TECHNICAL NOTE

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Validity Testing of Commercial Urine Cocaine Metabolite Assays: III. Evaluation of an Enzyme-Linked Immunosorbent Assay (ELISA) for Detection of Cocaine and Cocaine Metabolite

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ABSTRACT: A validity assessment study was performed on the Genetic Diagnostic Enzyme Immunoassay test kit, a new enzyme-linked immunosorbent assay (GDC ELISA) for detection of cocaine and cocaine metabolite in urine. A set of 290 urine specimens, comprised of clinical cocaine urines collected from 5 male subjects who had received single doses of intravenous cocaine, drug-free urines spiked with cocaine, cocaine metabolites, cocaine isomers, and other drugs of abuse, were assayed by GDC ELISA. The results were compared with results by gas chromatography/mass spectrometry (GC/MS) assay for benzoylecgonine. Concordance was high between the GDC ELISA assay and GC/MS and with results reported earlier for other commercial assays. Detection times and specificity of the GDC ELISA antibody were most similar to those of the Abuscreen® radioimmunoassay for cocaine metabolite. Overall, the assay produced no false negative or false positive results and appeared to be a reliable screening test for detection of cocaine and benzoylecgonine in human urine.

KEYWORDS: toxicology, cocaine, benzoylecgonine, urine, urine testing, ELISA

Recent exposure to cocaine can be revealed by urine testing for cocaine and its metabolites, benzoylecgonine and ecgonine methyl ester. Numerous commercial assays are now available for cocaine metabolite testing, some of which are used as the key initial screening test prior to confirmation of positive results in forensic testing of urine specimens. A comprehensive examination of eight of these screening assays indicated the presence of important differences in their accuracy and specificity when used for testing of clinical cocaine specimens obtained from subjects who were administered intravenous cocaine under controlled conditions [*1,2*].

We evaluated the Genetic Diagnostics Corporation (GDC) Cocaine Enzyme Immunoassay with the same specimens as used in our previous study of commercial cocaine metabolite

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assays [*1,2*]. This assay is a competitive enzyme-linked immunosorbent assay (ELISA) for the qualitative determination of cocaine and cocaine metabolites. The specimen set used in these evaluations consisted of urines collected from five male subjects who resided on our clinical ward and received single intravenous doses of cocaine. The set also included spiked control urines containing known amounts of cocaine, metabolites, cocaine isomers, cocaine derivatives, and other drugs of abuse. Test results were compared with assay results of the same specimens by gas chromatography/mass spectrometry (GC/MS) and to previously reported results by other commercial assays.

Materials and Methods

A frozen set of urine specimens identical to those used in a previous evaluation of commercial cocaine assays [*1,2*] was thawed and analyzed by the GDC ELISA assay (Genetic Diagnostics Corporation, Great Neck, New York). All tests were performed according to manufacturer's procedures. The specimens were analyzed in random order under blind conditions, and results were decoded only after completion of the entire study. Test results on the same specimens were compared to those obtained by GC/MS assay for benzoylecgonine [*3*], TDx® Cocaine Metabolite assay (Abbott Laboratories, Irving, TX), Coat-a-Count® Cocaine Metabolite radioimmunoassay (RIA) and Double Antibody radioimmunoassay (Diagnostics Products Corp., Los Angeles, California), Abuscreen® RIA for Cocaine Metabolite (Roche Diagnostics Systems, Nutley, New Jersey) and Emit® dau® Cocaine Metabolite assay (Syva Co., Palo Alto, California) as described previously [*1,2*].

Briefly, the GDC ELISA test procedure consisted of addition of 0.005 mL of positive (300-ng/mL benzoylecgonine) and negative standards and test specimens in duplicate to microtiter wells (96 well plates) coated with benzoylecgonine followed by addition of anti-benzoylecgonine antibody (first antibody). After incubation at room temperature for 30 min, the plate was washed to remove unbound antibody. An enzyme-labeled second antibody was then added and incubated for 30 min at room temperature. The plate was washed again and substrate added for color development. After 15 min, a solution of dilute hydrochloric acid was added to halt enzymatic action. Color development in the wells, which was inversely proportional to cocaine metabolite concentration, was determined with a microtiter plate reader equipped with a 450-nm filter. Negative assays appeared bright yellow, and positive assays displayed a pale yellow color. Specimen results on each plate were determined to be positive only if the average optical density of duplicate determinations of the specimens was equal to or less than the average optical density of the positive standards applied to that plate.

Results and Discussion

A total of 290 human urine specimens were analyzed by the GDC ELISA test for the qualitative determination of cocaine and cocaine metabolite. The specimen set was comprised of 148 clinical urines obtained from 5 male subjects (Subjects A to E) who had received single, 20-mg intravenous doses of cocaine (Subject A received a second dose of 40 mg of cocaine 24 h later) and 142 control urines containing known amounts of drug standard. Assay of the clinical cocaine urines by the GDC ELISA showed close agreement with earlier assay results by GC/MS for benzoylecgonine, as given in Table 1. A total of 72 of the 148 clinical cocaine urines tested positive (300-ng/mL cutoff) by the GDC ELISA assay, 61 of which contained benzoylecgonine at concentrations greater than 300 ng/mL by GC/MS assay. The remaining 11 positives contained benzoylecgonine in the concentration range of 20 to 300 ng/mL. No false positives (specimens testing positive by GDC ELISA which contained less than 20-ng/mL benzoylecgonine by GC/MS assay) were produced by the GDC ELISA assay. There were 76 negative results produced by the GDC ELISA assay; 72 of these contained less than

TABLE 1—*Concordance of positive/negative results by GDC ELISA assay of 148 clinical cocaine specimens with quantitative results of GC/MS assay for benzoylecgonine.*

	GC/MS Concentration, ng/mL					
GDC ELISA Result[a]	>300	299-200	199-150	149-100	99-20	<20
Positive	61	6	0	3	2	0
Negative	0	2	2	0	33	39

[a]Specimens were determined to be positive on the basis of the average response of duplicate tests compared to benzoylecgonine standard (300 ng/mL).

100 ng/mL of benzoylecgonine by GC/MS assay. Four of the negatives contained benzoylecgonine in the range of 150 to 300 ng/mL. No false negatives (specimens testing negative by GDC ELISA which contained 300 ng/mL or more of benzoylecgonine by GC/MS assay) were produced.

'A comparison of the GDC ELISA test results of the clinical cocaine specimens with other commercial immunoassay results reported previously is given in Table 2. The GDC ELISA results were most similar to the Abuscreen assay results. Only one specimen assayed differently in these two assays. The GDC ELISA detected a greater number of positives than did either the Emit dau or TDx assay, whereas it detected less positives than did the Coat-a-Count and Double Antibody assay. These results are consistent with specificity studies of these assays [*1,2*]. Both Coat-a-Count and Double Antibody assay show greater cross-reactivity with cocaine than with benzoylecgonine, whereas the Emit dau and TDx assays are highly specific for benzoylecgonine. The Abuscreen assay showed approximate equal crossreactivity with cocaine and benzoylecgonine. Results from the present study in testing drug standards indicated that the antibody of the GDC ELISA assay is equally cross-reactive with benzoylecgonine and cocaine. Both cocaine and benzoylecgonine displayed approximate equal percent inhibition of binding of standards in urine across a concentration range of 50 to 5000 ng/mL during testing. Consequently, the similarity in test results with clinical cocaine specimens by the GDC ELISA and Abuscreen assay appears to be a reflection of the similarity in the specificities of the antibodies of the two assays.

The specificity of the GDC ELISA assay also was tested with a variety of cocaine isomers, metabolites, derivatives, and other drugs of abuse added to control urine. Of these analytes, only *l*-cocaine and *l*-benzoylecgonine produced inhibition of binding sufficient to test positive (300-ng/mL cutoff). The following drugs tested negative at 5000-ng/mL standard concentration: *d*-Cocaine; *l*-pseudococaine; *d*-pseudococaine; *l*-ecgonine methyl ester; *l*-ecgonine; *l*-benzoylnorecgonine; *l*-pseudoecgonine methyl ester; *d*-pseudoecgonine methyl

TABLE 2—*Concordance of positive/negative (+/−) results from the GDC ELISA assay of 148 clinical cocaine urines with results from other commercial urine cocaine metabolite immunoassays.*[a]

	TDx		Coat-a-Count		Double Antibody		Abuscreen		Emit dau	
GDC ELISA	+	−	+	−	+	−	+	−	+	−
+	65	7	71	1	72	0	71	1	67	5
−	0	76	20	56	23	53	0	76	0	76

[a]All assays used a 300-ng/mL benzoylecgonine cutoff.

TABLE 3—*Detection times by GDC ELISA assay for cocaine metabolite in urines of four human subjects after intravenous cocaine administration.*

	Detection Times, h				
Specimen Type[a]	Subject B	Subject C	Subject D	Subject E	Mean ± S.E.
Last positive	50.2	36.6	51.7	36.3	43.7 ± 4.2
First negative	32.6	43.6	39.9	29.7	36.5 ± 3.2

[a]Last positive = the last cocaine metabolite positive specimen (cutoff = 300 ng/mL) detected after single-dose cocaine administration (20 mg). First negative = the first cocaine metabolite negative specimen detected after single dose cocaine administration.

ester; *l*-norcocaine; morphine; lidocaine; and phencyclidine. In addition, clinical urines collected immediately following administration of lidocaine, marijuana, and ibuprofen tested negative in the GDC ELISA assay.

Although assay drug detection times are dependent on many factors, both pharmacological and chemical, they are important to determine since they define the time "window" of detection that an assay is useful in detecting drug exposure and how soon after drug exposure a subject will test negative. The determination of detection times is meaningful only if the drug is administered under controlled conditions and the subject has no other access to the drug. Drug detection times also serve as benchmarks for inter-assay comparisons when tests are performed on the same specimens. The GDC ELISA assay detection times for Subjects B to E are given in Table 3. All urines were collected from these subjects following a single, 20-mg intravenous dose of cocaine. The subjects resided on a closed clinical ward under supervision during the study. The mean time from drug administration to detection of the last positive specimen by the GDC ELISA assay was 43.7 h. For comparison, the mean detection time for these subjects by other commercial assays [*1,2*] were as follows: TDx, 34.5 h; Emit dau, 35.8 h; Abuscreen, 43.2 h; Coat-a-Count, 52.9 h; and Double Antibody, 52.9 h. The mean time from drug administration to the first negative urine for the GDC ELISA assay was 36.5 h. Other commercial assay results [*1,2*] were as follows: TDx, 36.1 h; Emit dau, 36.5 h; Abuscreen, 36.5 h; Coat-a-Count, 43.0 h; and Double Antibody, 43.3 h. It is apparent that the similarity in the specificity of the GDC ELISA antibody to that found in the Abuscreen assay also is reflected in their similarity in detection times.

Overall, the GDC ELISA assay for cocaine and cocaine metabolite appears to be a reliable and accurate immunoassay test for cocaine exposure. When tested on drug standards, the test demonstrated high specificity for benzoylecgonine and cocaine with negligible crossreactivity toward a variety of cocaine metabolites, isomers, and other drugs of abuse. When tested on clinical cocaine specimens there were no false positive or false negative results produced in comparison with GC/MS assay for benzoylecgonine, and the assay demonstrated equivalent detection times to other commercial cocaine metabolite assays.

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