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Validity Testing of Commercial Urine Cocaine Metabolite Assays: I. Assay Detection Times, Individual Excretion Patterns, and Kinetics After Cocaine Administration to Humans

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ABSTRACT: A validity study of eight commercial urine assays for detection of cocaine metabolite was performed on clinical specimens collected from human subjects who received single 20-mg intravenous doses of cocaine hydrochloride. The specimens were collected under controlled conditions and analyzed in random order under blind conditions. Benzoylecgonine concentration in each specimen also was determined by gas chromatography/mass spectrometry (GC/MS). Mean times of detection of the last positive specimen ($\geq$300 ng/mL of benzoylecgonine equivalents) after cocaine administration varied among seven of the commercial tests from 16.9 to 52.9 h in the following ascending order: Toxi-Lab® < TDx® = EMIT® dau™ = EMIT® st™ < Abuscreen® < Coat-A-Count® = Double Antibody. In contrast, a commercial spot test (KDI Quik Test™) which was evaluated for detection of cocaine metabolite produced both false positives and false negatives for benzoylecgonine and was not considered to be a valid test for detection of cocaine metabolite. Half-lives of excretion of benzoylecgonine among four subjects varied from 5.9 to 7.9 h, and overall recovery of benzoylecgonine varied from 15.0 to 34.3% of the administered dose of cocaine.

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

According to the 1985 National Household Survey on Drug Abuse, over 12 million people in the United States used cocaine in the year before the survey [*1*]. Equally disturbing statistics on cocaine appear in the Drug Abuse Warning Network (DAWN) tabulation of Emergency Room Mentions. Average monthly "mentions" for cocaine rose from 815 in 1984 to 1852 for 1986 [*2*]. These data indicate that increasing numbers of users are unable to cope with the toxic side effects of cocaine and are requiring emergency medical intervention. In an

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attempt to combat the spread of abuse of cocaine and other psychoactive drugs into the workforce, many employers in private industry, the military, and the public sector have resorted to urine testing of job applicants and employees. Now, literally millions of drug tests are performed yearly in an effort to eliminate drugs from the worksite.

Concurrent with the increased use of drug testing over the past five years, numerous new assays for the detection of drugs have been developed and introduced into the marketplace. Presently, there are at least eight commercial tests for cocaine metabolite in urine. These assays are based on a variety of technologies including radioimmunoassay (RIA), enzyme immunoassay, fluorescent polarization immunoassay, and chromatographic methods. Accordingly, there is a critical need for systematic evaluation of the performance of these divergent tests on validated clinical specimens.

This paper details an assessment of the performance of eight commercial drug tests for detection of cocaine metabolite in clinical specimens. The specimens were collected under controlled conditions from five human subjects after cocaine administration. The purpose of this study was to determine the influence of pharmacologic factors upon assay results and also to make inter-assay comparisons of assay detection times after cocaine use. The dose of cocaine and the route of administration were the same for all subjects, allowing comparison of individual excretion patterns, kinetics, and assay detection times for cocaine metabolite. The specific concentration of benzoylecgonine in each specimen also was determined via a reference gas chromatographic/mass spectrometric (GC/MS) assay [*3*].

Materials and Methods

Subjects, Dosing, and Specimen Collection

Five healthy male volunteer subjects (A to E) with histories of intravenous cocaine abuse participated in the study. The characteristics of the subjects, their urine admission test results, and their reported last use of cocaine are given in Table 1. The subjects were cocaine-free at the time of the study as determined by urinalysis of their pre-drug specimens by EMIT® dau assay (300-ng/mL cutoff) for cocaine metabolite.

Single intravenous doses of 20 mg of cocaine hydrochloride were administered on the test day. One subject (A) received a second dose of 40 mg of cocaine hydrochloride on the second day. The subjects remained under observation throughout the study. Urine specimens were collected before cocaine administration and ad libitum following drug for varying periods. Each specimen was collected in a polypropylene bottle without additives and refrigerated immediately. Specimens were frozen within 48 h of collection. Freshly thawed clinical specimens and standardized drug urines containing known amounts of cocaine, benzoylecgonine, cocaine isomers and derivatives, and other drugs were decanted into polypropylene contain-

TABLE 1—*Subject characteristics, recent drug use, and drug preference.*

Subject	Age	Weight, kg	Cocaine Dose, mg	Urine Test[a]	Last Use	Drug Preference
A	25	64.8	20 40, Day 2	neg	14 days	cocaine/ marijuana
B	34	67.5	20	neg	7 days	marijuana
C	34	80.0	20	neg	13 days	cocaine
D	28	103.5	20	pos	11 days	cocaine
E	32	72.8	20	neg	2 months	marijuana

[a]Urine test for cocaine metabolite upon admission to clinical research ward. All subjects tested negative by EMIT dau for cocaine metabolite (300-ng/mL cutoff) immediately before participation in study.

ers. A complete specimen set was prepared for each assay. The samples were coded and organized in random order for analysis.

Assays

All assays were performed on freshly thawed and divided sets of clinical specimens. The specimens were assayed in random order under blind conditions, and all specimens and standards were analyzed by each method. 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); 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 [*3*]. Briefly, specimens were treated with internal standard (d_3-benzoylecgonine) and buffer and extracted with a Prep I extractor (DuPont Co.). The evaporated residue was derivatized as the propyl derivative, purified, and analyzed by GC/MS with selected ion monitoring. 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 to 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.

Kinetic Analyses

Kinetic parameters for excretion of benzoylecgonine in urine were determined by the sigma-minus method [*4*]. Subject A was not included in this analysis as a result of receipt of a second dose of cocaine 24 h after the first dose. Total cumulative amount of benzoylecgonine excreted in urine was determined after adjustment for change in molecular weight and is expressed as percent of the administered dose of cocaine (Table 2).

Results

Subject Characteristics and Pre-Drug Testing

The five male subjects who participated in the urinary excretion study of cocaine ranged in age from 25 to 34 years and weighed from 64.8 to 103.5 kg (Table 1). Three subjects reported

TABLE 2—*Half-life and amount of benzoylecgonine excreted in urine of human subjects after a 20-mg intravenous dose of cocaine hydrochloride.*

Subject	Half-Life, h	Percent Dose[a]
B	6.9	16.3
C	6.5	15.0
D	7.9	34.3
E	5.9	32.4
Mean ± SE	6.8 ± 0.4	24.5 ± 5.1

[a]Cumulative amount by GC/MS assay of benzoylecgonine excreted in urine adjusted for molecular weight change and expressed as percent dose of cocaine.

that cocaine was their first drug of choice, and two indicated a preference for marijuana. All subjects had used cocaine by the intravenous route. Four of the subjects reported use of cocaine within the last 14 days. Four of the five subjects tested negative for cocaine metabolite by EMIT dau assay on admission to the research ward and continued to test negative until time of cocaine administration. Subject D was positive for cocaine metabolite on admission but subsequently tested negative by EMIT assay (300-ng/mL cutoff) prior to cocaine administration. GC/MS assay of control specimens collected from the five subjects immediately prior to testing indicated that Subjects A, B, and C were not excreting detectable levels of benzoylecgonine prior to cocaine administration. Control specimens collected from Subjects D and E prior to drug administration had benzoylecgonine equivalent levels of 35 and 23 ng/mL, respectively (Table 3). These determinations were made near the 20-ng/mL cutoff for assay by GC/MS, and identification as benzoylecgonine could not be made by all three criteria established for benzoylecgonine-positive specimens.

Pre-drug specimens also were tested by commercial assays for cocaine metabolite and were negative or tested below cutoffs for all subjects by TDx, Toxi-Lab, EMIT dau and EMIT st assay. Toxi-Lab testing for cocaine was negative as well. Some pre-drug specimens for Subjects B and D were positive (>300-ng/mL benzoylecgonine equivalents) by Coat-A-Count RIA, Double Antibody RIA, and Abuscreen RIA (Figs. 1 to 3). The high background color in the KDI Quik Test produced by pre-drug specimens and validated drug-free urines collected from non-addicts caused substantial interference for detection of cocaine/cocaine metabolite. As a result, all pre-drug specimens tested positive for cocaine use by this assay (Table 3).

Individual Excretion Patterns and Detection Times

Following the intravenous administration of 20 mg of cocaine hydrochloride to five male human subjects, all initial specimens tested positive for cocaine metabolite by all commercial assays and by GC/MS assay for benzoylecgonine. The earliest specimen from any subject was obtained 48 min after dosing from Subject B and contained 1682 ng/mL of benzoylecgonine by GC/MS assay. Peak concentrations of benzoylecgonine by GC/MS ranged from 4000 to 9000 ng/mL and occurred within the first 12 h of cocaine administration. Four of the five subjects also excreted detectable levels of cocaine by Toxi-Lab assay in their first specimen. The longest interval after dosing that cocaine was detectable by Toxi-Lab was 9.3 h (Subject E).

The individual excretion profiles for cocaine metabolite by commercial assay and corresponding benzoylecgonine determination by GC/MS assay are shown in Figs. 1 to 5 and Table 3. The administration of a second intravenous dose of cocaine (40 mg) to Subject A 24 h after the first test dose resulted in an immediate increase in metabolite concentration. As a result of this second dose, Subject A tested positive throughout the collection period.

TABLE 3—*Qualitative detection of cocaine and cocaine metabolite by commercial assays compared with GC/MS assay for benzoylecgonine.*

Time, h	GC/MS Assay, ng/mL	EMIT st	Toxi-Lab		Quik Test	
			Benzoylecgonine	Cocaine	Reader 1	Reader 2
			SUBJECT A			
0	0	−	−	−	+	+
2.0	5627	+	+	+	+	+
5.0	9192	+	+	+	+	+
7.5	6374	+	+	+	+	+
9.5	2570	+	+	−	+	+
11.1	1914	+	+	−	+	+
12.6	2665	+	+	−	−	−
13.3	720	+	−	−	+	+
15.5	1003	+	+	−	+	+
24.4	1521	+	+	−	+	+
26.6	7782	+	+	+	+	−
31.2	11246	+	+	+	+	+
34.8	7807	+	+	−	+	+
37.0	6851	+	+	−	+	+
44.0	4914	+	+	−	+	+
			SUBJECT B			
−4.5	0	−	−	−	+	+
−2.5	0	−	−	−	+	+
−0.5	0	−	−	−	+	+
0.8	1682	+	+	+	+	+
3.1	4095	+	+	+	+	−
4.0	1296	+	+	+	+	+
5.4	1748	+	+	−	+	+
8.7	1415	+	+	−	+	+
10.6	1902	+	+	−	+	+
11.3	645	+	−	−	+	+
12.4	660	+	−	−	+	+
13.5	946	+	−	−	+	−
14.4	432	+	−	−	+	+
19.8	933	+	−	−	+	+

TABLE 3—*Continued.*

Time, h	GC/MS Assay, ng/mL	EMIT st	Toxi-Lab		Quik Test	
			Benzoylecgonine	Cocaine	Reader 1	Reader 2
23.5	346	+	−	−	+	+
26.3	513	+	−	−	+	+
29.3	360	+	−	−	+	+
31.2	219	+	−	−	+	+
32.6	41[a]	−	−	−	+	+
34.1	21[a]	−	−	−	+	+
36.5	70	−	−	−	+	+
36.8	51	−	−	−	+	+
37.6	53	−	−	−	+	+
39.4	34[a]	−	−	−	+	+
42.5	0	−	−	−	+	+
44.7	67[a]	−	−	−	+	+
47.1	41[a]	−	−	−	+	+
50.2	71[a]	−	−	−	+	+
53.2	56[a]	−	−	−	+	+
54.9	23[a]	−	−	−	+	+
56.3	0	−	−	−	+	+
57.2	0	−	−	−	+	+
58.5	0	−	−	−	−	−
60.4	22[a]	−	−	−	+	+
61.0	30[a]	−	−	−	+	+
62.0	0	−	−	−	+	+
62.4	0	−	−	−	+	+
64.8	0	−	−	−	+	+
67.6	0	−	−	−	+	+
69.4	0	−	−	−	+	+
			SUBJECT C			
−4.6	0	−	−	−	+	+
1.5	1184	+	+	+	+	+
5.9	4228	+	+	−	+	+
10.8	3828	+	+	−	+	+
20.0	1623	+	+	−	+	+

34.1	366	+	−	−	+	+	+
36.6	383	+	−	−	+	+	+
43.6	99	−	−	−	+	+	+
61.2	30[a]	−	−	−	+	+	+
67.6	29[a]	−	−	−	+	+	+
71.6	0	−	−	−	+	+	+
		SUBJECT D					
−4.6	0	−	−	−	−	−	−
−1.3	35[a]	−	−	−	−	−	−
2.8	5464	+	+	−	−	−	−
3.6	2126	+	+	−	−	−	−
6.0	7408	+	+	−	−	−	−
7.6	6350	+	+	−	−	−	−
8.4	1749	+	+	−	−	−	−
9.6	2036	+	+	−	−	−	−
11.6	1379	+	+	−	−	−	−
13.4	2308	+	+	−	−	−	−
14.6	1512	+	+	−	−	−	−
16.3	1570	+	+	−	−	−	−
17.1	965	+	+	−	−	−	−
17.8	598	+	+	−	−	−	−
19.5	512	+	+	−	−	−	−
21.5	277	+	+	−	−	−	−
22.6	248	+	+	−	−	−	−
24.0	691	+	+	−	−	−	−
25.3	597	+	+	−	−	−	−
26.9	615	+	+	−	−	−	−
29.3	347	+	+	−	−	−	−
32.4	536	+	+	−	−	−	−
34.0	422	+	+	−	−	−	−
36.5	312	+	+	−	−	−	−
39.1	224	+	+	−	−	−	−
39.9	59	−	−	−	−	−	−
43.9	131	−	−	−	−	−	−
47.4	158	−	−	−	−	−	−
49.7	128	−	−	−	−	−	−
51.7	108	−	−	−	−	−	−
52.9	44	−	−	−	−	−	−

TABLE 3—*Continued.*

Time, h	GC/MS Assay, ng/mL	EMIT st	Toxi-Lab		Quik Test	
			Benzoylecgonine	Cocaine	Reader 1	Reader 2
53.5	27[a]	−	−	−	+	+
54.6	46	−	−	−	+	+
56.5	59	−	−	−	+	+
58.0	32	−	−	−	+	+
59.1	34	−	−	−	+	+
59.9	0	−	−	−	−	−
62.0	57	−	−	−	+	+
63.5	29[a]	−	−	−	+	+
64.5	0	−	−	−	+	+
65.5	0	−	−	−	+	+
67.6	40[a]	−	−	−	+	+
70.5	48	−	−	−	+	+
			SUBJECT E			
−2.8	0	−	−	−	+	+
−0.8	23[a]	−	−	−	+	+
1.3	3302	+	+	+	+	+
4.8	5525	+	+	+	+	+
9.3	8185	+	+	+	+	+
14.5	1335	+	+	−	+	−
19.8	2058	+	+	−	+	+
23.7	680	+	−	−	+	+
27.9	1383	+	−	−	+	+
29.7	208	−	−	−	+	+
33.0	289	+	−	−	+	+
34.6	207	−	−	−	+	+
36.3	268	+	−	−	+	+
39.8	168	−	−	−	+	+
44.1	48	−	−	−	+	+
47.7	36[a]	−	−	−	+	+
50.3	54	−	−	−	+	+
51.2	49	−	−	−	+	+
53.8	0	−	−	−	+	+

55.8	40[a]	−	−	−	+	+
57.8	0	−	−	−	+	+
59.8	0	−	−	−	+	+
63.3	31	−	−	−	+	+
67.6	0	−	−	−	+	+
71.7	0	−	−	−	+	+
75.2	0	−	−	−	+	+
77.5	0	−	−	−	+	+
82.4	0	−	−	−	+	+
85.5	0	−	−	−	+	+
87.5	0	−	−	−	+	+
91.8	0	−	−	−	−	−
94.8	0	−	−	−	+	+
96.8	0	−	−	−	+	+
101.8	0	−	−	−	+	+
104.4	0	−	−	−	+	+
106.5	0	−	−	−	+	+
108.8	0	−	−	−	−	−
111.0	0	−	−	−	+	+
116.0	0	−	−	−	+	+

[a]These specimens did not meet the ion ratio criteria necessary for determination as positive for benzoylecgonine by GC/MS assay.

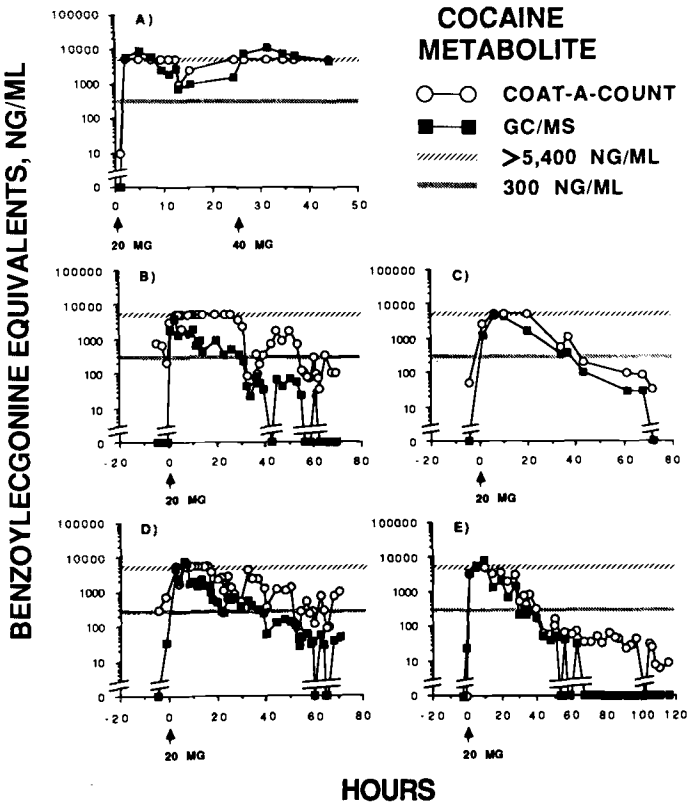

FIG. 1—*Coat-A-Count assay and GC/MS assay of cocaine metabolite in urine of Subjects A to E after intravenous cocaine administration. All specimens were analyzed in randomized order under blind conditions.*

For the remaining four subjects, cocaine metabolite concentrations peaked within hours of drug administration and declined rapidly to near detection limits over a period of approximately 48 to 72 h.

Differences between commercial assay performance were made on the basis of comparison of the commercial assay results to those produced by GC/MS assay. Of the four assays evaluated quantitatively, that is, TDx, Coat-A-Count, Double Antibody, and Abuscreen, the TDx results most closely paralleled the GC/MS data (Fig. 5), followed by Abuscreen (Fig. 3). Both the Coat-A-Count (Fig. 1) and the Double Antibody (Fig. 2) assays produced quantitative results for cocaine metabolite (benzoylecgonine equivalents) that were substantially higher than results by GC/MS assay for benzoylecgonine.

Comparison of commercial cocaine assays also was made on the basis of comparison of detection times of each assay to those produced by GC/MS assay for benzoylecgonine. The time to detection of the last cocaine metabolite positive specimen and the time to detection of the first negative specimen for all assays with the exception of the KDI Quik Test are shown in Table 4. The mean detection times by GC/MS assay for the last positive specimen and for the first negative specimen employing either a 150- or 300-ng/mL benzoylecgonine cutoff were not significantly different from the corresponding results by the seven commercial assays of Table 4 with the exception of Toxi-Lab. The mean detection time by Toxi-Lab to the

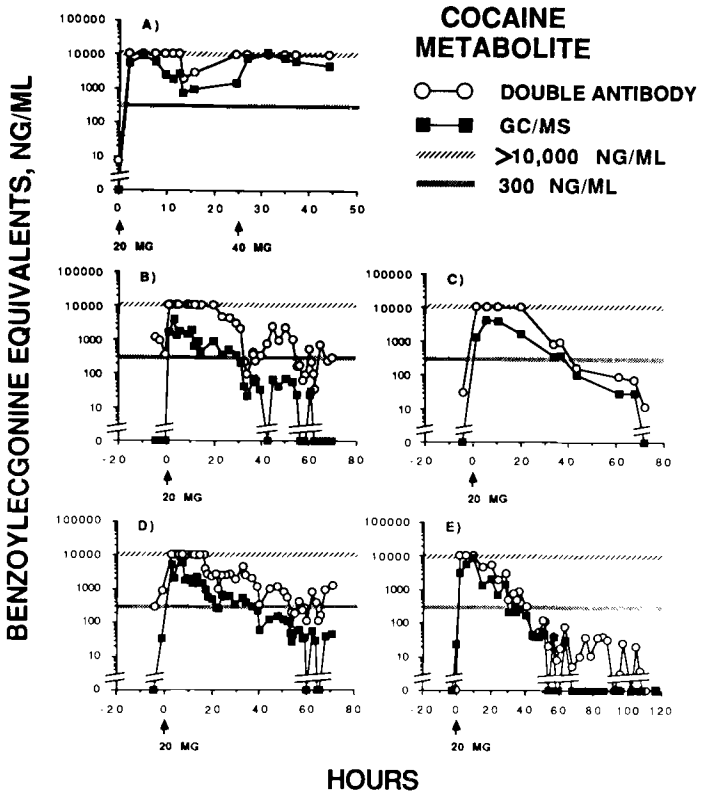

FIG. 2—*Double Antibody assay and GC/MS assay of cocaine metabolite in urine of Subjects A to E after intravenous cocaine administration. All specimens were analyzed in randomized order under blind conditions.*

last positive for benzoylecgonine was significantly ($p < 0.05$) shorter than by GC/MS assay.

Kinetics of Benzoylecgonine Excretion in Urine

The half-lives of elimination of benzoylecgonine in urine were determined for Subjects B through D following a 20-mg intravenous dose of cocaine hydrochloride (Table 2). The calculations were based on Benzoylecgonine concentrations determined by GC/MS assay. The half-lives varied from 5.9 to 7.9 h, with a mean ± SE (standard error) for the four subjects of 6.8 ± 0.4 h. The total amount of benzoylecgonine excreted in urine, expressed as percent dose of cocaine, varied from 15.0 to 34.3%, with a mean ± SE of 24.5 ± 5.1%.

Discussion

Commercial assay evaluations are best performed in the context of validity assessment studies. The "validity" of a drug testing method has been succinctly defined by Gorodetzky [5] as "its ability to detect a drug or its metabolites in biological fluids after human use." Implicit in this definition is the use of certified clinical specimens obtained over time following drug administration for validity assessment studies. "Spiked" specimens are not an ade-

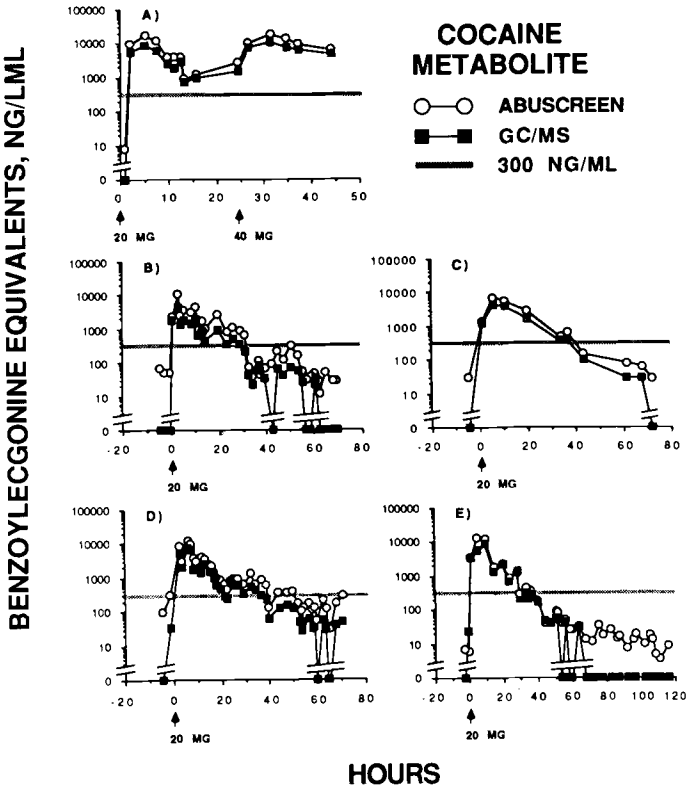

FIG. 3—*Abuscreen RIA and GC/MS assay of cocaine metabolite in urine of Subjects A to E after intravenous cocaine administration. All specimens were analyzed in randomized order under blind conditions.*

quate substitute. A validity study necessitates a consideration of pharmacological factors such as dose, route of administration, elapsed time after dosing, subject hydration, and individual variability in metabolism and excretion rates when assay performance of clinical specimens is being evaluated. A comprehensive validity assessment study must also include consideration of chemical factors such as sensitivity, specificity, and accuracy of the assay. In meeting current forensic science standards for drug testing, one must also evaluate the confirmation rate by a more specific technology, for example, GC/MS. Data on the systematic evaluation of each factor's importance in influencing the overall test outcome will enable health professionals to provide informed opinions and interpretations on the presence or absence of a drug of abuse in clinical specimens.

With the widespread use of urine testing for cocaine currently underway, validity data are critically needed on the commercial assays for detection of cocaine metabolite (benzoylecgonine). Although earlier reports have appeared in which the performance of cocaine and cocaine metabolite assays were compared [*6,7*], a number of new commercial assays have been introduced since these reports. Presently, there are no comprehensive assessments of these assays. In the present study, pharmacologic factors related to individual differences in metabolism and excretion rate of cocaine were assessed for eight commercial assays and by GC/MS. The clinical specimens were collected under conditions in which the dose and route of administration were maintained constant. Testing cocaine specimens that were col-

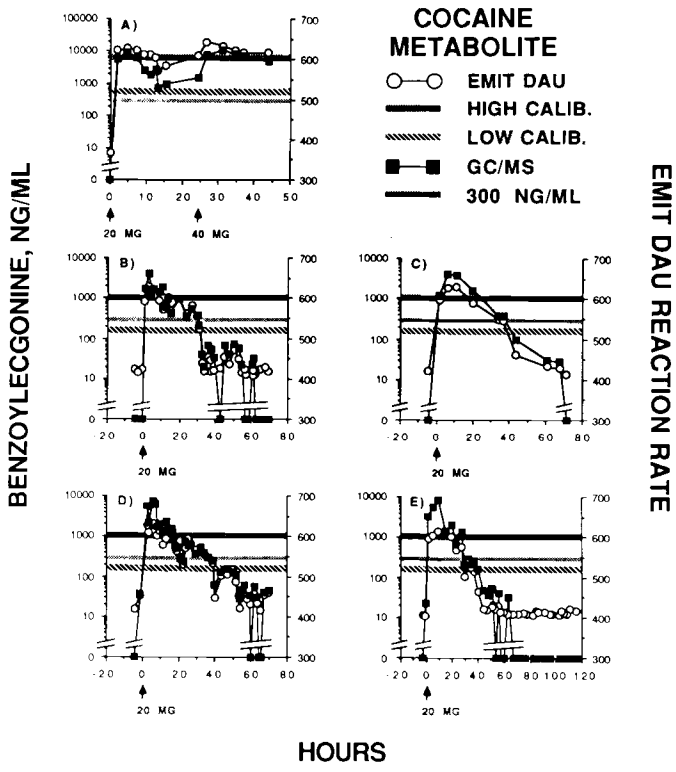

FIG. 4—*EMIT dau assay and GC/MS assay of cocaine metabolite in urine of Subjects A to E after intravenous cocaine administration. All specimens were analyzed in randomized order under blind conditions. The low calibrator concentration of benzoylecgonine was 300 ng/mL and the high calibrator concentration was 3000 ng/mL.*

lected over time after drug administration allowed determination of the maximum time for detection of the parent drug or metabolite or both by each assay. These measures of assay detection times are benchmarks for making inter- and intra-assay comparisons, given all the differences among the assays and the inter-subject variability. Detection times to the last positive specimen for benzoylecgonine by GC/MS assay (>300 ng/mL) varied by only 11.5 h with a 150-ng/mL cutoff and by 8.7 h with a 300-ng/mL cutoff (Table 4) among the four subjects, indicating the absence of large inter-subject differences in excretion of benzoylecgonine after a single intravenous dose of cocaine. These data also are consistent with the observation of a short elimination half-life of benzoylecgonine in urine. As a consequence of rapid metabolite elimination, similar time intervals were noted for detection of the last positive specimen and detection of the first negative specimen. In contrast, drugs with long half-lives of elimination will often display a pattern of excretion in which negative specimens can frequently be followed by the appearance of positive specimens late in the excretion phase. For example, the last positive specimen for marijuana metabolite can occur much later than the time to detection of the first negative specimen [*8*]. The rapid rate of elimination of benzoylecgonine also was reported by Ambre et al. [*9*] in subjects using cocaine by the intranasal route. They found that half-lives for benzoylecgonine excretion in urine varied from 4.0 to 6.0 h, similar to the present finding of 5.9 to 7.9 h after intravenous administration. These findings suggest that administration of cocaine by the intranasal or intravenous route does not influence the elimination rate of benzoylecgonine.

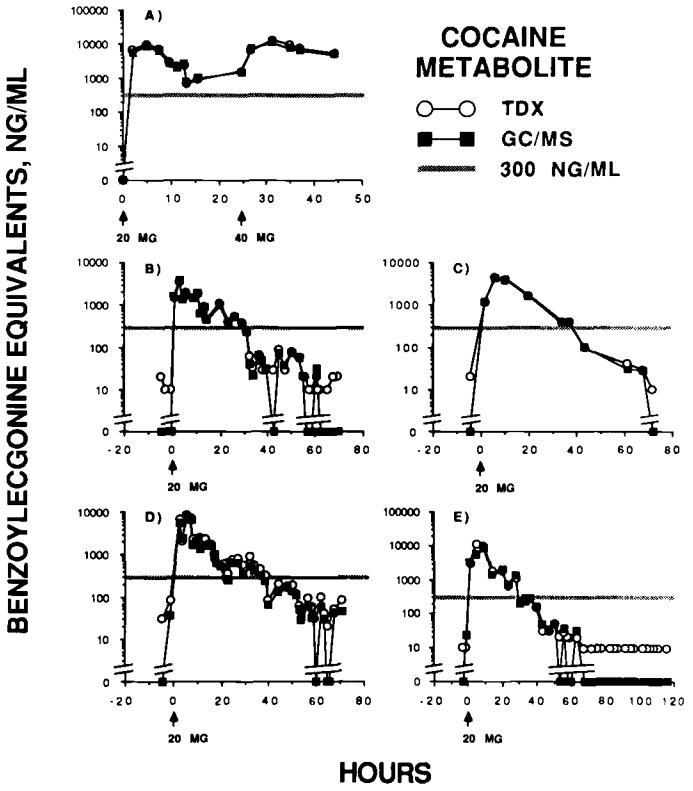

FIG. 5—*TDx assay and GC/MS assay of cocaine metabolite in urine of Subjects A to E after intravenous cocaine administration. All specimens were analyzed in randomized order under blind conditions.*

Differences in performance between the commercial assays were most apparent when comparing assays based on different technologies. Toxi-Lab, a thin-layer chromatography (TLC) procedure, had the shortest detection times for cocaine metabolite (Table 4). This was due to the relatively low sensitivity of the TLC method versus GC/MS or immunoassay. The time to detection of the last positive specimen by Toxi-Lab was one-half or less that by GC/MS assay (150-ng/mL cutoff) for benzoylecgonine. However, in contrast to the remaining assays, Toxi-Lab had the versatility to provide additional drug testing information. Only the Toxi-Lab assay provided data on the excretion of unmetabolized cocaine in urine. The sensitivity for cocaine by this method was approximately 150 ng/mL with a 5-mL specimen. The elimination of cocaine was rapid, and cocaine was not detected beyond 9.3 h. In an earlier study, Hamilton et al. [*6*] found that cocaine administered by the intranasal route (1.5 mg/kg) was generally detected in urine for 2 to 4 h by TLC and up to 12 h by gas chromatography. Similarly, Ambre et al. [*9*] reported detection by GC/MS of unchanged cocaine in urine up to 11.3 h after intranasal or intravenous cocaine.

Detection of benzoylecgonine or cocaine by the KDI Quik Test assay was unsuccessful as a result of the color interference from normal urine and also the lack of sensitivity of the test. The lack of validity of this test for detection of benzoylecgonine in urine has already been reported [*10*].

Similar or longer detection times were noted for the six immunoassay-based tests in comparison to GC/MS assay if the same cutoff was employed for comparison of assays, that is,

TABLE 4—*Detection times of cocaine metabolite and cocaine by commercial assay and of benzoylecgonine by GC/MS assay in clinical specimens after intravenous cocaine administration.*

Assay	Cutoff, ng/mL	Specimen Type[a]	Detection Time, h				
			Subject B	Subject C	Subject D	Subject E	Mean + SE
GC/MS	300	last pos	29.3	36.6	36.5	27.9	32.6 ± 2.3
		1st neg	31.2	43.6	21.5	29.7	31.5 ± 4.6
GC/MS	150	last pos	32.6	43.6	39.9	44.1	40.0 ± 2.7
		1st neg	31.2	36.6	47.4	39.8	38.8 ± 3.4
TDx	300	last pos	29.3	36.6	39.1	33.0	34.5 ± 2.1
		1st neg	31.2	43.6	39.9	29.7	36.1 ± 3.4
Toxi-Lab	NA[b]	last pos	10.6	20.0	17.1	19.8	16.9 ± 2.2
(Benzoylecgonine)		1st neg	11.3	34.1	17.8	23.7	21.7 ± 4.8
Toxi-Lab	NA	last pos	4.0	1.5	0	9.3	3.7 ± 2.0
(Cocaine)		1st neg	5.4	5.9	2.8	14.5	7.2 ± 2.5
Coat-A-Count	300	last pos	64.8	36.6	70.5[c]	39.8	52.9 ± 8.6
RIA		1st neg	32.6	43.6	51.7	44.1	43.0 ± 3.9
Double	300	last pos	64.8	36.6	70.5[c]	39.8	52.9 ± 8.6
Antibody RIA		1st neg	32.6	43.6	52.9	44.1	43.3 ± 4.2
EMIT dau	300	last pos	31.2	36.6	39.1	36.3	35.8 ± 1.7
		1st neg	32.6	43.6	39.9	29.7	36.5 ± 3.2
EMIT st	300	last pos	31.2	36.6	39.1	36.3	35.8 ± 1.7
		1st neg	32.6	43.6	39.9	29.7	36.5 ± 3.2
Abuscreen	300	last pos	50.2	36.6	49.7	36.3	43.2 ± 3.9
RIA		1st neg	32.6	43.6	39.9	29.7	36.5 ± 3.2

[a]last pos = detection time to the last cocaine or cocaine metabolite positive specimen. 1st neg = detection time to the first cocaine or cocaine metabolite negative specimen.

[b]NA = not applicable.

[c]These detection times are only estimates since no further specimens were collected.

300-ng/mL cutoff. Among these, TDx, EMIT dau, and EMIT st had very similar detection times to GC/MS; Abuscreen was somewhat longer, and Coat-A-Count and Double Antibody were longest. Use of a lower cutoff for the GC/MS assay, that is, 150 ng/mL, increased the mean detection time of this method by 7.4 h, or approximately one half-life (mean = 6.8 h, Table 2), as expected. Consistent with these findings, Van Dyke et al. [*11*] found detection times to the last positive specimen by EMIT dau (1000-ng/mL cutoff) varied from 15 to 46 h after intranasal administration of 1.5 to 1.7 mg/kg of cocaine hydrochloride to 16 surgical patients, but made no comparisons with other assays. The longer detection times of the Coat-A-Count, Double Antibody, and Abuscreen in the present study are likely due to differences in sensitivity and specificity of the commercial antibodies to cocaine constituents present in the clinical specimens. Each of these 3 assays crossreact with cocaine, traces of which could be present at times later than that detected by the Toxi-Lab assay.

This would also account for the substantially higher quantitative amounts of benzoylecgonine equivalents found in the clinical specimens by the latter three assays versus the amount of benzoylecgonine found by GC/MS assay. Hamilton et al. [*6*] noted similar differences in detection times between assays in studies with human subjects who received cocaine hydrochloride intranasally (1.5 mg/kg). They found that benzoylecgonine was detectable for 48 to 72 h by gas chromatography (400-ng/mL cutoff), EMIT dau (1000-ng/mL cutoff), and TLC (500-ng/mL cutoff) and for 120 h by RIA (25-ng/mL cutoff).

Detection times for a commercial assay which substantially exceed those by GC/MS assay (150-ng/mL cutoff) could present some problems in confirmation, particularly if the increased detection time of the commercial assay is a result of significant cross-reactivity with other cocaine-related material eliminated in urine at times when benzoylecgonine levels have declined below the sensitivity of the GC/MS assay. Analysis of a specimen under these conditions would result in a lack of confirmation of the initial positive finding by the RIA method. This situation occurred in the Coat-A-Count and Double Antibody determination of some of the pre-drug control specimens from Subjects B and D and also in the analysis of a few specimens collected late in the excretion phase after cocaine administration. Although the lack of confirmation by GC/MS may appear to indicate that the two immunoassays, Coat-A-Count and Double Antibody, produce "false" positive results for some specimens, it is probable that the reactivity was due to the presence of cocaine-related material in these specimens. However, when single specimens are being tested in a drug-testing laboratory, this type of finding will be difficult to interpret.

This investigation of validity parameters associated with pharmacologic variables and detection times indicated that substantial qualitative and quantitative differences do occur between commercial assays for detection of cocaine metabolite. It is essential that the differences and limitations of each methodology be clearly understood by those personnel involved in implementation of drug-testing programs and those involved in interpretation of drug-testing results.

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