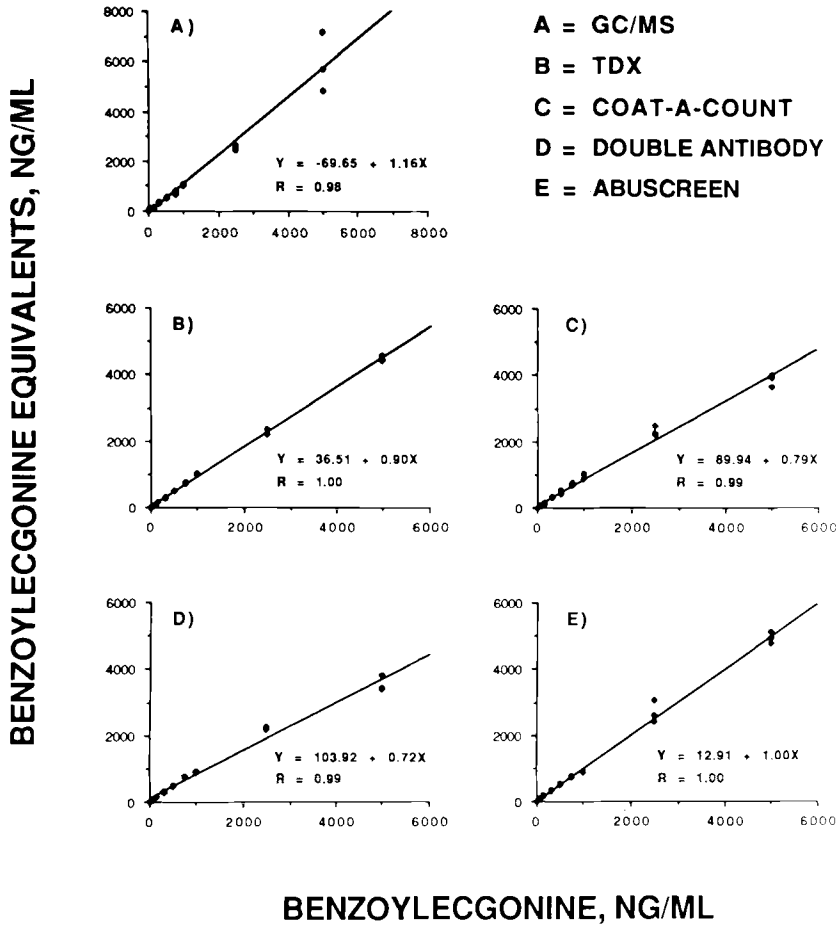


FIG. 2—*Benzoylecgonine standard curves by GC/MS assay and by commercial cocaine metabolite assays. Benzoylecgonine standard solutions were prepared in control urine and analyzed together with clinical specimens in randomized order under blind conditions.*

Coat-A-Count, Double Antibody, and Abuscreen. The pattern of cross-reactivity of the Coat-A-Count and Double Antibody assay with cocaine was similar in that both exhibited very high cross-reactivity (>5000% at 50-ng/mL concentration), high cross-reactivity to *l*-norcocaine (>60% at 500-ng/mL concentration), and low-to-moderate cross-reactivity to *d*-cocaine (7.4–19.3% at 5000-ng/mL concentration). Abuscreen displayed high cross-reactivity to *l*-cocaine (215% at 300-ng/mL), low cross-reactivity with *l*-norcocaine (5.1% at 5000-ng/mL concentration), and very low cross-reactivity with *d*-cocaine (<1%).

Cross-reactivity in the GC/MS assay was recorded only for *l*-cocaine, *d*-cocaine, and *l*-benzoylnorecgonine. These responses were likely due to slight hydrolysis of cocaine to benzoylecgonine and to a slight impurity of benzoylecgonine in the *l*-benzoylnorecgonine standard.

Assessment of cross-reactivities of analytes (Fig. 1) in the Toxi-Lab assay for detection of cocaine also was made. Both *l*-cocaine and *d*-cocaine were equally responsive in producing positive results, whereas other analytes tested did not interfere in the detection of cocaine.

TABLE 2—*Accuracy of assay determinations of benzoylecgonine concentration in standardized control urine.*

Benzoylecgonine Concentration, ng/mL	Assayed Concentration, ng/mL (Standard Deviation, $N = 3$)				
	GC/MS	TDx	Coat-A-Count	Double Antibody	Abuscreen
0	0	0	1.3	0	4.3
	(0)	(0)	(2.3)	(0)	(1.9)
50	73.7	46.7	66.6	45.5	64.1
	(24.6)	(5.8)	(2.6)	(5.6)	(8.5)
150	119.3	150.0	149.7	145.4	173.7
	(29.0)	(10.0)	(18.8)	(7.7)	(11.0)
300	328.3	286.7	313.2	284.8	323.4
	(19.3)	(5.8)	(13.6)	(10.8)	(9.9)
500	525.7	493.3	493.2	472.7	522.9
	(42.0)	(5.8)	(61.9)	(24.2)	(28.5)
750	747.3	740.0	724.4	737.8	736.6
	(60.1)	(10.0)	(46.1)	(14.9)	(23.2)
1000	1045.3	1016.7	922.3	906.3	899.7
	(36.2)	(11.5)	(81.8)	(26.1)	(25.0)
2500	2569.3	2306.7	2320.6	2205.2	2680.4
	(104.0)	(92.4)	(142.3)	(29.1)	(336.7)
5000	5904.7	4496.7	2536.0	3544.0	4940.3
	(1172.9)	(80.2)	(2201.4)	(231.8)	(163.9)

Other standard analytes which were tested for cross-reactivity but are not listed in Table 1 included morphine, lidocaine, and fencamfamine. Clinical specimens collected from human subjects within 24 h after lidocaine administration, ibuprofen administration, and marijuana use also were tested for cross-reactivity. Cross-reactivity was negligible (<1%) for each of the analytes or specimens in all assays.

Concordance of Results of Commercial Assay of Clinical Specimens with GC/MS Assay

The results of commercial assay of clinical specimens for cocaine metabolite by TDx, Coat-A-Count, Double Antibody, and Abuscreen are shown in Fig. 3 versus GC/MS assay for benzoylecgonine. Data points were not included for specimens with metabolite concentrations exceeding those of the standard curve. The solid line represents the best fit of the data by least squares linear regression analysis. Regression parameters for each of the quantitative assays are shown in Table 3 along with a similar regression analysis of the GC/MS assay of benzoylecgonine standards. Deviation from a slope of 1.0 and an intercept of 0.0 was considered an indication of possible relative bias and fixed bias, respectively, in reference to the GC/MS assay for benzoylecgonine. Comparison of the regression lines (solid lines, Fig. 3) for the commercial assays of clinical specimens to regression lines (dotted lines, Fig. 3) for commercial assay of benzoylecgonine standards in control urine indicated the potential for relative bias in each of the assays. The degree of relative bias increased in the four assays in the following order:

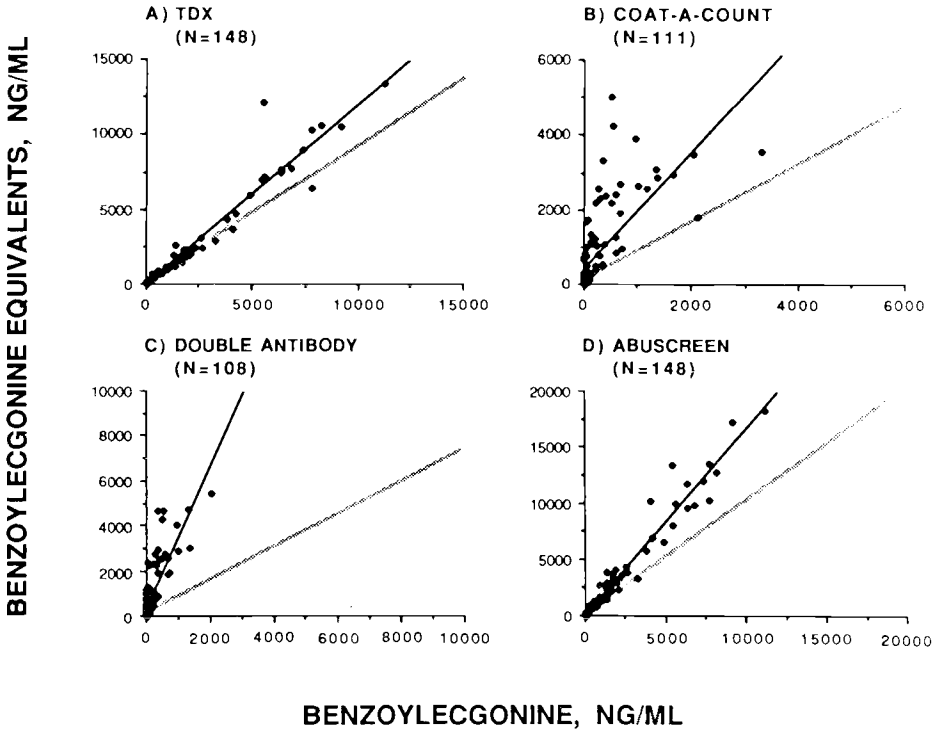


FIG. 3—*Methods comparison of commercial cocaine metabolite assays of clinical specimens versus GC/MS assay for benzoylecgonine. Solid line shows least-squares linear regression fit of data points. Dotted line shows least-squares linear regression fit for benzoylecgonine standard curve from Fig. 2.*

TDx < Abuscreen < Coat-A-Count < Double Antibody

The analysis of variance of results from each commercial assay (dependent variable) versus GC/MS (independent variable) indicated that >95% of the variance could be ascribed to regression (Table 3, percent regression sums of squares) for the TDx and Abuscreen assays and <5% was due to random variation (Table 3, percent residual sums of squares), whereas regression accounted for 49.9 and 67.5% of the variance in the Coat-A-Count and Double Antibody assays, respectively, with the remainder representing random variance. The linear regression analysis of GC/MS assay of benzoylecgonine standards, also shown in Table 3, indicated that the amount of variance contributed by this assay to the methods comparison analysis was relatively low (<4% random variation).

A complete tabulation of the concordance of positive/negative results for each of the commercial assays with GC/MS by concentration range is given in Table 4. Commercial tests were determined to be positive if the concentration of benzoylecgonine equivalents was ≥300 ng/mL or alternatively, for the qualitative tests, if the test result displayed characteristics specified by the manufacturer for a positive result. Generally, all specimens which contained ≥300 ng/mL of benzoylecgonine by GC/MS tested positive by commercial assay, with the exception of the Toxi-Lab assay, which displayed a 14.8% false negative rate, and the KDI Quik Test, which displayed a 4.7% false negative rate. Specimens which contained <20 ng/mL of benzoylecgonine by GC/MS generally were negative by commercial assay with the exceptions of Coat-A-Count, which displayed a 3.4% false positive rate, Double Antibody, which displayed a 4.1% false positive rate, and the KDI Quik Test, which displayed a 23.6% false positive rate.

Discussion

Although forensic drug testing for cocaine abuse is widespread and the analytical methods employed are varied, there is a paucity of data on comparison of commercial methods. In 1977, Wallace et al. [*8*] reported the evaluation of early versions of the EMIT dau and Abuscreen assays for detection of cocaine and benzoylecgonine added to control urine. On the basis of that evaluation, the authors concluded that the Abuscreen assay was more sensitive than EMIT dau and displayed more cross-reactivity with cocaine. Also in 1977, Hamilton et al. [*9*] reported comparison of methods for the detection of cocaine and benzoylecgonine in the urine of human subjects who had received 1.5 mg/kg of cocaine hydrochloride. This study included a comparison of EMIT dau, Abuscreen, GC, and thin-layer chromatography (TLC) for detection of benzoylecgonine and cocaine, where applicable. Detection times for benzoylecgonine were longest by Abuscreen, substantially exceeding detection times by the other methods examined. Since these early reports, there has been relatively little evaluation of commercial methods for detection of cocaine metabolite in urine. Recently, Clark and Hajar [*10*] compared the use of EMIT dau for detection of cocaine metabolite with TLC and GC methods for detection of cocaine and ecgonine methyl ester, a metabolite of cocaine [*11*], and concluded that detection of ecgonine methyl ester and cocaine was as sensitive a test for cocaine use as was EMIT dau. Also, Poklis [*12*] recently reported an evaluation of the TDx and EMIT dau assay for detection of cocaine metabolite in 120 clinic urines found positive for benzoylecgonine by high-performance, liquid chromatography (HPLC). It was found that 116 specimens were positive by both methods and 2 negatives were produced by each commercial method.

The present studies were designed to provide a comprehensive validity assessment of most existing commercial urine assays for detection of cocaine abuse. Based on the assumption that each of these assays could be used as the initial "screening" test and would be followed by a confirmation test by GC/MS, the factors considered most important to evaluate included sensitivity, specificity, and accuracy of each method as well as the concordance of

TABLE 3—*Linear regression statistics of cocaine metabolite assays of clinical specimens versus GC/MS and of GC/MS standard curve for benzoylecgonine.*

Assay Versus GC/MS (N)	Slope	Intercept	Correlation	%Regression SS[a]	%Residual SS[a]
TDx (148)	1.20	−18.31	0.98	95.1	4.9
Coat-A-Count (111)	1.56	426.87	0.71	49.9	50.1
Double Antibody (108)	3.14	347.13	0.82	67.5	32.5
Abuscreen (148)	1.64	43.99	0.98	96.3	3.7
BE STD Curve[b] (27)	1.16	−69.64	0.98	96.5	3.5

[a]Regression SS and %Residual SS = percent regression sums of squares (amount of variance accounted for by regression) and percent residual sums of squares (random variance).

[b]BE STD Curve = Benzoylecgonine standard curve.

TABLE 4—*Concordance of positive/negative results of commercial assays of 148 clinical specimens for cocaine metabolite with quantitative results of GC/MS assay for benzoylecgonine.*

Commercial Assay	Result, Pos/Neg[a]	GC/MS Concentration, ng/mL						%False Pos[b]	% False Neg[c]
		>300	299-200	199-150	149-100	99-20	<20		
TDx	+	61	4	0	0	0	0	0	...
	−	0	4	2	3	35	39	...	0
Toxi-Lab (BE)	+	39	0	0	0	0	0	0	...
	−	22	8	2	3	35	39	...	14.9
Coat-A-Count	+	61	8	2	2	13	5	3.4	...
	−	0	0	0	1	22	34	...	0
Double Antibody	+	61	8	2	3	15	6	4.1	...
	−	0	0	0	0	20	33	...	0
KDI Quik Test	+	54	8	2	3	35	35	23.6	...
(Reader No. 1)	−	7	0	0	0	0	4	...	4.7
Abuscreen	+	61	6	1	2	1	0	0	...
	−	0	2	1	1	34	39	...	0
EMIT dau	+	61	6	0	0	0	0	0	...
	−	0	2	2	3	35	39	...	0
EMIT st	+	61	6	0	0	0	0	0	...
	−	0	2	2	3	35	39	...	0

[a]Pos/Neg = determination of positive or negative test for cocaine metabolite with a concentration of benzoylecgonine equivalent ≥300 ng/mL or alternately, according to the specifications by the test manufacturer for a positive or negative test.

[b]Percentage of specimens testing positive by commercial assay which had <20 ng/mL of benzoylecgonine by GC/MS assay.

[c]Percentage of specimens testing negative by commercial assay which had ≥300 ng/mL of benzoylecgonine by GC/MS assay.

results with GC/MS assay. A summary of the conclusions of this validity assessment of the commercial assays is shown in Table 5. Generally, sensitivity did not appear to be a factor in the performance of the assays with the exception of Toxi-Lab, in which lower sensitivity for benzoylecgonine resulted in production of a substantial number of false negatives. This occurred primarily for specimens containing benzoylecgonine in the concentration range of 300 to 1000 ng/mL.

The specificity of the immunoassays examined varied considerably, particularly for cocaine. The TDx, EMIT dau, and EMIT st assays demonstrated very low cross-reactivities with cocaine, resulting in high concordance of results with GC/MS assay for benzoylecgonine. The low cross-reactivity of EMIT dau for cocaine was noted earlier by Wallace et al. [*8*]. The results for TDx cross-reactivity with cocaine are in agreement with the manufacturer's reported cross-reactivity of 1.1 to 1.4% and the report by Poklis [*12*] on the evaluation of the TDx Cocaine Metabolite Assay, but are in contrast to those reported by Baselt and Baselt [*13*], of 19.9%. The high specificity of the TDx assay in our studies also resulted in accurate quantitative determinations (low bias) of benzoylecgonine in reference to the GC/MC assay. In contrast, the accuracy of the Abuscreen was somewhat reduced (moderate bias), and there was evidence of substantial bias in the Coat-A-Count and Double Antibody assays, most likely as a result of their cross-reactivities with cocaine and cocaine-related metabolites. Although the introduction of this type of bias in the quantitative determination of cocaine metabolite by the immunoassays will occasionally result in the production of an initial false positive, as indicated in Table 5 for the Coat-A-Count and Double Antibody assays, it is likely that these specimens are "true positives" in that they presumably contained cocaine-related constituents not detected by GC/MS. It may be that the enhanced sensitivity of these assays as a result of their cross-reactivity with cocaine would be an advantage in certain testing situations, such as saliva and hair testing.

With the exception of cocaine and norcocaine, a minor cocaine metabolite, the cross-reactivities of the assays of Table 5 toward other cocaine metabolites was generally less than 10%. A summary by Baselt [*14*] of existing cross-reactivity data for cocaine metabolites in the Abuscreen and EMIT dau assay is consistent with the present findings, with the exception of reporting 47% cross-reactivity for benzoylnorecgonine in contrast to the present findings of 1.3%. Norcocaine also was reported to have a higher cross-reactivity (33 versus 5.1%) than in the present study.

Cross-reactivities toward the optical isomer of cocaine, *d*-cocaine, were low for all assays, including those assays which displayed substantial cross-reactivity with cocaine (*l*-cocaine). Geometric isomers (pseudo-isomers) of cocaine and ecgonine methyl ester also failed to show any substantial cross-reactivity in the assays examined in the present study.

Overall, the potential usefulness of each of the assays of Table 5 for screening for cocaine metabolite was considered to be high, with the exception of the KDI Quik Test assay. This latter assay was not a reliable indicator for the presence of cocaine-related metabolites in urine. Also, use of Toxi-Lab in screening for benzoylecgonine would result in a substantial number of false negatives. The significance of these findings is that most of the existing commercial cocaine metabolite assays examined can be used in a reliable manner for detection of cocaine use in human specimens, but that individual assay performance characteristics (cocaine metabolite detection times, sensitivity, specificity, accuracy, and confirmation rate by GC/MS) vary considerably and should be considered when implementing cocaine testing procedures or interpreting test results.

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TABLE 5—*Summary of validity assessment of commercial assays for cocaine metabolite.*

Assay	Cutoff, ng/mL	Sensitivity,[a] ng/mL	Specificity[b]	Bias[c]	False[c] Positives	False[c] Negatives	Screening[d] Utility
TDx	300	30	high	low	no	no	high
Toxi-Lab	NA[e]	1000	high/none[f]	NA	no	yes	limited sens, detects cocaine
Coat-A-Count	300	25	high cross-reactivity with cocaine	substantial	yes	no	high, detects cocaine
Double Antibody	300	6	high cross-reactivity with cocaine	substantial	yes	no	high, detects cocaine
KDI Quik Test	NA	unknown	none	NA	yes	yes	none
Abuscreen	300	5	cross-reacts with cocaine	moderate	no	no	high, detects cocaine
EMIT dau	300	NA	high	NA	no	no	high
EMIT st	300	NA	high	NA	no	no	high

[a]Manufacturer's sensitivity specifications.
[b]Specificity of benzoylecgonine.
[c]As defined in Table 4.
[d]Overall validity assessment of assay as a urine screen for the detection of cocaine use.
[e]NA = not applicable.
[f]No specificity between optical isomers.

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