Immunoassay for Detection of Cocaine/Metabolites in Oral Fluids

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Abstract

There is growing interest in the use of alternate biological fluids for drug testing. An advantage of oral fluids is that collection can be made from individuals under direct observation without undue embarrassment or invasion of privacy. This study evaluated the STC Cocaine Metabolite MICRO-PLATE EIA for use in detection of cocaine and metabolites in oral fluids. Intra- and interassay precision of the EIA was < 10%. The EIA was cross-reactive to benzoylecgonine (100%), cocaine (> 12.9%), and cocaethylene (13.8%), but did not demonstrate detectable cross-reactivity with other commonly encountered medicants. Evaluation of a series of potential adulterants of oral fluids indicated that common household chemicals and foodstuffs did not alter the outcome of EIA testing for cocaine metabolite. Analysis by EIA and by gas chromatography–mass spectrometry (GC–MS) of oral fluids and urine specimens collected from current drug users in treatment programs and subjects participating in research studies involving controlled dosing of cocaine provided assessment of the clinical sensitivity and specificity of the STC Cocaine Metabolite EIA. Analysis of the data by means of receiver operating characteristic (ROC) plot indicated that the optimal cutoff concentration for the oral fluids EIA was 10 ng/mL. In comparison to GC–MS (10-ng/mL combined cutoff concentration for cocaine and benzoylecgonine), the EIA (10-ng/mL cutoff concentration) demonstrated a sensitivity, specificity, and accuracy of 95%, 82%, and 88%, respectively. The oral fluids EIA was slightly less sensitive than the urine EIA (300-ng/mL cutoff concentration) for the detection of cocaine metabolite with a sensitivity, specificity, and accuracy of 73%, 85%, and 88%, respectively. Overall, testing of oral fluids for cocaine metabolite with the STC Cocaine Metabolite MICRO-PLATE EIA appears to offer a viable alternative to urine for detection of recent cocaine use.

Introduction

Oral fluid is a complex mixture of fluids and solids originating from the parotid glands, submandibular glands, sublingual glands, minor mucous glands, gingival crevicular fluid, and cellular debris. In addition, oral fluids may contain bacteria, nasal secretions, and residues of ingested fluids and substances. The primary contributors to this mixture are saliva secretions from the various glands. Hence, many authors have used other terms for oral fluids such as "saliva" and "mixed saliva" (1,2). The composition of oral fluids can change rapidly in response to stimulation. The parotid gland normally contributes about 20% of the total volume of unstimulated saliva. At high stimulated flow rates, the parotid gland becomes the predominant secretor, and its contribution can rise to 50% (3).

Cocaine and many other drugs of abuse have been measured in varying concentrations in oral fluids following different routes of administration. Several reviews regarding the occurrence of drugs of abuse in oral fluids, analysis, and the concentration relationships between "saliva" and plasma have been published (4–10). Disposition of cocaine into oral fluids appears to occur through a combination of mechanisms including (1) passive diffusion from blood across acinar cell membranes into saliva ducts followed by secretion into the mouth and (2) direct deposition and sequestration in the oral cavity during drug administration by oral, smoked, and intranasal routes. The concentrations of cocaine and metabolites (benzoylecgonine and ecgonine methyl ester) deposited into saliva and subsequently into oral fluids by passive diffusion are determined by their solubility, pK_a, degree of plasma protein binding, and saliva flow rate (11). To enter saliva by passive diffusion, drugs must be lipid-soluble, non-ionized, and unbound (free fraction of drug in plasma). Because of these factors, cocaine is frequently detected in saliva in higher concentrations than found in simultaneously collected plasma specimens (12). Benzoylecgonine and ecgonine methyl ester are less lipid-soluble than cocaine and are generally found in lower concentrations than found in simultaneously collected plasma specimens. In addition to passive diffusion from blood, direct deposition of cocaine into oral fluids occurs when cocaine is administered by the smoking and intranasal routes. Shortly after ingestion, the amount of cocaine present in oral fluids as a result of direct deposition and sequestration can greatly exceed the amount contributed by passive diffusion (13). However, cocaine deposition

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into oral fluid through this mechanism clears rapidly in 1–2 h after administration. Thereafter, "saliva"/plasma ratios were comparable to those obtained after intravenous cocaine administration.

Methods for the detection of cocaine and metabolites in oral fluids have become a topic of considerable interest because of the inherent advantages over other biological specimens. The single, most important advantage in the use of oral fluids is in the ease of collection. Specimens can generally be collected in a matter of minutes under direct observation and without embarrassment to the donor. An additional advantage may also be that adulteration of oral fluids is likely to be more difficult than adulteration of urine specimens. Also, detection times for cocaine and/or benzoylecgonine may be longer than expected based upon estimated half-lives of approximately 1 h and 4.5 h for cocaine and benzoylecgonine, respectively. For example, Cone and Weddington (14) reported detection of cocaine in oral fluids by a sensitive immunoassay method for 5–10 days. Benzoylecgonine has also been reported to be detectable in oral fluids for 24 h (15). Consequently, the use of oral fluids for routine detection of recent cocaine use may represent a feasible alternative to urine testing.

The goal of the present study was the validation of a commercial immunoassay method for detection of cocaine and metabolites in oral fluids. Specimens were collected from drug-free human subjects and from cocaine users with the Intercept™ Oral Specimen Collection Device (Epitope, Inc., Beaverton, OR). This device consists of a treated, absorbent, cotton-fiber pad affixed to a nylon stick. The collection pad is impregnated with a mixture of salts and gelatin that creates an increased osmotic pressure when placed in contact with oral mucosal cells. Placement of the collection pad in contact with the gingival mucosa between the lower gum and cheek enhances the flow of oral fluids including mucosal transudate onto the absorptive pad. After several minutes, the collection pad was placed into a vial containing a buffer solution. The pad collects approximately 400 µL of oral fluid, which is diluted into 800 µL of buffer in the transport tube. Specimens were tested for cocaine metabolites with a solid-phase, competitive enzyme immunoassay (EIA). Analytical precision, sensitivity, and specificity were determined for the EIA system. Clinical sensitivity and specificity of the EIA were determined by comparison of results to analysis by gas chromatography–mass spectrometry (GC–MS).

Materials and Methods

Chemicals

All buffer and reagent chemicals were obtained from the Sigma/Aldrich Chemical Co. (St. Louis, MO). Test kits were supplied by STC Technologies, Inc. (Bethlehem, PA).

Specimen collection and storage

Oral fluids were collected from human subjects with the Intercept Oral Specimen Collection Device. This device consists of an absorbent, cotton-fiber pad affixed to a nylon stick and a plastic specimen vial containing a preservative solution. The fiber pad is impregnated with a mixture of salts and gelatin which creates a hypertonic environment and an increase in osmotic pressure wherever it contacts oral mucosal cells. The pad was placed between the lower gum and cheek during collection of oral fluids. Following a collection period of approximately 2.0 min, the pad was placed into the vial containing the preservative solution, and the vial was sealed. Specimens were stored at room temperature and shipped to the laboratory for analysis.

The clinical sensitivity and specificity of the EIA were determined by testing specimens from chronic cocaine users as well as volunteer subjects receiving single doses of cocaine. Informed consent was obtained from all subjects. Specimens were collected from cocaine-experienced subjects participating in research at Johns Hopkins School of Medicine, Baltimore, MD (99 subjects); patients in treatment at the Hooper Detoxification Center, Portland, OR (50 subjects); and research volunteers at the National Institute on Drug Abuse, Intramural Research Program, Baltimore, MD (10 subjects). In the first study (Study 1), a total of 149 matched Intercept oral fluid and urine specimens were collected randomly from cocaine-experienced subjects (N = 99) and patients when visiting the clinic for treatment (N = 50). Oral fluid specimens were analyzed using the STC EIA at the Gulf Coast Regional Blood Center, Houston, TX, and all urine specimens were analyzed by GC–MS. In the second study (Study 2), 163 oral fluid specimens and 156 matching urine specimens were collected in a controlled-dosing study in which 10 subjects were administered single, intravenous doses of cocaine. Timed oral fluid specimens were collected over 131 h by expectoration into plastic vials. Urine specimens were collected at each time point unless the subject could not void a specimen. Saliva flow was stimulated by use of sour candy. All specimens were frozen until time of analysis. Aliquots of oral fluid specimens were applied to Intercept collection devices and analyzed by the STC EIA and GC–MS at STC Diagnostics. Urine specimens were screened by EMIT using a 300-ng/mL cutoff and confirmed by GC–MS using a 150-ng/mL cutoff.

EIA procedures

The STC Cocaine Metabolite MICRO-PLATE EIA (STC Diagnostics) is a competitive immunoassay for the detection of cocaine and cocaine metabolites in oral fluid collected with the Intercept collection device. To each microplate well, 50 mL of sample eluate (N = 3), control, or calibrator was added along with 50 µL of labeled enzyme and allowed to incubate at room temperature in the dark for 30 min. After competitive binding of cocaine metabolite and enzyme-labeled hapten to the antibody fixed onto the EIA well, the wells were washed six times with deionized water. Substrate reagent (100 µL, 3,3',5,5'-tetramethylbenzidine) was added, and the microplate was again incubated at room temperature in the dark for 30 min. The reaction was then stopped by adding 100 µL of stopping reagent (1M sulfuric acid), and the color was measured at 450 nm with a Bio-Tek EL312 reader. The absorbance measured was inversely proportional to the quantity of cocaine and cocaine metabolite in the specimen. The calibrators (N = 3) in the assay

kit were included on every plate and consisted of oral fluid control buffered matrix containing benzoylecgonine at 0, 5, 10, and 50 ng/mL.

GC–MS procedures

Oral fluid eluates were analyzed for cocaine, ecgonine methyl ester, and benzoylecgonine by GC–MS with selective-ion monitoring (a model 5890A GC coupled with a model 5970 mass selective detector, Hewlett-Packard, Palo Alto, CA). A J&W, 0.25-mm o.d., 3-m column was used for the GC. One milliliter of each specimen was treated with deuterated analogues of cocaine, ecgonine methyl ester, and benzoylecgonine (25 ng/mL) and extracted with solid-phase columns (#ZSDAU020, World Wide Monitoring). Ecgonine methyl ester and benzoylecgonine were derivatized with pentafluoropropionic anhydride in the presence of pentafluoropropanol. The following ions were monitored for each analyte: cocaine, *m/z* 182, 272, 303; cocaine-d_3, *m/z* 185, 275, 306; ecgonine methyl ester, *m/z* 182, 314, 345; ecgonine methyl ester-d_3, *m/z* 185, 317, 348; benzoylecgonine, *m/z* 300, 316, 421; and benzoyecgoine-d_3, *m/z* 303, 319, 424. The approximate retention times were as follows: ecgonine methyl ester, 3.4 min; cocaine, 6.2 min; and benzoylecgonine, 5.7 min. The limit of quantitation (LOQ) was defined as the lowest concentration at which 100% of the diluted standards yielded all qualifier ions within ± 20% and retention times within ± 2% of the target concentration. The LOQs (N = 6 replicates) for cocaine and benzoylecgonine in oral fluids were 4.0 ng/mL and 7.0 ng/mL, respectively. The intra-assay precision was cocaine (11%), benzoylecgonine (2%), and ecgonine methyl ester (10%).

Sensitivity, specificity, and accuracy

The number of true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN) was determined at specified cutoff concentrations by comparison of the results from analysis of clinical specimens with the following assays: (1) STC Cocaine Metabolite MICRO-PLATE EIA for cocaine and cocaine metabolites in oral fluids (cutoff concentration = 10 ng/mL benzoylecgonine equivalents); (2) STC Cocaine Metabolite MICRO-PLATE EIA for cocaine metabolites in urine (cutoff concentration = 300 ng/mL benzoylecgonine equivalents); (3) GC–MS analysis of cocaine and benzoylecgonine in oral fluids (cutoff concentration = 10 ng/mL of combined total concentration of cocaine and benzoylecgonine); and (4) GC–MS analysis of benzoylecgonine in urine (cutoff concentration = 150 ng/mL). In comparisons of EIA results with GC–MS, a result was considered a true positive or a true negative if both results were in agreement. A result in which the EIA was positive and the GC–MS result was below the cutoff calibrator was considered a false positive. A result in which the EIA was negative and the GC–MS result was equal to or greater than the cutoff calibrator was considered a false negative. In comparisons of analysis of oral fluids by EIA to analysis of urine specimens by EIA, the result of the urine assay was arbitrarily designated as the correct result. Sensitivity, specificity, and accuracy were calculated as follows:

Sensitivity = TP/(TP+FN)

Specificity = TN/(TN+FP)

Accuracy = (TP+TN)/(TP+TN+FP+FN)

Receiver operating characteristic (ROC) plot

The optimum cutoff concentration for the STC Cocaine Metabolite MICRO-PLATE EIA was determined by use of an ROC plot (16). EIA results (5-, 10-, 20-, and 50-ng/mL cutoff concentrations) of oral fluid specimens obtained in the controlled-dosing study (N = 163) were compared to GC–MS results (10-ng/mL cutoff concentration, sum of cocaine and benzoylecgonine). GC–MS results were considered to be accurate. The EIA ROC plot was constructed by plotting sensitivity versus 1 – specificity.

Results

EIA limit of detection (LOD)

The LOD of the STC Cocaine Metabolite MICRO-PLATE EIA was defined as the mean absorbance (A_0) at zero-drug concentration minus three times the noise (SD) (LOD = A_0 – 3 SD) (17). The detection limit for benzoylecgonine was determined by obtaining the average absorbance value for 12 drug-free Intercept devices and then subtracting 3 SDs of the average. The absorbance value minus three SDs was then extrapolated from the log-log standard curve and represents the sensitivity of the assay. The minimum detectable concentration for benzoylecgonine was 0.95 ng/mL.

Precision of EIA

The precision of the STC Cocaine Metabolite MICRO-PLATE EIA was assessed by the addition of standard concentrations of benzoylecgonine to the Intercept preservative solution followed by assaying over a period of 20 days. The intra-assay precision was determined by analyzing each concentration 16 times per run for 4 runs in 1 day. Interassay precision was determined by analyzing 2 samples at each concentration in 2 separate runs per day for 20 days. The results of testing are listed in Table I.

Specificity of EIA

Compounds that demonstrated no cross-reactivity at concentrations of 10,000 ng/mL with the STC Cocaine Metabolite MICRO-PLATE EIA are listed in Table II. The cross-reactivity of

Table I. Precision of the STC Cocaine Metabolite EIA

Benzoylecgonine (ng/mL)	Intra-assay %CV* (N = 64)†	Interassay %CV (N = 80, 20 days)‡
0	6.7	5.8
5	5.8	8.7
10	6.3	9.2
20	6.5	9.1

* CV, coefficient of variation.
† N = 64 indicates that 16 devices were analyzed on one day at each concentration × 4 runs.
‡ N = 80, 20 days indicates that 2 devices at each concentration were analyzed × 2 runs/day × 20 days.

cocaine and related metabolites compared to benzoylecgonine are listed in Table III.

Table II. Compounds Exhibiting No Cross-Reactivity with the STC Cocaine Metabolite EIA when Tested at 10,000-ng/mL Concentration

Acetylsalicylic acid	Gentisic Acid	Nicotine
Amitriptyline	Glutethimide	Nicotinic acid
Amobarbital	Hexobarbital	Norethandrolone
Aprobarbital	Hydrocodone	Oxycodone
Ascorbic Acid	Hydromorphone	Oxymorphone
Barbital	Ibuprofen	Phencyclidine
Butabarbital	Imipramine	Penicillin
Butalbital	Ketamine	Pentobarbital
Caffeine	Levorphanol	Phendimetrazine
Cannabidiol	Maprotiline	Phenobarbital
Cannabinol	Methylenedioxy-amphetamine	Phentermine
Codeine		Phenylbutazone
D-Amphetamine	Methylenedioxymeth-amphetamine	Phenylpropanolamine
D-Methamphetamine		Pseudoephedrine
Dimethylaminoan-tipyrine	Meperidine	Quinine
	Mephobarbital	Secobarbital
Diphenhydramine	Meprobamate	Δ^8-Tetrahydro-cannabinol
Doxepin	Methaqualone	
Ephedrine	Morphine	Theophylline
Erythromycin	Naphthalene	Trimipramine
Ethchlorvynol	Acetic Acid	
Fenfluramine	Niacinamide	

Table III. Cross-Reactivity of Cocaine and Metabolites with the STC Cocaine Metabolite EIA

Compound	Concentration (ng/mL)	%Cross-Reactivity
Benzoylecgonine	10	100.0
Cocaethylene	100	13.8
Cocaine	100	12.9
Ecgonine	1000	2.2
Ecgonine methyl ester	10,000	0.2

Table IV. Effect of Potential Adulterant on Response of the STC Cocaine Metabolite EIA

Substance	Manufacturer	Final specimen eluate concentration	EIA result (adulterant only)	EIA result (adulterant & BZE)*
Sugar water	Domino Pure Cane Sugar	145 mg/mL	Negative	Positive
Toothpaste	P&G Crest (original)	25 mg/mL	Negative	Positive
Cranberry juice	Ocean Spray	25%	Negative	Positive
Baking soda	Arm & Hammer	25 mg/mL	Negative	Positive
Orange juice	Tropicana 100% Pure	25%	Negative	Positive
Cola	Coca-Cola	25%	Negative	Positive
Cough syrup	Nyquil Adult Nighttime	0.05 g/mL	Negative	Positive
Antiseptic	Listerine (Warner-Lambert)	25%	Negative	Positive
Water	Deionized Water	25%	Negative	Positive

* Benzoylecgonine (BZE) was added to provide a final eluate concentration of 20 ng/mL.

Stability of benzoylecgonine in stored specimens

Oral fluid specimens were collected from 15 self-reported cocaine-negative subjects with the Intercept collection device. Oral fluid eluates were combined into a total of 12 specimen pools. Each pool was confirmed to be free of cocaine metabolite by EIA. Benzoylecgonine was added to achieve final concentrations of 0, 10, and 50 ng/mL in each of four pools. Specimens were stored at –20°C, 4°C, 23°C, or 37°C for 21 days. In addition, a portion of each subject pool was added to specimen pads and stored at –20°C, 4°C, 23°C, or 37°C for 21 days. Eluates were tested in the STC Cocaine Metabolite MICRO-PLATE EIA (10-ng/mL benzoylecgonine cutoff concentration) at 0, 7, 14, and 21 days. No qualitative changes in specimen responses were observed during the 21-day storage period for the specimens containing 0 and 50 ng/mL benzoylecgonine. The signal strengths (absorbance) of the specimens containing 10 ng/mL benzoylecgonine were subjected to an analysis of variance to determine if significant changes ($p < 0.05$) had occurred over the 21 days of storage. No significant changes in absorbance had occurred.

Adulteration testing of EIA

Common foodstuffs and household chemicals were added to Intercept specimens in the absence and presence of benzoylecgonine (final eluate concentration of 20 ng/mL) and tested with the STC Cocaine Metabolite MICRO-PLATE EIA (10-ng/mL benzoylecgonine cutoff concentration). As shown in Table IV, there were no false positives with any of the products in the absence of benzoylecgonine. All specimens tested positive when benzoylecgonine was present.

Effect of specimen pH on EIA

Oral fluid specimens were collected from self-reported cocaine-negative subjects with the Intercept collection device. Specimens were pooled and confirmed negative by EIA. All negative specimen pools were combined into a single specimen pool that had a pH of 7.03. This pool was divided into 10 aliquots. The pH of nine individual aliquots was adjusted with NaOH or HCl to the following pH levels: 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, and 9.0. The pH of one aliquot was not adjusted. Each aliquot was further divided into three equal subaliquots. Benzoylecgonine was added to achieve final concentrations of 0, 10, and 50 ng/mL in each aliquot. The unadjusted pH sample was tested in duplicate, and the remaining samples were tested in triplicate with the STC Cocaine Metabolite MICRO-PLATE EIA (10-ng/mL benzoylecgonine cutoff concentration). At all pH levels, specimens without benzoylecgonine tested negative, and specimens containing 50 ng/mL of benzoylecgonine tested positive. Specimens containing 10 ng/mL benzoylecgonine tested positive at all pH levels except 7.5. The pH 7.5 specimen tested negative but was within 0.01 absorbance units of the 10-ng/mL cutoff calibrator sample. The percent coefficients of variation (%CV) for responses across the entire pH range tested

for specimens containing benzoylecgonine at 0, 10, and 50 ng/mL were 10.2%, 7.3%, and 6.9%, respectively.

Clinical sensitivity and specificity

Oral fluid specimens collected from cocaine users (combined N = 312 specimens) were analyzed with the STC Cocaine Metabolite MICRO-PLATE EIA and by GC–MS for cocaine and benzoylecgonine. GC–MS concentrations of cocaine and benzoylecgonine in each oral fluid specimen were summed for comparisons to other assays. For example, an oral fluid specimen that contained 6 ng/mL of cocaine and 5 ng/mL of benzoylecgonine by GC–MS was considered positive at a cutoff concentration of 10 ng/mL. However, it should be noted that for some specimens, the values were below the LOQ of the method. Therefore, the result is an estimation of concentration. Matched urine specimens (combined N = 305) were analyzed with the STC Cocaine Metabolite MICRO-PLATE EIA and by GC–MS for benzoylecgonine. The sensitivity and specificity of the STC Cocaine Metabolite MICRO-PLATE EIA for analysis of oral fluids and urine is tabulated in Table V. Comparisons were made between the oral fluids EIA and GC–MS, between oral fluids EIA and urine EIA, and between the urine EIA and GC–MS.

Table V. Clinical Sensitivity and Specificity of the STC Cocaine Metabolite EIA for Cocaine and Cocaine Metabolites in Oral Fluids and the STC Cocaine Metabolite EIA for Cocaine Metabolites in Urine

		Oral fluids, GC–MS (10 ng/mL total concentration)	
		+	–
Oral fluids (N = 312)	+	132	31
EIA (10 ng/mL)	–	7	142
Sensitivity		95%	
Specificity		82%	
Accuracy		88%	

		Urine, EIA (300 ng/mL)	
		+	–
Oral fluids (N = 305)	+	142	17
EIA (10 ng/mL)	–	52	94
Sensitivity		73%	
Specificity		85%	
Accuracy		77%	

		Urine, GC–MS (150 ng/mL)	
		+	–
Urine (N = 305)	+	194	0
EIA (300 ng/mL)	–	31	80
Sensitivity		86%	
Specificity		100%	
Accuracy		90%	

ROC curve analysis

Figure 1 illustrates the ROC curve analysis for 163 oral fluid specimens by the STC Cocaine Metabolite MICRO-PLATE EIA compared to GC–MS results (sum of cocaine and benzoylecgonine concentrations). The GC–MS results were considered to be an accurate indicator of the true presence or absence of cocaine and benzoylecgonine. From the ROC curve analysis, the maximum sensitivity and specificity were obtained at a cutoff concentration of 10 ng/mL. The false-negative rate at 10 ng/mL was 4/163 (2%), but rose rapidly at higher cutoff concentrations (e.g., 21/163 [13%]) at 20 ng/mL. At 10 ng/mL, the sensitivity was 96% and specificity was 87%.

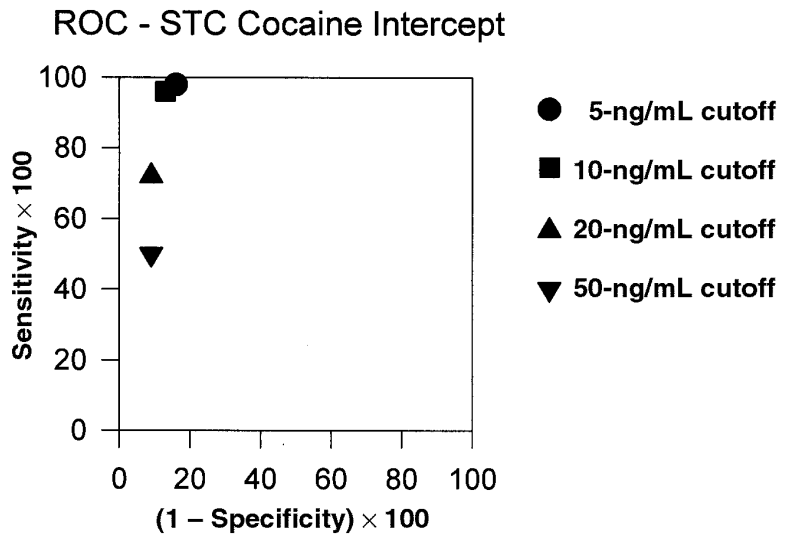

Figure 1. ROC plot for the STC Cocaine Metabolite MICRO-PLATE EIA compared to GC–MS analysis of 163 oral fluid specimens. The data points for each cutoff value (5, 10, 20, and 50 ng/mL) are indicated on the plot.

Discussion

Immunoassays have become a mainstay in forensic testing for drugs of abuse because of their ability to be performed rapidly, accurately, and inexpensively with automated equipment. They are a requirement of the United States Department of Health and Human Services (DHHS) *Mandatory Guidelines for Federal Workplace Drug Testing Programs*, which states that "[the] initial test shall use an immunoassay which meets the requirements of the Food and Drug Administration for commercial distribution" (18). The chief function of an immunoassay is to accurately identify negative specimens that contain no drug or drug at a concentration below an administrative cutoff level. Specimens that initially test positive by immunoassay (presumptive positives) then become the focus of further testing. A natural result of this process is that negative specimens are eliminated from more time-consuming, expensive, analytical procedures. In addition, positive specimens are subjected to a minimum of two different test procedures that greatly improve the accuracy of the test result.

Although the federal workplace drug-testing program in the U.S. is currently designed for testing only urine, considerable interest has evolved over the last decade in the use of alternate biological specimens (e.g., oral fluids, sweat, and hair specimens) (19). In particular, oral fluids testing for cocaine and cocaine metabolites offers specific advantages over urine testing in that the collection procedure should be easier and less embarrassing and the specimen should be more difficult to tamper with, substitute, or adulterate. Direct observation of urine collection is expressly forbidden in the DHHS program unless "…there is reason to believe that a particular donor may alter or substitute the specimen…" (18). In contrast, direct observation of oral fluids collection by the collector should not be considered an invasion of privacy by the donor. Consequently, the integrity of specimens and chain-of-custody procedures should be considerably easier to ensure with oral fluids than urine collection. Another potential advantage of oral fluid testing for cocaine could be realized in testing programs that are directed toward "fitness for duty." Because cocaine and cocaine metabolite concentrations in oral fluids frequently demonstrate direct relationships to blood concentrations (12), positive findings can be interpreted as evidence of recent drug use. Some inference might even be made regarding residual, psychoactive drug effects from recent cocaine use on the basis of a confirmed oral fluid test result. However, some prior studies have shown cocaine to be detectable for 5 to 10 days in chronic users (14). Thus, any conclusions regarding recent use from oral fluids must be studied further.

Development of an EIA for detection of cocaine and metabolites in a new biological matrix such as oral fluids requires consideration of similarities and differences between the new biological matrix compared to existing urine-screening assays. Although benzoylecgonine is the target analyte in urine testing, oral fluids may contain a combination of cocaine, benzoylecgonine, and ecgonine methyl ester. Cocaethylene, an active metabolite of cocaine and ethanol, could also be present. With the exception of ecgonine methyl ester, the presence of these analytes in combination or individually could be detected by the STC Cocaine MICRO-PLATE EIA. Consequently, GC–MS confirmation procedures for each of these analytes may be necessary. After very recent use (1–2 h), cocaine may be the predominant analyte present in oral fluids, whereas after 2 h or with multiple dosing, benzoylecgonine may be predominant (15,20). Concentrations of cocaine and metabolites in oral fluids will generally be lower than concentrations found in urine making lower EIA cutoff concentrations necessary on an operational basis. For example, in the present study, the STC Cocaine Metabolite MICRO-PLATE EIA performed optimally at 10 ng/mL for detection of cocaine use as compared to 300 ng/mL in urine.

An oral fluids EIA for detection of cocaine use should generally exhibit comparable clinical sensitivity, specificity, and accuracy to urine testing methods. In the current study, the STC Cocaine Metabolite MICRO-PLATE EIA demonstrated a clinical sensitivity of 95% and specificity of 82% in testing of the overall population (N = 312). In addition, an overall accuracy rate of 88% for detection of cocaine and cocaine metabolites in oral fluids in comparison to GC–MS was observed (Table V). An accuracy rate of 77% was also obtained in comparison of EIA analysis of oral fluids with EIA analysis of simultaneously collected urine specimens. These results are quite comparable to an accuracy rate of 90% obtained by comparison of EIA analysis of urine specimens to GC–MS analysis.

Although dilution and adulteration of urine specimens are common problems in urine testing (21–23), the prospect of adulteration of oral fluids is unclear. Flushing and dilution may sometimes be effective in the reduction of cocaine metabolites in urine below detection limits (23), but this approach is not likely to be effective in oral fluids testing. Excessive water ingestion is not likely to affect cocaine analyte concentrations in plasma or in oral fluids. Other commonly employed methods of adulteration include the addition of a toxic substance directly to the specimen. For oral fluids collection, some individuals may attempt to adulterate the specimen by holding liquids in their mouths. This opportunity to adulterate an oral fluid specimen could be substantially reduced by rinsing the oral cavity with water prior to collection, instituting a brief waiting period before collection, and by observing the collection process. Also, evaluation of different household products and foodstuffs is needed to determine if their presence in the oral cavity influences test outcome. An examination of several common products (Table IV) indicated that their presence did not produce either false-positive results or false-negative results in the oral fluids EIA.

Conclusions

Analysis of oral fluids by the STC Cocaine Metabolite MICRO-PLATE EIA provided a rapid and sensitive method for the detection of cocaine and cocaine metabolites. Data from method validation showed acceptable intra- and interassay precision and accuracy. The EIA demonstrated cross-reactivity to benzoylecgonine and cocaine sufficient for detection of cocaine use with an accuracy equivalent to that obtained by urinalysis. The assay was highly selective, and no cross-reactivity was demonstrated with other common medications. An evaluation of potential adulterants showed no interference in EIA test outcome. In a study of oral fluid and urine specimens simultaneously collected from current cocaine users, the EIA was slightly less sensitive than urine (73% sensitivity). Overall clinical sensitivity and specificity of the EIA compared to GC–MS were 95% and 82%, respectively. The performance of the oral fluids EIA in combination with GC–MS confirmation suggest that oral fluids testing may be an attractive alternative to urine testing for cocaine use.

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