Technical Note

Oral Fluid Testing for Drugs of Abuse: Positive Prevalence Rates by Intercept™ Immunoassay Screening and GC–MS–MS Confirmation and Suggested Cutoff Concentrations*

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Abstract

Draft guidelines for the use of oral fluid for workplace drug testing are under development by the Substance Abuse and Mental Health Services Administration (SAMHSA) in cooperation with industry and researchers. Comparison studies of the effectiveness of oral fluid testing versus urine testing are needed to establish scientifically reliable cutoff concentrations for oral fluid testing. We present the results of the first large scale database on oral fluid testing in private industry. A total of 77,218 oral fluid specimens were tested over the period of January through October 2001 at Lab*One* (Lenexa, KS). Specimens were screened by Intercept immunoassay at manufacturer's recommended cutoff concentrations for the five SAMHSA drug categories (marijuana, cocaine, opiates, phencyclidine, and amphetamines). Presumptive positive specimens were confirmed by gas chromatography–tandem mass spectrometry. A total of 3908 positive tests were reported over the 10-month period, representing a positive rate of 5.06%. Of the five drug categories, marijuana and cocaine accounted for 85.75% of the positives. The pattern and frequency of drug positives showed remarkable similarity to urine drug prevalence rates reported for the general workforce according to the Quest Diagnostics' Drug Testing Index over the same general period suggesting that oral fluid testing produces equivalent results to urine testing. The data on oral fluid testing also revealed a surprisingly high 66.7% prevalence of 6-acetylmorphine confirmations for morphine positives suggesting that oral fluid testing may be superior in some cases to urine testing. Comparison of oral fluid drug concentrations to SAMHSA-recommended cutoff concentrations in Draft Guidelines indicated that adoption of the screening and confirmation cutoff concentrations of Draft Guidelines #3 would produce the most consistent reporting results with the exception of amphetamines. Consequently, it is suggested that the final Guidelines adopt the screening and cutoff concentrations listed in Draft Guidelines #3 with the exception of lowering the amphetamines cutoff concentrations (screening/confirmation) to 50/50 ng/mL for amphetamine and methamphetamine.

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Introduction

In 1986, the United States federal government established a comprehensive urine drug-testing program for federal workers. Five general classes of drugs of abuse were included in the guidelines (1). The program has been highly successful using mandated cutoff concentrations and established methods for collection, transport, screening, confirming and reporting of tested specimen results. Since the program's inception a number of changes and modifications have continued to refine the guidelines and in some cases expand the list of target drugs, their metabolites, and the test cutoff limits (2).

In parallel to the testing programs for urine, counter efforts have been taken by drug users to adulterate specimens and to alter positive test results. Deliberate adulteration can be accomplished by several means including substitution, in vivo adulteration, and in vitro adulteration. Some adulterated urine specimens can be successfully tested, and in some cases, the nature of the adulterant can be identified. Many laboratories now

routinely test specimens for pH, specific gravity, and creatinine, and specific criteria have been derived for substituted specimens (3). In addition, substantial efforts have been made to identify specific chemicals known to be present in adulterants (4,5). The most problematic adulterants are ones that either extract the drug from the specimen or destroy the drugs and leave no identifiable element to show that the sample was tampered. Because over 70% of positives found in workplace testing are marijuana, many adulterants are targeted for marijuana metabolite. This is an ongoing problem for urine drug-testing programs as new adulterants appear frequently and are advertised broadly on the Internet.

The use of oral fluid for drug testing appears to offer some advantages over urine in overcoming specimen adulteration. Specimens can be readily collected under observed conditions without invasion of privacy, thus precluding substitution or deliberate adulteration (6). The Substance Abuse and Mental Health Services Administration (SAMHSA) in cooperation with industry and researchers has developed draft guidelines for drug testing of oral fluid in the workplace. Various drafts have been presented on the Internet for comment and evaluation. The most recent draft (Draft #4) was released to the public in September 2001. It is expected that the final draft guidelines will go through a period of public comment, followed by final regulations that will be put into practice.

The final selection of screening and confirmation cutoff concentrations will be critical in establishing the usefulness of oral fluid as a viable alternative to urine. The concentration of target drugs or metabolites found in oral fluid is considerably lower than found in urine. Secondly, it is suspected that the windows of detection in oral fluid will be shorter than urine. By appropriate selection of cutoff concentrations, it should be possible to approximately match the performance of urine testing in detection of recent drug use.

This report presents a survey of drug concentrations found in oral fluid specimens analyzed over a recent ten month period for the five common classes of drugs covered by the SAMHSA guidelines. Specimens were collected in private workplace testing programs and analyzed by immunoassay and gas chromatography–mass spectrometry–mass spectrometry (GC–MS–MS). The rate of confirmed positive specimens from oral fluid testing is compared to the rate of confirmed positive specimens reported in workplace urine drug testing programs. In addition, an evaluation of cutoff concentrations of confirmed positive specimens was made based on SAMHSA draft guidelines.

Methods

Oral fluid collection

A total of 77,218 oral fluid specimens (non-regulated) were collected primarily in workplace drug testing programs from across the United States. The specimens were tested at Lab*One* (Lenexa, KS) for the 5-panel of drugs of abuse over a 10-month period (January–October 2001). Testing included screening and quantitative confirmation of marijuana (THC), cocaine metabolite (benzoylecgonine), opiates (morphine, 6-acetylmorphine, and codeine), phencyclidine, and amphetamines (amphetamine and methamphetamine). Oral fluid specimens were collected with the Intercept DOA Oral Specimen Collection Device (OraSure Technologies, Bethlehem, PA) according to manufacturer's instruction. Briefly, the collection device consists of a treated, absorbent cotton fiber pad affixed to a nylon stick and a preservative solution (0.8 mL) in a plastic container. With this device, an average of 0.4 mL of oral fluid is collected. The collection device pad was placed between the lower gum and cheek for 2–5 min, then placed in the preservative solution. The resulting total volume was approximately 1.2 mL (0.4 mL specimen and 0.8 mL preservative solution). Consequently, the oral fluid specimen was diluted by a factor of 3. Drug and metabolite concentrations of positive specimens were multiplied by 3 to account for dilution of the oral fluid specimen.

Immunoassay

Oral fluid specimens were analyzed with the Intercept MICRO-PLATE Enzyme Immunoassay (OraSure Technologies, Bethlehem, PA) by Lab*One* (Lenexa, KS) following manufacturer's procedures. Details of the assay for THC, cocaine, and opiates have been described (6–8). Each specimen was analyzed in singlicate. Quality control samples (below cutoff control; above cutoff control) were utilized in all cases. Mean responses of specimens were compared to the mean response of the calibrator (N = 4). Specimens with absorbance less than or equal to the calibrator were considered positive and specimens with responses greater than the calibrator were considered negative.

Oral fluid specimens were also analyzed for IgG content to insure that a valid specimen was collected. IgG was measured with the IgG Intercept MICRO-PLATE EIA by OraSure Technologies according to manufacturer's instructions. The immunoassay antibody is specific for human-derived IgG. Mean responses of specimens were compared to the mean response of the IgG calibrator (0.5 mg/mL). Specimens with IgG concentration equal to or greater than the calibrator were considered to be valid specimens for testing. (A valid specimen is defined as a human-derived oral fluid specimen with adequate volume for testing as demonstrated by Parry [9].)

Confirmation methods

All presumptive positive oral fluid specimens were confirmed by quantitative GC–MS–MS by Lab*One* (Lenexa, KS). The methods for confirmation testing for THC and opiates have been described elsewhere (6,8). For amphetamines, benzoylecgonine, PCP, 6-acetylmorphine (6-AM), and THC, specimens were extracted by liquid–liquid extraction, derivatized (if needed), and analyzed by GC–MS–MS. All GC–MS–MS procedures were developed and validated internally by the laboratory. Limits of quantitation (LOQ) and intra-assay precision data (%CV) for the assays (200% controls, N = 10) were as follows: THC, LOQ = 0.15 ng/mL, %CV = 12.8; benzoylecgonine, LOQ = 0.6 ng/mL, %CV = 9.9; morphine, LOQ = 6 ng/mL, %CV = 5.8; codeine, LOQ = 3 ng/mL, %CV = 5.8; 6-AM, LOQ = 0.6 ng/mL, %CV = 12.4; PCP, LOQ = 0.3 ng/mL, %CV = 12.0; amphetamine, LOQ = 30 ng/mL, %CV = 3.8; methamphetamine, LOQ = 12

ng/mL, %CV = 4.2. Precision data (%CV) for 50% controls (N = 10) were as follows: THC, 14.5; benzoylecgonine, 13.6; morphine, 11.7; codeine, 11.5%; 6-AM, 8.4; PCP, 7.4; amphetamine, 7.1; and methamphetamine, 5.6.

Results and Discussion

Oral fluid test results

A total of 77,218 oral fluid specimens were collected under workplace drug testing conditions (private sector, non-regulated) and tested in Lab*One* for the basic five-drug panel (marijuana, cocaine, opiates, PCP, and amphetamines). Specimens were initially screened with the Intercept MICRO-PLATE enzyme immunoassay (EIA); presumptive positive specimens were then confirmed by GC–MS–MS. Specimens that screened positive for opiates were tested for morphine and codeine. Specimens that confirmed positive for morphine were also tested for 6-acetylmorphine. The initial and confirmatory test cutoff concentrations utilized in the assays are shown in Table I.

Table I. Cutoff Concentrations Utilized for Testing Oral Fluid Specimens by Intercept (Whole Saliva) and GC–MS–MS Confirmation Assay

Assay	Cutoff Concentration Oral Fluid (ng/mL)
Initial test (Intercept)	
THC (parent drug and metabolite)	3
Cocaine metabolites	15
Opiate metabolites	30
Phencyclidine	3
Amphetamines	120
Confirmatory Test	
THC (parent drug)	1.5
Benzoylecgonine	6
Morphine	30
Codeine	30
6-Acetylmorphine	3
Phencyclidine	1.5
Amphetamine	120
Methamphetamine	120

Table II. Overall Confirmed Positive Rates for Oral Fluid Specimens in LabOne over a 10-Month Period (January–October 2001)*

Oral Fluid Specimens (N = 77,218)	Number of Specimens	% Positive
Confirmed positive tests	3908	5.06
THC (parent)	2486	3.22
Cocaine	865	1.12
Opiates	175	0.23
Phencyclidine	21	0.03
Amphetamine/Methamphetamine	361	0.47

* 6-Acetylmorphine was only tested for morphine positives and are not included in the overall total number of positives.

Of the 77,218 oral fluid specimens tested, 3908 confirmed positive results were reported. This is an overall positive rate of 5.06% and a negative rate of 94.94% for the oral fluid specimens. The confirmed positive specimens consisted primarily of THC and cocaine; these two drug groups accounted for 85.75% of the total positives. The frequency of positives was in the following order: THC > cocaine metabolite > amphetamines > opiates > PCP. The number and percentage positive by drug group is shown in Table II.

These data are specific to collection of oral fluid by the Intercept DOA Oral Specimen Collection Device. One limitation of this study is the lack of an exact volume for the oral fluid specimen. The device collects an average of approximately 0.4 mL of oral fluid. The specimen was then placed in a container with a preservative solution (0.8 mL) giving a total volume of approximately 1.2 mL. Concentrations of drug in oral fluid were adjusted for the dilution and are reported as approximate concentrations occurring in oral fluid. In an earlier study (7), it was reported that the mean volume of oral fluid collected by the device from 83 normal (drug-free) individuals was 0.38 ± 0.19 (SD) with a range of 0.05 to 0.8 mL. Thus, oral fluid concentrations are subject to some variation depending upon the efficiency of the device and to individual variations in oral fluid production. This variation in specimen volume is not unlike the situation present in urine collection. It is commonly accepted that there is enormous variability associated with urine production by individuals. Urine output is directly related to individual fluid intake, renal output, and collection conditions.

The oral fluid drug prevalence rate found in the current study is remarkably similar to urine drug prevalence rates reported for the general workforce according to the Quest Diagnostics' Drug Testing Index. Although these data arise from different populations, different analytical methods, and different cutoff concentrations (as appropriate for different biological matrices), the end product is equivalent, that is, detection of drug use. Table III provides a comparison of positive drug prevalence rates by oral fluid workplace testing, by federally mandated urine testing, and by general U.S. workforce urine testing. The Quest Diagnostics' data are based on combined totals of > 1 million and > 5.2 million urine workplace drug tests for federally mandated employees (safety sensitive) and the U.S. general workforce, respectively, performed between January and December 2001. Generally, the positivity rates by drug category for oral fluid specimens were similar to that reported for the general workforce urine drug testing program. The overall positivity rate for oral fluid testing was 5.06% compared to 4.46% for the general workplace. The positivity rates for federally mandated urine drug testing were similar to the general workforce data with one major exception; THC positivity rates were approximately 84% higher in the general workforce than in the federal programs.

The pattern of positive test results per drug category for oral fluid specimens was also similar to that reported for the general workforce. THC and cocaine accounted for a total of 85.77% of

the total positive results by oral fluid testing and 86.55% by general workforce testing. The largest differences between oral fluid and the general workplace urine drug testing program occurred in the cocaine and amphetamines categories. Cocaine and amphetamines positivity prevalence rates for oral fluid testing were approximately 60% higher than for urine testing suggesting that these drugs are more efficiently accumulated in oral fluid relative to urine. THC, opiates, and PCP positivity prevalence rates were approximately equivalent to urine testing. Generally, positivity prevalence rates by drug category for oral fluid testing were higher than those observed in federally mandated urine drug-testing programs with the exceptions of opiates and PCP which were slightly lower.

The similarity in prevalence rates for oral fluid testing and urine testing in the general workforce suggests that testing with either type of specimen produces equivalent results. This was not entirely expected given that oral fluid is presumed to have a somewhat shorter window of drug detection as compared to urine. This suggests other possibilities for the similarity in positivity rates. Some of the positives found in oral fluid are most likely from individuals who took drugs a short time before giving their sample. Urine testing of these same individuals within several hours after their drug use might have resulted in negative results. For example, Niedbala et al. (6) reported a lag time of 4–6 h between administration of smoked marijuana and the appearance of positive urine specimens. Similarly, Heists et al. (10)reported lag times that ranged from 2.3 hours to 8.0 hours. In comparison, oral fluid specimens collected from the same individuals were immediately positive after drug use. Another possibility is that some individuals adulterated their urine specimens thereby defeating the urine drug testing laboratories' ability to detect a particular drug of abuse. A third possibility is that some individuals are routine drug users and it would not matter if one tested oral fluid or urine since there is a constant presence of drug in both body fluids. Regardless of the explanation, it is clear that the value of oral fluid for drug detection is at least equivalent and in some cases may be superior to urine drug testing.

In the current study, a total of 48 oral fluid specimens were positive for morphine. Approximately 66.7% (N = 32) of these specimens tested positive for 6-acetymorphine by GC–MS–MS (Table IV). These results are surprising when one considers the short detection window for 6-acetylmorphine in urine. Following single-dose heroin administration, 6-acetylmorphine is detectable in urine by GC–MS (10-ng/mL cutoff concentration) for approximately 3.3 h (11). In contrast, morphine was detectable in urine at a cutoff concentration of 300 ng/mL for approximately 26 h following administration of 6 mg of heroin. The high prevalence of 6-acetylmorphine in oral fluid together with morphine could be explained by frequent and/or recent use of heroin by the subjects prior to specimen collection. It is also possible that the excretion of 6-acetylmorphine in oral fluid is a more efficient process than the excretion of morphine. 6-Acetylmorphine is more lipid-soluble than morphine and thus, may more readily cross biological membranes. Evidence of this phenomenon was reported by Jenkins et al. (12). It was noted that following intravenous heroin administration saliva/plasma (S/P) ratios of 6-acetylmorphine tended to increase over time and were generally > 1, whereas morphine S/P ratios were < 1. Higher S/P ratios were reported following heroin administration by the smoked route than by the intravenous route suggesting the possibility that residues of heroin and 6-acetylmorphine were deposited in the oral cavity during the smoking process. Consequently, there are likely to be numerous factors involved in the explanation of the unusually high prevalence of 6-acetylmorpine associated with morphine positives found in the current study.

Establishing appropriate cutoff concentrations is an essential step in the development of guidelines for oral fluid testing. Table IV delineates the concentration ranges of the 3908 confirmed positive results and illustrates how the effect of different cutoff concentrations from Draft Guidelines #3 and #4 affect positivity rates of the current data set. It is recognized that these comparisons are subject to some limitations. The specimens were screened by Intercept EIA at their recommended manufacturer's cutoff concentrations and confirmed by GC–MS–MS. Further, the cutoff concentrations utilized in confirmation assays were based on internal validation studies by the laboratory. Consequently, the determination of a positive drug test result is directly linked to the performance characteristics of the screening and confirmation assays.

The recent draft guidelines (Draft #4) by SAMHSA altered screening and confirmation cutoff oral fluid concentrations for THC, PCP, and amphetamines from the previous draft (Draft #3). The increase in the recommended THC confirmation cutoff concentration from 2 ng/mL to 4 ng/mL by Draft #4 would have resulted in 30.2% of the positive specimens being reported as negative. As a result, a total of 750 positive THC oral fluid specimens would pre-

Table III. Comparison of Positive Drug Prevalence Rate by Oral Fluid Testing to Federally Mandated and General Workforce Urine Drug-Testing Programs According to Quest Diagnostics' Drug Testing Index

Drug Category	Positivity Prevalence Rate: Oral Fluid Drug Testing	Drug Testing Index: Federally Mandated Urine Drug Testing*	Drug Testing Index: General Workforce Urine Drug Testing*
	January–October 2001 (N = 77,218)	January–December 2001 (N > 1,000,000)	January–December 2001 (N > 5,200,000)
THC	3.22	1.72	3.17
Cocaine	1.12	0.60	0.69
Opiates	0.23	0.26	0.29
PCP	0.03	0.05	0.02
Amphetamines	0.47	0.29	0.29
Total	5.06	2.92	4.46

* Urine test data according to Quest Diagnostics' Drug Testing Index for workplace drug tests performed January to December, 2001 by Quest Diagnostics (data source can be found at http://www.questdiagnostics.com/brand/business/b_bus_lab_emp_drugtesting_index.html).

Table IV. Comparison of Concentrations of Oral Fluid Specimens Screened and Confirmed Positive (N = 3908 specimens) to SAMHSA Draft Cutoff Concentrations*

Drug/Metabolite	Oral Fluid Concentration (Confirmatory Test, ng/mL)	# Positives	% Positive	SAMHSA Draft Initial/ Confirmatory Test Cutoff Concentrations (ng/mL)		% Positive Specimens Below SAMHSA Confirmation Test Cutoff Concentration	
				Draft #3	Draft #4	Draft #3	Draft #4
THC	1.5–1.9	85	3.4	4/2	4/4	3.4	30.2
THC	2–3.9	665	26.8				
THC	4–49.9	1516	61.0				
THC	50	220	8.8				
Benzoylecgonine	6 to 7.9	78	9.0	20/8	20/8	9.0	9.0
Benzoylecgonine	8–19.9	201	23.2				
Benzoylecgonine	20	586	67.8				
Codeine†	30 to 39.9	16	12.6	40/40	40/40	12.6	12.6
Codeine†	40	111	87.4				
Morphine	30 to 39.9	6	12.5	40/40	40/40	12.5	12.5
Morphine	40	42	87.5				
6-Acetylmorphine	3–3.9	4	12.5	4/4	4/4	12.5	12.5
6-Acetylmorphine	4	28	87.5				
PCP	1.5 to 1.9	2	9.5	4/2	10/10	9.5	57.1
PCP	2–3.9	7	33.3				
PCP	4–9.9	3	14.3				
PCP	10	9	42.9				
Amphetamine	120–159.9	37	29.6	160/160	50/50	29.6	NA‡
Amphetamine	160	88	70.4				
Methamphetamine	120–159.9	16	6.8	160/160	50/50	6.8	NA‡
Methamphetamine	160	220	93.2				
Total	–	3908	–	–	–	–	–
Total # of positives below SAMHSA confirmation cutoff concentration	–	–	–	–	–	240	862
%Positives below SAMHSA confirmation cutoff concentration	–	–	–	–	–	6.1	22.1

* 6-Acetylmorphine was only tested for morphine positives and are not included in the overall total number of positives.
† Codeine only.
‡ NA = not applicable; Intercept EIA cutoff concentration was 120 ng/mL.

sumably been reported negative as a result. The increase in PCP cutoff concentrations (initial/confirmation) from 4/2 ng/mL to 10/10 ng/mL would have resulted in 57.1% of the positive specimens being reported as negative. Thus, a total of 12 positive PCP specimens would have reported negative. For amphetamines, the Intercept EIA cutoff concentration was 120 ng/mL; therefore, it is uncertain how large the effect would be by the lowering of the cutoff concentration from 160 ng/mL to 50 ng/mL. However, use of the 160/160 ng/mL cutoff concentrations instead of 120/120 ng/mL would have resulted in 29.6% of the amphetamine positives and 6.8% of the methamphetamine positives being reported as negative. These results

suggest that the recommended increases in confirmation cutoff concentrations for THC and PCP by Draft #4 guidelines would have a deleterious effect on detection of positive oral fluid specimens, whereas the recommended lowering of the amphetamines cutoff concentration would have a favorable effect on detection rates.

Overall, utilization of the confirmation cutoff concentrations in SAMHSA Draft Guidelines #3 would have resulted in 6.1% positive tests being reported negative, whereas 22.1% would have been negative by the cutoff concentrations of Draft Guidelines #4 (excluding 6-acetylmorphine positives). Accordingly, the most consistent results would be produced by maintaining the cutoff concentrations of Draft Guidelines #3, but adopting the suggested change of amphetamines to 50/50 ng/mL. This would have resulted in 4.8% of the positive specimens (N = 187) being reported as negative. Consequently, it is suggested that the final Guidelines adopt the cutoff concentrations listed in Draft Guidelines #3 with the exception of lowering the amphetamines cutoff concentrations to 50/50 ng/mL for amphetamine and methamphetamine.

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