Journal of Clinical Toxicology
ISSN: 2161-0495

Smiley et al., J Clin Toxicol 2019, 9:2

Research Article Open Access

A Comparison of Urine and Oral Fluid Drug Testing

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Received date: March 05, 2019, **Accepted date:** March 25, 2019, **Published date:** April 01, 2019

Abstract

Urine has become a staple for drug screening; however limitations due to the inconvenience of the collection process and a lack of integrity due to possible adulteration, substitution, and diversion have paved the way for other matrices such as oral fluid. A clinical study was conducted to compare the use of oral fluid versus urine for compliance with monitoring rehabilitation, pain, behavioural health, and internal medicine patients. Patients (n=142) undergoing drug monitoring at 25 clinics within 12 states provided paired oral fluid and urine specimens. The oral fluid specimens were collected with Quantisal® saliva collection devices the same day as the urine collection. All specimens were analyzed by validated high sensitivity liquid chromatography- tandem mass spectrometry procedures (LC-MS/MS) using AB Sciex 6500 LCMS systems for 26 drugs and/or metabolites. Of the 142 paired specimens, there was an agreement of 52.1% where 244 (7.66%) analytes were positive in both matrices and 2677 (84%) analytes were negative in both matrices, with a Cohen's Kappa coefficient of 0.501 indicating there is a 'moderate' agreement. The analyte detected most frequently in both urine and oral fluid was buprenorphine, followed by amphetamine. In urine, higher rates of detection occurred with hydromorphone, norbuprenorphine, and oxazepam, while oral fluid saw higher rates of detection with methamphetamine, heroin metabolite 6-Monoacetylmorphine (6MAM), and morphine. Factors that are responsible for the difference of analytes detected between the two matrices include the length of detection in urine, the lower cut offs in the oral fluid analysis due to lower concentrations of drugs, and the physiological factors that cause detection rates to differ between the two matrices for certain drug classes. The authors conclude oral fluid drug testing may be an alternative to urine drug testing when illicit drug testing or recent drug use is the primary goal of drug testing.

Keywords: Oral fluid drug testing; Urine drug testing; LC-MS/MS; Matrix; Detection time; Cut-offs; Illicit drugs; Prescription monitoring

Abbreviations: U: Urine; OF: Oral Fluid; DHHS: Department of Health and Human Services; LC-MS/MS: liquid Chromatography-Tandem Mass Spectrometry; MPA: Mobile phase-A; MPB: Mobile phase-B; SEM: Standard Error of the Mean; SN: Sensitivity; SP: Specificity; TP: True Positive; FP: False Positive; FN: False Negative; TN: True Negative; 6MAM: 6-Monoacetylmorphine

Introduction

Drug testing has become essential in today's society with substance abuse increasing daily, worldwide, and the need for prescription drug monitoring in the fields of pain management, behavioural health, and internal medicine, among others [1]. The most common test matrix is urine [2]. As the need for drug testing has expanded, there have been advances in other drug monitoring techniques and matrices, such as oral fluid testing [3]. The growing popularity in oral fluid as a desirable matrix has opened the discussion of its concordance with urine drug testing.

Oral fluid drug testing has many advantages over urine testing, the major advantage being that it uses a less invasive collection process to benefit the patient [4]. Furthermore, it limits the potential for adulteration, substitution, and diversion due to the ability to directly observe collections, and minimizes the concerns surrounding specimen integrity [3].

Limitations in recent and past publications of this nature include single subset patient populations, for example, pain patients [4], or are limited to certain drug classes [5]. In this clinical study, we set you to determine the concordance of urine and oral fluid drug testing collected on patient's same day while expanding on patient populations included in the data (i.e., pain, treatment, internal medicine, etc.) as well as only including one urine and oral fluid specimen per patient. For the purposes of this study, concentrations greater than or equal to the validated cut off level were detected by liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS).

Materials and Methods

Participants and specimen collection

This study was approved by Aspire IRB Santee, CA and was conducted over a 4-month period from August 2016 to November 2016, with specimens collected from 25 clinics across 12 states and shipped to Precision Diagnostics (San Diego, CA). Among the clinics, clinical specialties included 11 addiction/rehabilitation clinics, 6 internal or family medicine clinics, 4 pain management clinics, and 4 behavioural health clinics where both urine and oral fluid samples were collected on the same day from each patient. The time between each collection could not be determined as collection was completed within the individual clinics. The randomly selected specimens were analysed from 142 patients undergoing prescription drug management programs within addiction, pain, behavioural, or internal medicine practices. Only one urine and oral fluid specimens pair collected same day from each patient was used in the analysis to prevent bias. Collection and storage of urine and oral fluid specimens were completed and regulated at the corresponding physician offices and sent to Precision Diagnostics. Oral fluid specimens were collected with

Quantisal® saliva collection devices per the Quantisal® manufacturing instructions. The collector was placed under the patient's tongue until the indicator turned blue. Then the collector was placed in the buffer solution of the transport tube and packaged for transport. The analyses of analytes in each specimen tested were limited to those deemed medically necessary by the requesting physician.

Laboratory analyses

The urine specimens were prepared and analysed using the previously published method by Krock et al. [6]. A high-sensitivity LC-MS/MS method was developed and validated capable of detecting low concentrations of up to 71 drugs and metabolites important for monitoring medication adherence and substance use disorder for the urine specimens. The test results were analysed on logarithmic distributions fitted with a trend line to estimate the required cut-off level necessary to capture the normal distribution of each drug and metabolite. An LC-MS/MS method was developed and validated, capable of quantitating up to 33 drugs and metabolites important for monitoring medication adherence and substance use disorder for the oral fluid specimens. A Shimadzu (Kyoto, Japan) 20 series binary pump system, well-plate auto sampler, and temperature-controlled column oven was paired with a Sciex (Framingham, MA) 6500 triple quadruple mass spectrometer. LC-MS grade water was obtained from a Sartorius (Bohemia, NY) ultrapure water system, LC-MS grade methanol, acetonitrile and ammonium hydroxide were obtained from EMD Millipore (Billerica, MA), and LC-MS grade formic acid was obtained from Covachem (Loves Park, IL). A Phenomenex (Torrance, CA) Kinetex® phenyl-hexyl column with dimensions of 50 × 4.6 mm and 2.6 µm particle size was used for chromatographic separation. The binary pump system delivered a mixture of the mobile phases at the proportion and flow rate displayed in Table 1. Mobile phase-A (MPA) was 0.1%formic acid in LC-MS grade water and Mobile Phase- B (MPB) was LC-MS grade methanol containing 0.1%formic acid.

Time	Event	Parameters
0	Pump B Conc.	5
2.2	Pump B Conc.	40
4.5	Pump B Conc.	95
5	Pump B Conc.	95
5.2	Pump B Conc.	5
6	Controller	Stop

Table 1: The gradient of mobile phase delivered during oral fluid analysis allowed separation of the isobaric compounds and interferents and provided a high organic phase portion to clean the column before the next analysis.

The mass spectrometers used the following settings common to all analytes; curtain gas of 35 L/min, collision gas of 10 L/min, positive mode Ion Spray voltage of 2500 V, source temperature of 450°C, ion source gas 1 of 60 L/min and ion source gas 2 of 50 L/min. Two transitions for each analytes were optimized for declustering potential, collision cell energy and exit potential. Analytes and internal standards were obtained from Cerilliant (Round Rock, TX). Four-point calibration curves were prepared from the cut-off level to 25 times the cut-off level for each analytes. Two quality control samples were analysed with each batch of specimens to ensure acceptability of results. Samples were prepared by an automated solid phase extraction procedure, outlined here. Automated sample handling tips filled with 10 mg of cation exchange resin (DPX Technologies, Coloumbia, SC) were wetted with a mixture of 50%each methanol and deionized water, by volume. A mixture of stable isotope internal standards was added to the specimens and drawn into the solid phase extraction tips. Unbound material was rinsed away with deionized water and the analytes were eluted into a 96-well plate with 5% ammonium hydroxide in acetonitrile. This plate was dried to completeness using a MiniVap (Porvair Sciences, Wrexham, Wales, UK) drying station set to 40 L/min of nitrogen and heated to 45°C. Prior to analysis, the specimens were reconstituted with 100 µL of a mixture of 20% methanol and 80% water by volume, with 0.1% formic acid. Five µL of prepared specimen was injected onto the analytical column for LC-MS/MS analysis and a flow diversion valve sent the first 1.3 minutes of eluent to waste. Results were analyzed using Indigo Bio Automation's (Indianapolis, IN) ASCENT software. A four-point calibration curve was used with a linear fit and 1/x weighting. Calibrator acceptability was within ± 20% of the expected concentration with an R^2 value of greater than 0.98. The area ratio of the analyte to a deuterated internal standard was used to account for ion suppression. All analytes had a signal to noise calculation of greater than 10 at the lower limit of quantitation. The precision and accuracy of the assay was evaluated over five days for both intraday and interday variability and all analytes were within 20% CV. Recovery was determined to be within ± 20% for all analytes.Drugs and metabolites detected in urine and oral fluid are listed in Table 3 along with the associated cut off levels used as developed and validated per Krock et al. [6].

Data analyses

For each analyte, the mean, Standard Error of the Mean (SEM), and the concentration range were determined in Table 4.The Sensitivity (SN), how likely to detect analytes in both urine and oral fluid, and Specificity (SP), how likely to have a negative in urine or oral fluid were determined and used to evaluate the validity of oral fluid drug testing as an alternative or replacement for urine drug testing [7,8]. The equations used to determine sensitivity and specificity using the previously published method in Fenn Buderer [9], where True Positive (TP) is the number of urine and oral fluid positives; False Positive (FP) is the number of urine negatives and oral fluid positives; False Negative (FN) is the number of urine positives and oral fluid negatives; True Negative (TN) is the number of urine and oral fluid negatives (Table 5). Furthermore, the predictive values, both positive, proportion and negative were determined using the determine TP, FP, FN, and TN values resulted in Table 5.

Urine and oral fluid in this study are considered the 'judges' operating independently from the analytes or 'categories,' to determine the percent agreement and ultimately the reliability of the results, or Cohen's Kappa [10]. Cohen's Kappa values less than 0 are considered 'poor', 0-0.20 are 'slight', 0.20-0.40 are 'fair', 0.40-0.60 are 'moderate', 0.60-0.80 are 'substantial,' and 0.80-1.00 are in almost 'perfect agreement [11]. The agreement between the two 'judges' was determined by the proportion in which the units agree, or po, and the proportion of expected agreement, or pe; where po and pe are found per Cohen [10]. (Table 2) The same analysis was completed for all analytes and as well as two subsets of drugs set out by the Department of Health and Human Services (DHHS) for federal workplace practice.

		Urine		**Total N**
		P1	P2	
Oral Fluid	P2	P1 P1	P2 P1	∑ (P1P1+ P2P1)
	P2	P1 P2	P2 P2	∑ (P1P2+ P2P2)
		∑ (P1P1+ P1P2)	∑ (P2P1+ P2P2)	∑ (P1P1+ P2P2)

Table 2: Cohen's Kappa values drugs and metabolites in oral fluid and urine.

Drug (Metabolite)	**Oral Fluid Cut off levels (ng/mL)**	**Urine Cut Off Levels (ng/mL)**
Alprazolam	1	5
Amphetamine	2	25
Buprenorphine	0.03	5
(Norbuprenorphine)	5	5
Carisoprodol	2	10
(Meprobamate)	2	100
Clonazepam	0.5	5
Cocaine	1	5
Fentanyl	0.05	1
(Norfentanyl)	0.2	2
6-monoacetylmorphine (Heroin metabolite)	0.5	5
Hydrocodone	1	5
(Hydromorphone)	0.5	5
Hydromorphone	0.5	5
Lorazepam	1	10
Methadone	1	50
(EDDP)	1	100
Methamphetamine	1	50
Morphine	1	50
(Hydromorphone)	0.5	5
Naloxone	2	10
Oxycodone	1	10
Temazepam	0.5	10
(Oxazepam)	1	10
Tetrahydrocannabinol (THC)	2	25
Tramadol	1	25
Zolpidem	1	1

Table 3: Cut off levels for drugs and metabolites in oral fluid and urine.

Results

Prevalence and concentration

The prevalence and concentration of 26 drugs and metabolites were evaluated for 142 patients, where a urine and oral fluid specimen was collected in concordance. The analyses of 142 patients in urine and oral fluid specimens submitted from August 2016 to November 2016 were confirmed by LC-MS/MS and are listed in Table 4. Analytes that could not be tested for in the oral fluid matrix were not included for the purposes of this study. For example, results for many metabolites such as noroxycodone, norhydrocodone, alphahydroxyalprazolam, 7-aminoclonazepam, and nordiazepam were excluded from this study as they are not currently incorporated into the LC-MS/MS oral fluid testing.

Urine (ng/mL) †				
Drugs/Metabolites	N	Mean ± SEM	Median	Range
Amphetamines				
Amphetamine	23	2912.7 ± 888.4	834	25-15631
Methamphetamine	19	10664.5 ± 4909.7	1635	58.3-84453.1
Benzodiazepines				
Alprazolam	13	125.3 ± 34.1	114	2-368
Clonazepam	8	16 ± 4.1	9	14427
Lorazepam	5	511.2 ± 212.6	321	45-1328
Oxazepam	10	620.4 ± 315.7	271	66-2933
Temazepam	8	370.7 ± 135	221	71-1057
Cannabis				
THCA	14	216.6 ± 55.8	147.5	23-673
Carisoprodol				
Carisoprodol	1	-	-	-
Meprobamate	2	113429.5 ± 79335.6	113429.5	1232-225627
Cocaine				
Cocaine Metabolite	14	6167.5 ± 3053.2	51	5-28222
Opiates				
Codeine	6	497.6 ± 212.4	480	23-1338
Morphine	18	21784.5 ± 5467	16503	410-79252
Hydrocodone	17	3488.9 ± 1472.6	773	6-21509
Hydromorphone	30	475.8 ± 157.9	88	14-2738
Oxycodone	21	10743.5 ± 7853.7	1660	301-131810
Opioids				
Buprenorphine	48	665.5 ± 271.9	100.5	1857108
Norbuprenorphine	46	459.7 ± 80.2	280	167799
Fentanyl	3	21.3 ± 11.5	12	17958
Norfentanyl	4	180.5 ± 78.1	164.5	2-391
Methadone	12	3191.8 ± 699.6	3006	1096-8123
EDDP	12	8060.1 ± 1356	6544	1987-15749

Tramadol				
Tramadol	8	9825.7 ± 2256.4	8333	137-18012
Other				
6-monoacetylmorphine (Heroin metabolite)	5	405.8 ± 172.2	358	3-1005
Naloxone	42	674.2 ± 145.5	329	355961
Zolpidem	4	117.7 ± 61.8	51	11-291
† patients excluded from this table: patients with out of range creatinine (<20 mg/dl), patients suspected of contamination of the oral cavity and patients suspected of pill shaving				

Table 4a: Prevalence, mean, median, and range of drug and metabolite concentrations in urine and oral fluid.

Oral Fluid (ng/mL) †				
Drugs/Metabolites	**N**	**Mean ± SEM**	**Median**	**Range**
Amphetamines				
Amphetamine	31	753.2 ± 297.9	86.03	2.19-7179.48
Methamphetamine	35	2568 ± 1075.1	76.3	1-22625.68
Benzodiazepines				
Alprazolam	15	12.7 ± 8.4	3.77	1.15-109.14
Clonazepam	9	6.1 ± 4.2	1.27	0.61-33.3
Lorazepam	2	1.6 ± 0.1	1.6	1.45-1.75
Oxazepam	2	1.6 ± 0	1.63	1.63-1.63
Temazepam	4	2.7 ± 1.4	2.685	0.66-4.71
Cannabis				
THC	28	196.3 ± 101.4	38.31	3.5-1917.5
Carisoprodol				
Carisoprodol	2	44.1 ± 19.1	44.085	17.04-71.13
Meprobamate	6	1095.9 ± 544.9	195.34	4.74-2829.73
Cocaine				
Cocaine Metabolite	15	248.6 ± 120.2	108.03	1.64-1367.2
Opiates				
Codeine	11	18.4 ± 9.2	2.895	1.22-79.62
Morphine	25	123.6 ± 72.2	15.64	1.29-1431.26
Hydrocodone	19	493.5 ± 230.3	128.895	1.06-3404.16
Hydromorphone	10	12.9 ± 9.8	2.11	0.55-86.26
Oxycodone	26	370.1 ± 174.2	109.62	1.02-2984.76
Opioids				
Buprenorphine	46	311 ± 90.3	50.005	0.36-1892.72
Norbuprenorphine	11	10 ± 1.7	8.37	5.01-19.89

Fentanyl	6	14.2 ± 6.1	12.96	0.16-37.99
Norfentanyl	3	1.5 ± 0.7	1.1	0.38-3.08
Methadone	17	343.5 ± 57.8	370.79	1.22-724.6
EDDP	13	6 ± 1.9	4.3	1.08-23.06
Tramadol				
Tramadol	6	235.2 ± 118.6	203.155	2.16-532.21
Other				
6-monoacetylmorphine (Heroin metabolite)	14	120.6 ± 94.6	10.39	1.83-1205.16
Naloxone	20	80.2 ± 20.3	60.55	2.51-275.08
Zolpidem	3	16.2 ± 6.7	17.5	1.36-29.63
† patients excluded from this table: patients with out of range creatinine (<20 mg/dl), patients suspected of contamination of the oral cavity and patients suspected of pill shaving				

Table 4b: Prevalence, mean, median, and range of drug and metabolite concentrations in urine and oral fluid.

From the results listed in Table 4, in urine, norbuprenorphine, hydromorphone, naloxone, oxazepam and temazepam tested positive most prevalently while their oral fluid counterparts were negative for these analytes. In oral fluid, methamphetamine, heroin metabolite (6MAM), amphetamine, morphine, codeine and cocaine tested positive most prevalently in oral fluid while their urine counterparts were negative for these analytes.

In urine when methadone, methamphetamine, carisoprodol, and meprobamate were positive, the oral fluid counterparts were also positive. In oral fluid, if naloxone, norbuprenorphine, zolpidem, and lorazepam were positive, the urine counterparts were also positive. It was also noted when temazepam and oxazepam were positive in urine, the oral fluid counterparts were always negative.

In many cases, the concentrations found in urine were 10 to 100 times greater compared to oral fluid concentrations. Per the methods used in Cohen (1960), the Pearson product moment correlation coefficient were determined for 6 analyte data sets (morphine; r=0.305, oxycodone; r=0.052, buprenorphine; r=0.079, amphetamine; r=0.51, methamphetamine; r=0.22, and naloxone; r=0.09). This is the measure of strength of a linear relationship between two subsets where r=1 is a perfect correlation, and r=0 indicated no correlation [12]. From these results, there was a positive relationship for amphetamine, morphine, and methamphetamine. Figure 1 demonstrates an example of this relationship for amphetamine concentrations in urine and oral fluid.

Agreement between oral fluid and urine

The overall agreement between the urine and oral fluid samples is shown in Table 6. This table included all n=142 patients with no exclusions. Based on statistical references the agreement of all analytes listed in Table 5, between urine and oral fluid was found as 52.1% [10,13,14]. These values fall into range with Heltsley et al. study of comparison, however the Cohen's Kappa of 0.501, indicated a lesser strength, 'moderate,' between urine and oral fluid results compared to Heltsley et al. study [4]. All 26 drug analyte results were evaluated using urine and oral fluid as the two raters.

Two subsets of the data per SAMHSA's Workplace Program Guidelines were also examined using the same methodology. The first subset examined were the analytes set out in the Department of Health and Human Services (DHHS) which included amphetamine, methamphetamine, THC, cocaine, codeine, morphine, and hydromorphone. The agreement and Cohen's Kappa was 73.1% and 0.682 respectively. The second subset was the extended DHHS, added in hydrocodone and oxycodone which resulted in an agreement of 68.5% and Cohen's Kappa of 0.644. Both subsets were found to have a 'substantial' agreement between urine and oral fluid (Table 6) [10,15].

Discussion

This study compared the use of urine and oral fluid drug testing for licit and illicit drugs in the population of addiction, internal medicine, behavioural medicine, and pain patients, with the potential for oral fluid matrix to be used as an alternative to the standard urine analysis. All urine and oral fluid specimens were analysed by LC-MS/MS.

There was 'moderate' agreement found between the test results in the oral fluid and urine specimens as a 52.1% agreement and 0.501 Cohen's Kappa coefficient between analytes was calculated. This suggests that there is a 'moderate' relation between urine and oral fluid drug testing across all observed analytes in this study, indicating oral fluid to be a substitute, but not replacement to urine drug testing [10].

There are many factors that can be attributed to the observed differences between urine and oral fluid drug testing. Pharmacokinetic factors involved in the two matrices can have a large effect on the concordance of results. Many drugs are subject to 'phase 1' drug metabolism and undergo some degree of metabolism before entering 'phase II' in which the drugs are rendered more polar enabling the final products of drug metabolism to be excreted in urine [16,17]. Once bound or glucuronidated, the polarity and charge on the drug or metabolites make it hard to passively diffuse into oral fluid for detection [1,16,17]. This phenomenon is particularly observed in benzodiazepines which are highly protein bound and weakly acidic allowing for ease of detection with urine rather than oral fluid drug tests [1].

Alternatively, oral fluid samples tend to detect drugs in the unbound or free form. Therefore, initial confirmatory tests target parent drugs.

For example, the carboxy metabolite of THC has almost no presence in oral fluid, hence oral fluid testing targets the parent drug THC, whereas urine detection involves the detection of the carboxy metabolite, THC-COOH [1,18]. There are some exceptions to this rule of thumb, like the conversion of cocaine to its metabolite, benzoylecgonine which is very well captured in oral fluid [1,18]. A main distinction of oral fluid testing is its variability in pH and the influence it plays in the detection of certain analytes [1,16]. Weak bases are detected in higher concentrations and for longer time in oral fluid. Depending on the pKa and lipophilicity, these analytes are subjected to 'Ion trapping' due to the difference of pH between blood and oral fluid. The low pH in oral fluid allows weak bases to ionize, increasing oral fluid drug concentrations [16,17].

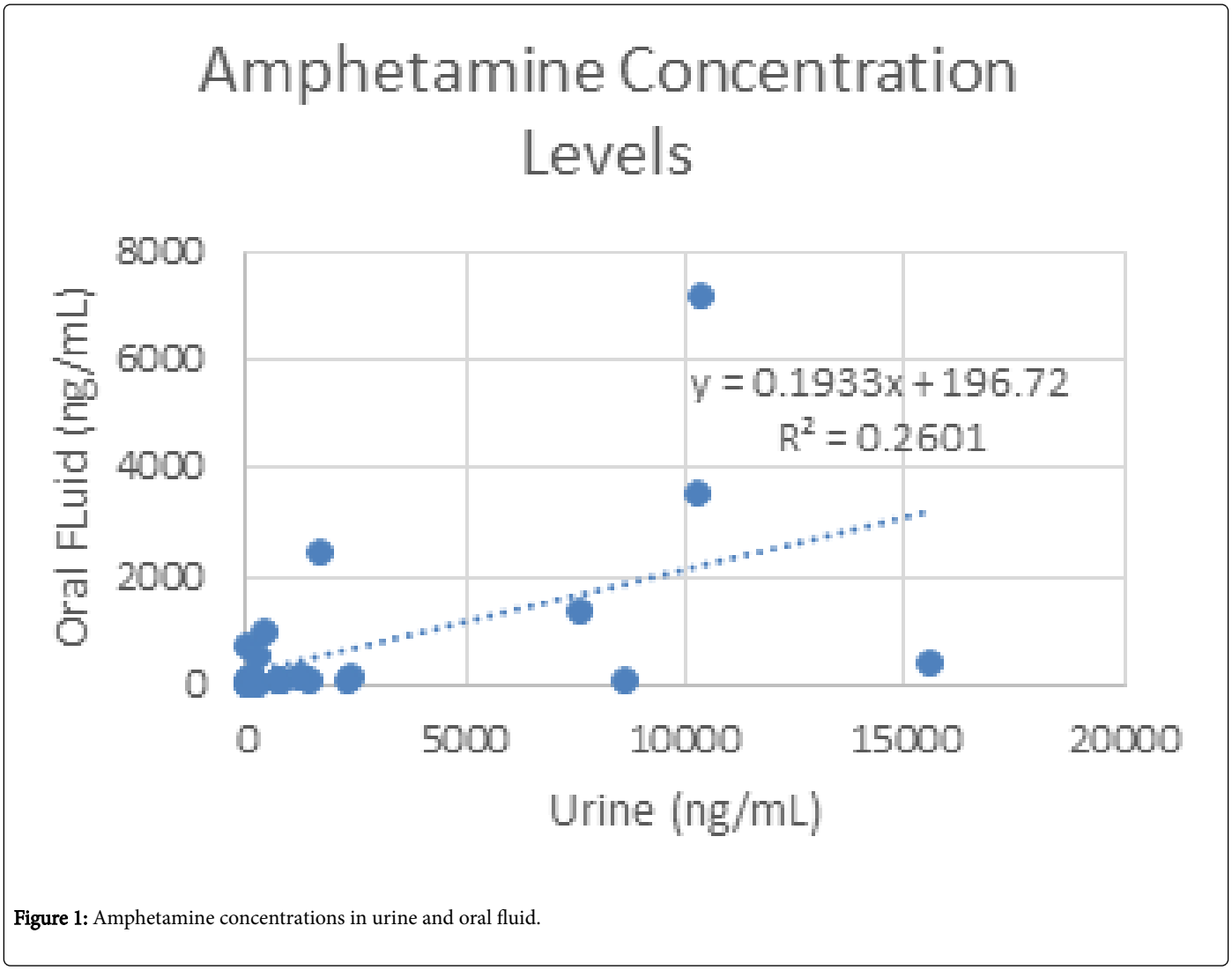

Figure 1: Amphetamine concentrations in urine and oral fluid.

Drug/Metabolites	#U Positive and of Positive (TP)	#U Negative and of Positive (FP)	#U Positive and of Negative (FN)	#U Negative and of Negative (TN)	Total
Alprazolam	10	5	3	124	142
Amphetamine	21	10	2	108	141
Buprenorphine	42	4	6	86	138
Carisoprodol	1	1	0	82	84
Clonazepam	4	5	4	129	142
Cocaine (Benzoylecgonine)	8	7	6	121	142

Codeine	2	9	4	127	142
EDDP	11	1	1	128	141
Fentanyl	2	4	1	95	102
6-monoacetylmorphine (Heroin metabolite)	3	11	2	126	142
Hydrocodone	14	5	3	120	142
Hydromorphone	7	3	23	109	142
Lorazepam	2	0	3	137	142
Meprobamate	2	4	0	78	84
Methadone	12	4	0	125	141
Methamphetamine	19	17	0	104	140
Morphine	15	10	3	114	142
Naloxone	20	0	22	48	90
Norbuprenorphine	9	2	37	90	138
Norfentanyl	2	1	2	97	102
Oxazepam	0	2	10	130	142
Oxycodone	19	7	2	114	142
Temazepam	1	3	7	131	142
THCCOOH (U)/THC (OF)	12	2	3	7	24
Tramadol	3	3	2	90	98
Zolpidem	3	0	0	57	60
Total	244	120	146	2677	3187
Total (%)	7.66%	3.77%	4.58%	84.00%	-
Total	244 (7.66%)	120 (3.77%)	146 (4.58%)	2677 (84.00%)	-
Abbreviations: U= urine; OF= oral fluid					

Table 5: Test agreement between urine and oral fluid.

		Validity		Agreement				Predictive Value	
Specimen Drug Set	**#Compa-risons**	**Sensitivity (%)**	**Specificity (%)**	**Agreement (%)**	**Agree-ment Expected by Chance (%)**	**Cohen's Kappa (%)**	**Strength of Agreement**	**Positive (%)**	**Negative (%)**
DHHS	873	67.2 (62.4-74.5)	92.2 (91.4-93.1)	73.1	15.4	68.2 (68.0-68.3)	"Substantial"	59.1 (58.5-60.0)	94.4 (92.6-96.3)
DHHS (Extended)	1157	71.2 (66.8-78.7)	92.9 (92.3-93.7)	68.5	11.7	64.35 (22.6-23.7)	"Substantial"	62.6 (61.6-63.7)	95.3 (93.8-96.9)
All Drugs	3187	62.3 (60.1-66.0)	95.7 (95.1-96.3)	52.1	4	50.1 (49.9-50.2)	"Moderate"	67.0 (66.2-67.5)	94.8 (93.7-96.0)
DHHS: Department of Health and Human Services									

Table 6: Validity, agreement, and predictive value.

The detection of an analytes can also be dependent on many factors involving the individual patient/subject such as dose, time of dose, and route of administration [1-3]. In this study, the dose and therefore time of dose of a patient relies on limited knowledge. Consequently, an

analyte positive in urine and negative in oral fluid could be due end elimination of a particular drug. For example, results in this study show a large prevalence of hydromorphone detection in urine than in oral fluid. Although pharmaceutically available, in urine hydromorphone is commonly detected as a metabolite of morphine or hydrocodone use [19]. Depending on last use, hydromorphone may not present in oral fluid unless taken in free form i.e., Dilaudid [1]. Detection of a drug may also be affected by the route of administration, for instance, drugs detected in oral fluid may be due to residual contamination of the oral cavity due to recent use after smoking or snorting a certain drug [18].

The most prevalent positives in urine with negative results in oral fluid consisted of nor buprenorphine, hydromorphone, naloxone, oxazepam and temazepam (Table 5). With the higher prevalence of drug metabolites detected in urine, these results suggest, that urine drug testing can be better used when monitoring drug elimination over time due to differences in detection time between urine and oral fluid, along with its ability to detect metabolites of the parent licit and illicit drugs. Results of this study, indicates urine analysis as fit for monitoring medication compliance and licit drug use, while also being successful in determining illicit drug use such as methamphetamine, THC, and cocaine (Table 5).

For those analytes positive in oral fluid and negative in urine, it is possible the drug had not had the allotted time needed for metabolism to then excretes the analytes in urine or may be due to contamination of the oral cavity, indicating recent use [18]. Oral fluid also tends to have a high prevalence of illicit drugs, like methamphetamine, due to the lower pH in the oral cavity causing ion trapping of the positively charged drug [11,18]. Results for the illicit drugs in this study: heroin metabolite (6MAM), methamphetamine, cocaine, and THC, all tested positive at a higher incidence in oral fluid than in urine (Table 5). This suggests that the oral fluid matrix can be used for monitoring illicit drug use and is a good indicator of recent drug use. Based on these results, it can be concluded that oral fluid testing would be successful in the treatment and rehabilitation environments, monitoring patient's illicit use of drugs. Additionally, since we see a very high concordance of buprenorphine and methadone, prescribed medications used to treat opioid addiction, oral fluid testing can be considered a successful alternative to urine monitoring [18].

In this study there was a stronger agreement between the drugs indicated in the DHHS and 'extended' DHHS subsets showing substantial agreements of 73.1%, with a Choen's Kappa of 68.2% and 68.5%, with a Choen's Kappa of 64.35%, respectively. Due to the drugs evaluated: amphetamine, methamphetamine, THC, cocaine, codeine, morphine, hydromorphone, and the addition of hydrocodone and oxycodone in the extended subset, there was a strong agreement between urine and oral fluid as seen in Table 5. These drugs capture well in both matrices as they involve illicit drugs, and parent drugs. Without the addition of metabolites for the opioids involved, the agreement between urine and oral fluid is strengthened. This supports the conclusion that oral fluid can act as a successful alternative, but not a true replacement to urine drug testing especially in the realm of pain medicine.

Conclusion

Due to the diverse patient population in this study, a high rate of positives within the pool of analytes was anticipated. This study differs from previous studies in the fraction of the test subjects still using illicit drugs. Additionally, the specimens used in this study consisted of the first urine and oral fluid specimens collected on the same day for each patient to help rule out any bias of including multiple specimens for the same patient. Lastly, when compared to other studies comparing urine and oral fluid drug matrices, the cut off levels are much lower than the industry standard cut off levels allowing for higher rates of positives [6] (Table 3).

The comparison of urine and oral fluid testing resulted in a lower Cohen's Kappa coefficient meaning a 'moderate' agreement between urine and oral fluid matrices. This can be attributed to the many different factors in which equivalence between urine and oral fluid is not always expected; route of administration, free drug versus protein bound drug, lower cut off levels in oral fluid testing, inability to monitor adulteration, substitution, or diversion with the urine samples, and the difference in detection windows. In conclusion, analyses of urine and oral fluid specimens collected from addiction, family medicine, pain, and behavioural clinics concurrently provided a 'moderate' agreement between the two matrices, and that oral fluid testing can be used as a substitute to the gold standard of urine but may not act in full as a replacement.

References

1. Drummer OH (2006) Drug testing in oral fluid. Clin Biochem Rev 27: 147-159.
2. Christo PJ, Manchikanti L, Ruan X, Bottro M, Hansen H, et al. (2011) Urine drug testing in chronic pain. Pain Physician 14: 132-143.
3. Niedbala RS, Kardos KW, Fritch DF, Kardos S, Fries T, et al. (2001) Detection of marijuana use by oral fluid and urine analysis following single-dose administration of smoked and oral marijuana. J Anal Toxicol 25: 289-303.
4. Heltsley R, Depriest A, Black DL, Robert T, Marshall L, et al. (2011) Oral fluid drug testing of chronic pain patients. I. Positive prevalence rate of licit and illicit drugs. J Anal Toxicol 35: 529-540.
5. Petrides AK, Melanson SEF, Kantartjis M, Le RD, Demetriou CA, et al. (2018) Monitoring opioid and benzodiazepine use and abuse: Is oral fluid or urine the preferred specimen type? Clinica Chimica Acta 481: 75-82.
6. Krock K, Pesce A, Ritz D, Thomas R, Cua A, et al. (2017) Lower Cutoff for LC-MS/MS Urine Drug Testing Indicates Better Patient Compliance. Pain Physician 20: E1107-E1113.
7. Caplan YH (2014) In disposition of toxic drugs and chemicals in man, 10th Ed Biomedical Publications, Seal Beach. J Anal Toxicol 39: 417.
8. Verstraete AG (2004) Detection times of drugs of abuse in blood, urine, and oral fluid. Ther Drug Monit 26: 200-205.
9. Buderer F, Nancy M (1996) Statistical Methodology. I. Incorporating the Prevalence of Disease into the Sample Size Calculation for Sensitivity and Specificity. Acad Emerg Med 3: 895-900.
10. Cohen J (1960) A coefficient of agreement for nominal scales. Educ Psychol Meas 20: 37-46
11. Bosker WM, Huestis MA (2009) Oral fluid testing for drugs of abuse. Clin Chem 55: 1910-1931.
12. https://statistics.laerd.com/statistical-guides/pearson-correlation-coefficient-statistical-guide.php.
13. Watkins MW, Pacheco M (2000) Interobserver agreement in behavioral research: Importance and calculation. J Behav Educ 10: 205-212.
14. Viera AJ, Garrett JM (2005) Understanding interobserver agreement: The Kappa Statistic. Family Med 37: 360-363.
15. Landis JR, Koch GG (1977) The measurement of observer agreement for categorical data. Biometrics 33: 159-174.
16. Allen KR (2011) Screening for drugs of abuse: Which matrix, oral fluid or urine. Ann Clin Biochem 48: 531-541.
17. Verstraete AG (2004) Detection times of drugs of abuse in blood, urine, and oral fluid. Ther Drug Monit 26: 200-205.

18. Vindenes V, Yttredal B, Oiestad EL, Waal H, Bernard JP, et al. (2011) Oral fluid is a viable alternative for monitoring drug abuse: Detection of drugs in oral fluid by liquid chromatography-Tandem mass spectrometry and comparison to the results from urine samples from patients treated with methadone or buprenorphine. J of Anal Toxicol 35: 32-39.

19. Sobolesky PM, Smith BE, Pesce AJ, Fitzgerald RL (2018) Interpretation of pain management testing results using case examples. J Appl Lab Med 2: 610-621.