

Assessment of the use of oral fluid as a matrix for drug monitoring in patients undergoing treatment for opioid addiction

Frank Kunkel, MD; Elizabeth Fey, MS; Damon Borg, PhD; Richard Stripp, PhD; Christine Getto, BS

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ABSTRACT

Drug testing is an important clinical tool that is available to physicians who are assessing the effectiveness of drug treatment as well as patient compliance to the administered program. While urine has traditionally been the matrix of choice for drug monitoring, oral fluid, a filtrate of the blood, has shown great promise as an alternative matrix for such applications. Oral fluid collection can be accomplished without the need for highly trained medical staff through the use of a simple, non-invasive oral fluid collection device, which obtains an adequate sample in only a few minutes. There has been a significant amount of research performed on the use of oral fluid for forensic toxicology application; however, more studies assessing the use of oral fluid drug testing are required to validate its ability to achieve clinical drug monitoring goals. Testing for various drugs in oral fluid may yield a different result when compared to the same drugs in urine, requiring an assessment of the utility of oral fluid for such practices. The purpose of this study was to examine the application of oral fluid drug testing in patients undergoing buprenorphine treatment for opioid dependence. A retrospective analysis of drug testing results obtained from 6,928 patients (4,560 unobserved urine collections and 2,368 observed oral fluid collections) monitored for heroin metabolite, amphetamine, benzodiazepines, buprenorphine, tetrahydrocannabinol, cocaine, codeine, hydrocodone, hydromorphone, methadone, morphine, oxycodone, and oxymorphone was completed. Results of this statistical exercise indicated that patients undergoing observed oral fluid collection tested positive more frequently than those unobserved urine collections for several illicit drugs and prescription medications targeted. Oral fluid was shown to detect illicit drug use as well as noncompliance in this patient population under the studied conditions more often than the urine specimens.

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INTRODUCTION

Urine is commonly used as the primary specimen for the detection of illicit drugs in workplace drug testing, substance abuse treatment programs, and also for the monitoring of licit pharmaceuticals to ensure compliance with treatment protocols. The analysis of urine samples primarily targets the drug metabolite(s) in the body. Detection of these compounds is facilitated by the relatively high concentration of analyte present in the urine, in addition to a reasonably long detection window (days). However, urine drug testing is limited by the high risk of adulteration and the

fact that results solely indicate past exposure to the detected compounds. Urine is not an acceptable matrix for determining impairment, pharmacological effects, or an estimation of dose based on measured drug concentration. Currently, blood is the preferred matrix for both assessing impairment based on biologically active drug levels in an individual in addition to determining the minimum dose consumed to produce a corresponding drug concentration in the body. Because of the ease at which urine can be adulterated or substituted paired with the invasive and costly blood collection techniques, improved methods of drug testing are desirable.

Oral fluid is composed of mixed saliva from multiple glands and other constituents in the mouth including plasma, electrolytes (K^+ , Na^+ , Cl^- , and bicarbonate), enzymes, and DNA. The first studies reporting the detection of drugs of abuse in oral fluid were published more than 20 years ago; however, over the last few years, oral fluid has steadily gained acceptance as a matrix for drug testing. The Substance Abuse and Mental Health Services Administration (SAMSHA) has accepted the recommendations of its technical advisory committee, the Drug Testing Advisory Board (DTAB), to include oral fluid testing in its mandatory guidelines and to expand opiate testing to include oxycodone, oxymorphone, hydrocodone, and hydromorphone. Furthermore, oral fluid is becoming a more widely used specimen in pharmacokinetic studies, therapeutic drug monitoring, and for the detection of illicit drugs.¹ Oral fluid is a matrix well suited for the pain management industry because it is based on simple, noninvasive, collection procedures. Oral fluid allows for the measurement of the free-fraction of drugs circulating in the body. Thus, the measured drug concentration in oral fluid represents the pharmacologically active portion of drug in plasma, in contrast to urine which contains an accumulation of excreted drugs. Oral fluid testing can therefore be used as a valuable tool for the monitoring of patient compliance, potential drug-drug interactions, or suspected drug abuse. Furthermore, oral fluid testing has the potential to allow for the accurate determination of a plasma drug level based on a stable ratio of drug partitioning between oral fluid and plasma. In theory, this practice would allow compliance with specific medication dosages to be evaluated through oral fluid drug testing.²

The principal advantages of oral fluid drug testing include an observed collection, information regarding very recent drug use due to drug incorporation rapidly after ingestion, and a better reflection of the concentration of drug in the blood.³ Furthermore, oral fluid may have detectable concentrations of both parent drug and metabolite(s), whereas urine generally contains high concentrations of metabolites.⁴ Perhaps most importantly, in the patient population studied is that urine samples are at much greater risk of adulteration or substitution compared to oral fluid, as the oral fluid sample is donated in the presence of the collector, providing greater assurance of specimen validity. Although the opportunity for subversion exists with oral fluid collection,

the likelihood of such practice is greatly diminished compared to urine collection.

As with any new technology, there are strengths and limitations to using oral fluid for drug testing. Drug concentrations are generally lower in oral fluid than in urine due to a lower rate of drug incorporation into the matrix. As a result, more sensitive analytical techniques may be required for drug screening and confirmation testing in oral fluid. The advantages of oral fluid drug testing along with the advances in technology have permitted this alternative matrix to progress in the field as a viable drug testing matrix.⁵ The aqueous nature of oral fluid, pharmacokinetic drug properties including pK_a values, lipophilicity, and plasma protein binding are main factors contributing to the variability in oral fluid drug incorporation rates between drug classes and respective metabolites.⁶ Furthermore, patient metabolic rate, collection time relative to last dose, and frequency of drug use can influence the window of detection of drugs in oral fluid as relative to urine.⁷ Interpersonal variation in oral fluid volume production as well as salivary pH can further impact drug incorporation rates.⁸ The objectives of this study were to first compare positivity rates for various illicit drugs and prescription medications through retrospective data mining of previously analyzed unobserved urine samples and observed oral fluid samples and second, to examine the potential oral fluid holds as an emerging alternative matrix for adherence monitoring for high-risk patients enrolled in substance abuse treatment programs.

MATERIALS AND METHODS

Specimen collection

All data analyzed in this study were retrospectively explored through data mining of de-identified analytical results. Datasets that were evaluated were free of patient name, postal or e-mail address, telephone or fax numbers, social security numbers, medical record numbers, health plan numbers, certificate or professional license numbers, VIN or license plate numbers, biometric identifiers, IP address, or any personal identifying information. Specimens were originally obtained for clinical drug monitoring from patients enrolled in a buprenorphine clinic to treat opioid addiction. The urine samples were collected into approved specimen cups in a designated collection facility and sent to Quest Laboratories for

routine pain management sample analysis by liquid chromatography tandem mass spectrometry (LC/MS-MS). The oral fluid samples were collected with Quantisal™ oral fluid collection devices (Immunoanalysis Corporation, Pomona, CA). The device collected 1 mL of neat oral fluid (± 10 percent) from the donor as indicated by a blue color change on the collection adequacy indicator. The donor provided an oral sample by placing the collector swab under his or her tongue until the indicator window turned blue. The donor did not eat or drink for 15 minutes prior to the collection time. The swab was then placed in 3 mL of oral fluid extraction buffer (included with collection device), capped for transport, and shipped to Sterling Healthcare for sample analysis.

ANALYTICAL TECHNIQUES

Standards and reagents

Analytical standards amphetamine, alprazolam, clonazepam, diazepam, nordiazepam, oxazepam, temazepam, lorazepam, 6-acetylmorphine, morphine, codeine, hydrocodone, hydromorphone, oxycodone, oxymorphone, methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), buprenorphine, norbuprenorphine, tetrahydrocannabinol (THC), cocaine, and benzoylecgonine and deuterated internal standards were obtained from Cerilliant Corp (Round Rock, TX). Buffered oral fluid matrix was obtained from Immunoanalysis Corporation. Chemical reagents including dichloromethane, isopropyl alcohol, methanol, ammonium hydroxide, water, formic acid, and sodium phosphate were purchased from VWR International (Bridgeport, NJ). All solvents were high-performance liquid chromatography grade or better.

Calibrators and quality control

Three working calibration standards were prepared in methanol from stocks at concentrations of 10,000 ng/mL, 1,000 ng/mL, and 100 ng/mL. Three working quality control standards were prepared in methanol from separate stocks at concentrations of 10,000 ng/mL, 1,000 ng/mL, and 100 ng/mL. A deuterated internal standard was prepared in methanol from stocks at a concentration of 1,000 ng/mL. All working standards were stored at -20°C when not in use. Calibration and quality control specimens were prepared by spiking aliquots of buffered oral fluid

with the appropriate volume of working calibration or quality control material. Calibration curves were generated over the range of 0.25-1,000 ng/mL. Quality control samples were included in each batch of analyzed specimens at concentrations of 10, 50, 100 ng/mL.

Extraction

All specimens underwent a sample preparation step to extract analyte(s) of interest from the respective biological matrices. For oral fluid analysis, mixed mode (reverse phase cationic exchange) solid-phase extraction (SPE) columns (Trace-B, TB-335M SPEware, San Pedro, CA) were placed into a positive pressure manifold and conditioned with methanol (1 mL) and deionized (DI) water (1 mL). Samples were applied to conditioned columns and allowed to flow by gravity. Columns were sequentially washed with DI water (3 mL), 0.1 M acetic acid (3 mL), and 25 percent methanol (3 mL). The SPE columns were dried under nitrogen pressure (50 psi; 10 minutes). Analytes were eluted with freshly prepared dichloromethane:isopropyl alcohol:ammonium hydroxide (80:18:2, v:v; 2 mL). To the eluent, 10 μL of an acetone:sulfuric acid (3:1) mixture was added to convert analytes from their free-base form to the less volatile ionic species. The extracts were evaporated to dryness under nitrogen and reconstituted in 100 μL mobile phase A (0.1 percent formic acid in water) and transferred to conical autosampler vials for LC/MS-MS analysis.

Liquid chromatography

An Agilent Technologies 1290 liquid chromatograph (LC) equipped with Zorbax Eclipse Plus C-18 columns (2.1 mm \times 50 mm \times 1.8 μm) was used for chromatographic separation of the targeted analytes. The LC columns were maintained at 50°C in the thermostated column compartment. The column compartment was configured with a 10-port valve and two LC columns to enable alternating column regeneration. Mobile phases consisted of 0.1 percent formic acid in DI water (A) and 100 percent methanol (B). The mobile phase flow rate was set at 0.7 mL/min. Initial mobile phase conditions were held at 0 percent B for 0.1 minutes then increased to 75 percent B over 5 minutes. The mobile then returned to initial starting conditions for a final run time of 5.5 minutes. All analyte retention times were

determined to be within ± 5 percent of calibrator retention times.

Mass spectrometry

An Agilent Technologies 6460 triple quadrupole mass spectrometer (QQQ) with a Jetstream electrospray source was operated in positive ion mode with the following common parameters: nitrogen drying gas temperature 350°C, nitrogen sheath gas temperature 400°C, nitrogen drying gas flow 10 L/min, nitrogen sheath gas flow 11 L/min, nebulizer pressure 45 psi, capillary voltage 4,000 V, and nozzle voltage 1,000 V. Table 1 summarizes analyte-specific multiple reaction monitoring (MRM) transitions, collision cell energies, fragmentator voltages, and retention times. One MRM transition served as a quantifier transition and a second MRM transitions served as a qualifier transition. All qualifier ion ratios were determined to be within ± 20 percent of calibrator qualifier ion ratios. All analyte specific parameters were optimized using individual methanolic standards and analysis in either full scan or product ion monitoring modes. Analytical cutoffs of the targeted analytes in both matrices are illustrated in Table 2.

RESULTS

Figure 1 illustrates the positivity observed for the various drugs analyzed from patients enrolled in a large substance abuse treatment practice. Patients provided either an unobserved urine ($n = 4,560$) or an observed oral fluid ($n = 2,368$) for routine analysis. Anonymous data mining to reexamine the clinical results allowed for the generation of positivity rates of the various drugs of interest in the two matrices. Differences in positivity rates were observed for 6-acetylmorphine, amphetamine, benzodiazepines, buprenorphine, THC, cocaine, codeine, hydrocodone, methadone, morphine, oxycodone, and oxymorphone, as shown in Table 3. Hydromorphone was the only drug/metabolite studied that showed a higher positivity rate in urine compared to oral fluid. The greatest difference in positivity observed between the two matrices was seen with buprenorphine and heroin metabolite, which showed 22 times greater positivity and five times greater positivity in oral fluid compared to urine, for buprenorphine and heroin metabolite detection, respectively. The distribution of positivity rates observed in the two matrices is illustrated in Figure 1.

DISCUSSION

This statistical exercise served as a collection process comparison of routinely collected urine and oral fluid samples obtained from high-risk substance abuse patients at a buprenorphine clinic. Evaluation of the unobserved and observed sample collection illustrated the potential oral fluid holds as an optimal alternative matrix for compliance monitoring of patients suffering from addiction, undergoing buprenorphine treatment. Unobserved urine samples were collected from 4,560 patient encounters and were analyzed for a targeted panel of commonly abused therapeutic and illicit drugs. Likewise, 2,368 patients from the same high-risk clinic provided observed oral fluid samples for analysis with the same drug-testing panel. Comparisons were made between the urine and oral fluid positivity rates for the drugs and metabolites listed in Table 2 based on the measured concentration of greater than cutoff.

Statistics on the relapse rate of patients treated for substance use disorders are on the order of 40-60 percent.⁹ Negative urine drug testing results may be the product of substituted or adulterated urine submitted for drug testing to mask the patient's continued use of illicit or nonprescribed medications. Oral fluid donated in the presence of the collector resulted in higher positivity rates compared to urine samples for majority of drugs tested in this statistical exercise. This suggests that observed oral fluid collection may likely reduce the risk of adulteration or substitution commonly seen with urine drug testing. Additionally, the disagreement in positive results observed between the two matrices may also be explained by the differences in urine and oral fluid drug incorporation rates, as recently consumed drugs appear first in the oral cavity prior to metabolism and urinary excretion.¹⁰ Regardless, in the high-risk substance abuse population evaluated, oral fluid revealed a greater percentage of positive toxicology results that may have been overlooked had urine been the testing matrix. The knowledge of the confirmed presence of these drugs is crucial to implement effective patient care. Thus, the greater oral fluid positivity rates observed hints at increased use of oral fluid for the monitoring of substance abuse patients. Specifically, those highly addicted individuals in treatment programs where there is a significant threat of sample substitution.

A large proportion of patient data reanalyzed in this study were being treated with buprenorphine

Table 1. Oral fluid LC/MS-MS MRM transitions

Compound name	Precursor ion	Product ion	Ret time, min	Fragmentor	Collision energy
6-Acetylmorphine	328.2	165 (152)	2.03	160	40 (76)
6-Acetylmorphine d6	334.2	165	2.03	165	36
Alprazolam	309.1	281.1 (205.1)	3.53	140	24 (36)
Alprazolam d5	314.2	286.2	3.53	140	24
Amphetamine	136.1	119.1 (91.1)	1.79	90	0 (12)
Amphetamine d5	141.1	124.1	1.79	75	4
Benzoylcegonine	290.2	168.1 (82.1)	2.54	120	16 (32)
Benzoylcegonine d3	293.2	85.1	2.54	128	28
Buprenorphine	468.4	396.3 (187.3)	3.18	220	55 (55)
Clonazepam	316.1	270.2 (214.1)	3.39	175	24 (40)
Clonazepam d4	320.1	274.1	3.38	175	24
Cocaine	304.2	182.1 (82.1)	2.66	125	16 (28)
Cocaine d3	307.2	85.1	2.65	130	28
Codeine	300.3	165 (152)	1.76	140	44 (76)
Codeine d3	303.3	152	1.75	150	72
Diazepam	285.2	257.1 (222.1)	3.68	170	20 (24)
Diazepam d4	290.2	227.1	3.67	175	28
EDDP	278.2	249.2 (219.1)	3.09	170	20 (44)
EDDP d3	281.3	234.1	3.08	165	32
Hydrocodone	300.3	199.1 (128)	1.95	155	28 (60)
Hydrocodone d3	303.3	199.1	1.94	150	28
Hydromorphone	286.3	185 (128)	1.15	150	28 (68)
Hydromorphone d3	289.3	185	1.14	155	28
Lorazepam	321.1	275 (229)	3.5	105	20 (32)
Methadone	310.2	265.1 (105.1)	3.33	110	20 (28)
Methadone d9	319.3	268.2	3.32	115	12
Morphine	286.3	165 (152)	0.83	145	68 (44)
Morphine d6	292.3	152	0.82	155	72
Norbuprenorphine	414.3	164.9 (114.9)	2.99	220	80 (80)
Nordiazepam	271	165 (140)	3.59	140	28 (28)
Nordiazepam d5	276.1	140	3.58	140	28
Oxazepam	287.1	269.1 (241.1)	3.5	125	12 (20)

Table 1. Oral fluid LC/MS-MS MRM transitions (continued)

Compound name	Precursor ion	Product ion	Ret time, min	Fragmentor	Collision energy
Oxazepam d5	292.1	246.1	3.5	120	20
Oxycodone	316.3	298.1 (241.1)	1.86	120	16 (28)
Oxycodone d6	322.4	304.2	1.85	130	16
Oxymorphone	302.3	284.1 (227)	0.98	130	16 (24)
Oxymorphone d3	305.3	287.1	0.97	125	16
Temazepam	301.1	255.1 (177)	3.56	105	20 (44)
Temazepam d5	306.2	260.1	3.55	115	20

for opioid addiction. The successful detection of buprenorphine in oral fluid demonstrates oral fluid testing as a convenient process to monitor buprenorphine adherence, and the drastically higher detection of the drug in oral fluid compared to urine signifies the potential oral fluid holds for buprenorphine therapy monitoring. Furthermore, the lower positivity rate of buprenorphine detected in urine indicates that substitution or adulteration of samples is likely commonplace in this patient population. Negative urine buprenorphine drug testing results are an unintended consequence of the adulteration process in an attempt to mask illicit or nonprescription drug positives. Therefore, negative urine drug testing results in patients prescribed buprenorphine regularly should be a flag for clinicians to question the authenticity of the specimen submitted for analysis.

In addition to buprenorphine, the positivity rates of commonly abused therapeutic and illicit drugs were greater in the oral fluid testing matrix. In particular, the substantially greater positivity rate of opioids in oral fluid is impactful, as many of the patients included in the study were enrolled at the clinic to receive therapy to overcome drug abuse, specifically opioid addiction. Heroin metabolite (6-MAM) was detected more than five times as often in oral fluid samples compared to urine. Heroin is quickly metabolized to 6-MAM and then undergoes further deacetylation to morphine; thus making both metabolites common indicators of heroin use.¹¹ However, after heroin intake, the short half-life of 6-MAM leads morphine to be the more commonly detected marker of heroin use in urine. This leads to complicated interpretation of morphine positives in

urine as a positive result can signify metabolism of 6-MAM or the ingestion of morphine or codeine.¹² The greater percentage of 6-MAM positives observed in the oral fluid specimens suggests that a large proportion of the morphine positives observed were likely due to heroin consumption. The ability to improve interpretation of heroin use through oral fluid's advantageous ability to better detect 6-MAM furthers the promise that the alternative matrix holds as the specimen of choice for substance abuse drug monitoring programs.

Additionally, prescription opioids with established histories of diversion and abuse, such as oxycodone and hydrocodone, showed an eightfold and 13-fold increase in positivity rate, respectively, in oral fluid. Furthermore, while it is established that metabolites of hydrocodone and oxycodone are poorly incorporated into oral fluid,¹⁰ positive results for hydromorphone and oxymorphone observed are in part due to the metabolism of these parent compounds. In addition, illicit use of nonprescribed hydromorphone or oxymorphone would account for the remaining positive results. Hydromorphone was the only drug tested that showed lower positivity in oral fluid compared to urine. This is likely explained by the poor incorporation of hydrocodone metabolites into oral fluid. Additionally, lower detection rates of hydromorphone in oral fluid have previously been observed when a dose is initiated or changed also explaining potential disagreement between the two matrices.⁷

Moreover, greater oral fluid positivity rates were also noted for other routinely abused drugs, such as stimulants (amphetamine) and sedatives (benzodiazepines). Amphetamine was detected six times

Table 2. LC/MS-MS oral fluid and urine cutoffs based on analyte

Drug	Oral fluid cutoff, ng/mL	Urine cutoff, ng/mL
Amphetamine	2.5	250
Benzodiazepines		
α-Hydroxyalprazolam	N/A	25
Alprazolam	1	N/A
7-Aminoclonazepam	N/A	25
Clonazepam	1	N/A
Diazepam	1	50
Nordiazepam	1	50
Oxazepam	1	50
Temazepam	1	50
Lorazepam	1	50
α-Hydroxymidazolam	N/A	50
α-Hydroxytriazolam	N/A	50
Hydroxyethylflurazepam	N/A	50
6-MAM	4	10
Morphine	2.5	50
Codeine	2.5	50
Hydrocodone	2.5	50
Hydromorphone	2.5	50
Oxycodone	2.5	50
Oxymorphone	2.5	50
Methadone	2.5	100
EDDP	2.5	100
THC	2	15
Cocaine	8	N/A
Benzoylcegonine	8	100
Buprenorphine	0.25	2
Norbuprenorphine	0.25	2

as often in oral fluid than in urine, while benzodiazepines as a class showed more than a twofold increase in oral fluid positivity. Positivity rates of other traditional drugs of abuse such as THC and

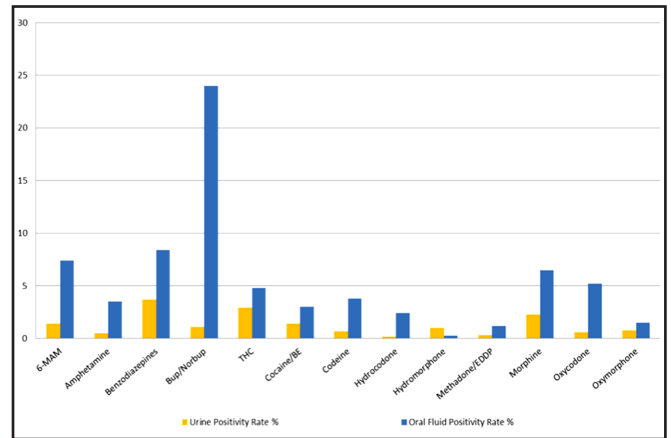


Figure 1. Comparison of urine vs oral fluid drug testing positivity rates in a substance abuse patient population. Urine, n = 4,560 and oral fluid, n = 2,368.

Table 3. Unobserved urine vs observed oral fluid collection positivity (percent)

Drug	Urine positivity rate, percent	Oral fluid positivity rate, percent
Amphetamine	0.5	3.5
Benzodiazepines	3.7	8.4
6-Acetylmorphine	1.4	7.4
Morphine	2.3	6.5
Codeine	0.7	3.8
Hydrocodone	0.18	2.4
Hydromorphone	1	0.25
Oxycodone	0.6	5.2
Oxymorphone	0.79	1.5
Methadone/EDDP	0.3	1.2
Buprenorphine/norbuprenorphine	1.1	24
THC	2.9	4.8
Cocaine/benzoylcegonine	1.4	3
Urine and oral fluid specimens are not paired.		

cocaine averaged a twofold increase in oral fluid over urine.

Interpretation of the data obtained in this statistical exercise is limited as the urine and oral fluid samples were not simultaneously collected. Despite

lack of paired specimens, the differences observed in positivity rate between the two matrices strongly illustrate the growing potential oral fluid holds as a simple, convenient, difficult to adulterate specimen type for high-risk patients.

Initial results from ongoing studies correlating positivity rates in paired samples from several matrices (including urine and oral fluid) suggest that alternate matrix drug testing provides great value when attempting to obtain an accurate assessment of patient drug use.

CONCLUSION

Oral fluid is a convenient alternative matrix used in monitoring patients for illicit and nonprescribed drugs in substance abuse treatment programs, while concurrently holding great potential to improve compliance assessment with maintenance treatment regimens. Oral fluid has distinct advantages over urine including ease of collection and a very low risk of substitution or adulteration. The ability of oral fluid to reveal higher positivity rates of commonly abused licit and illicit drugs than urine further illustrates its advantage over traditional urine drug testing.

The detection of both illicit drug use and noncompliance to a greater extent in oral fluid in this patient population establishes oral fluid as a promising matrix for the pain management setting, especially substance abuse programs where sample adulteration is at higher risk. Specifically, the dramatic increase in buprenorphine positivity observed in oral fluid samples compared to urine stresses the potential oral fluid drug testing holds for substance abuse programs prescribing buprenorphine for opioid addiction.

Frank Kunkel, MD, Accessible Recovery Services, Pittsburgh, Pennsylvania.

Elizabeth Fey, MS, Sterling Healthcare, Huntington, New York.

Damon Borg, PhD, Sterling Healthcare, Huntington, New York.

Richard Stripp, PhD, Sterling Healthcare, Huntington, New York.

Christine Getto, BS, Executive Assistant, Accessible Recovery Services, Inc., Pittsburgh, Pennsylvania.

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