



Oral fluid collection: The neglected variable in oral fluid testing

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Abstract

The potential to use oral fluid as a drug-testing specimen has been the subject of considerable scientific interest. The ease with which specimens can be collected and the potential for oral fluid (OF) drug concentrations to reflect blood–drug concentrations make it a potentially valuable specimen in clinical as well as forensic settings. However, the possible effects of the OF collection process on drug detection and quantification has often been overlooked. Several studies have documented that drug-contamination of the oral cavity may skew oral fluid/blood drug ratios and confound interpretation when drugs are smoked, insufflated or ingested orally. OF pH is predicted to have an effect on the concentration of drugs in OF. However, in a controlled clinical study, the effect of pH was less than that of collection technique. Mean codeine OF concentrations in specimens collected a non-stimulating control method were 3.6 times higher than those in OF collected after acidic stimulation. Mean codeine concentrations were 50% lower than control using mechanical stimulation and 77% of control using commercial collection devices.

Several factors should be considered if a commercial OF collection device is used. In vitro collection experiments demonstrated that the mean collection volume varied between devices from 0.82 to 1.86 mL. The percentage of the collected volume that could be recovered from the device varied from 18% to 83%. In vitro experiments demonstrated considerable variation in the recovery of amphetamines (16–59%), opiates (33–50%), cocaine and benzoylecgonine (61–97%), carboxy-THC (0–53%) and PCP (9–56%). Less variation in collection volume, volume recovered and drug recovery was observed intra-device. The THC stability was evaluated in a common commercial collection protocol. Samples in the collection buffer were relatively stable for 6 weeks when stored frozen. However, stability was marginal under refrigerated conditions and poor at room temperature. Very little has been published on the efficacy of using IgG concentration, or any other endogenous marker, as a measure of OF specimen validity. Preliminary rinsing experiments with moderate (50 mL and 2 × 50 mL) volumes of water did not reduce the OF IgG concentration below proposed specimen validity criteria. In summary, obvious and more subtle variables in the OF collection may have pronounced effects on OF–drug concentrations. This has rarely been acknowledged in the literature, but should be considered in OF drug testing, interpretation of OF–drug results and future research studies.

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1. Introduction

Several fluids combine to constitute what is commonly referred to as ‘saliva’. These fluids are excreted by the major salivary glands, minor salivary glands and gingival crevices.

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A mixture of the fluids from these sources is variously referred to as ‘whole saliva’, ‘mixed saliva’, ‘oral fluid’ or ‘oral fluids’ [1]. Strictly speaking, ‘saliva’ is collected from a specific salivary gland and is free from other materials while ‘oral fluid’ is a mixture of saliva from the glands and crevices and contains other materials that may be present in the mouth, such as shed mucosal cells and food residues [2]. Unfortunately, in the scientific literature, the important distinction between saliva and oral fluid has rarely been made.

Oral fluid (OF) performs a host of activities related to digestions and cleansing of the oral cavity by moistening the mucus membranes of the upper GI tract and supplying enzymes needed for digestion. It contains plasma electrolytes such as potassium, sodium, chloride and bicarbonate and many other plasma constituents such as enzymes, immunoglobulins and DNA [3]. The total volume of oral fluid produced by an adult may be in excess of 1000 mL/day with typical flows of 0.05 mL/min while sleeping, 0.5 mL/min while spitting and 1–3 mL/min or more while chewing [4].

Perhaps the most overlooked aspect of OF–drug testing and the interpretation of OF–drug test results has been the potential effects of specimen collection. Therefore, the following article provides examples of considerations from the literature as well as previously unpublished supporting data.

Several factors may affect drug transfer into saliva and OF and, consequently, drug detection and whether drug concentrations in these fluids correlate with drug concentrations in other body fluids such as blood or plasma. Factors affecting drug disposition into OF include pK_a , physical size, degree of protein binding and lipophilicity of the drug. Parent drugs, and not metabolites, are usually found in oral fluid because they are more lipid soluble and, therefore, pass more easily through the capillary and acinar cell membranes into the OF. Drug–protein binding and OF pH are also the factors. The binding of drugs to plasma proteins varies from drug-to-drug, but remains fairly consistent between individuals. However, only free drug diffuses into OF. pH affects the transfer of both acidic and basic drugs from the plasma into the saliva [5]. Mathematical models have been developed for predicting saliva/plasma (S/P) drug concentration ratios for both acidic and basic drugs to aid in interpretation [6,7]. When drugs are ingested orally or by smoking (heroin, methamphetamine, PCP, marijuana and cocaine) they may be detected in high concentrations in OF following recent use due to residual drug in the oral cavity. Under these circumstances, interpretation is affected because the drug concentration found in the OF may not reflect the blood–drug concentration [3,8]. In addition, OF composition and flow can be affected by many factors including oral diseases [9,3].

A variety of methods are available for collecting saliva and OF. Some collection methods yield non-stimulated (or unstimulated) saliva or OF. Non-stimulated saliva can be collected by the draining method that is performed by

allowing saliva to drip from the mouth into a collection container [8]. Various techniques have been developed to stimulate saliva production. The simplest involves tongue, cheek or lip movements without the use of an external stimulus [7,10]. Chewing paraffin wax, parafilm[®], teflon, rubber bands, gum base or chewing gum are referred to as ‘mechanical’ methods of stimulating production [11,12]. Lemon drop or citric acid can be placed in the mouth to provide a gustatory stimulation of saliva production [7,8,11]. Following stimulation, saliva can be spit, suctioned, absorbed or swabbed for collection [8]. Some collection techniques combine stimulation and collection of the saliva, or OF, using absorbent materials such as cotton balls or cotton rolls. After the absorbent material becomes saturated, the collection device is removed from the mouth and the saliva or OF is recovered by centrifugation or by applying pressure [13,14]. There are several potential problems associated with stimulating saliva production. Parafilm[®] has been shown to absorb some drugs and metabolites and, therefore, affect test results [15]. Also, paraffin contains compounds that may affect chromatographic analyses—again potentially affecting drug-testing accuracy [15]. Stimulation may change the salivary composition, thereby, potentially affecting saliva–drug or OF–drug concentrations [12,16]. Citric acid stimulation changes saliva pH and may alter saliva–drug concentrations (see discussion below). Citric acid and cotton have also been shown to alter immunoassay drug test results [7,11].

Several devices have been marketed to collect OF. Commercial devices included Oral Diffusion Sink[®] [17,18], Proflow Sialometer[™] [10], Orasure[®] [19], Salivette[®] [11] and Intercept[®], Finger Collector[®] and ORALscreen[®] [4]. These devices have been advocated for saliva collection when testing for ethanol, steroids, abused drugs and many therapeutic drugs. However, the efficacy and limitations of commercial collection devices has been largely under-addressed in the scientific literature.

2. The effect of oral contamination

Early researchers focused primarily on the detection of therapeutic drugs in saliva and the use of saliva as a diagnostic specimen for clinical purposes [7,20]. However, many drugs of interest in forensic settings have been reported in OF: ethanol; methamphetamine, amphetamine and other sympathomimetic amines; barbiturates; benzodiazepines; codeine, heroin and its metabolites; cocaine and its metabolites and cannabinoids and PCP [21]. One key question that clinical and forensic investigators asked was: “Do OF concentrations of abused drugs reflect blood concentrations?” Because oral fluid is a filtrate of the blood, oral fluid–drug concentrations should reflect blood–drug concentrations and mathematical models have been developed to predict S/P drug concentration ratios [6]. Thompson et al. reported that plasma and saliva cocaine concentrations

correlated significantly ($p < 0.001$) in a study in which three doses of cocaine were administered intravenously [22]. However, Thompson and Cone found that concentrations of THC in saliva were initially higher than those in plasma suggesting “oral contamination” [23]. Other authors have reported passive and active oral contamination with THC [24,25]. Research has also suggested that the presence of THC in saliva is solely due to oral contamination during drug ingestion. Cone demonstrated that saliva concentrations of heroin and its metabolites, 6-acetyl morphine and morphine, were ‘highly elevated’ over plasma concentrations for the first hour after intranasal heroin administration—again suggesting oral contamination [21,26]. Jenkins compared heroin concentrations in saliva and blood after administering the drug intravenously and by smoking [27]. Two subjects were administered heroin through smoking and also by IV. After smoking, peak OF heroin concentrations ranged from 3534 to 20,580 ng/mL, while peak heroin concentrations were 6–30 ng/mL following the IV dose. Saliva to blood (S/B) concentration ratios were >5 at all time points following smoking, but were always <2 following IV administration. The authors attributed the differences in S/B ratios to contamination of the saliva and oral cavity from smoking.

In a controlled clinical study designed to determine if S/P ratios of codeine were predictable, subjects were given a single 30 mg dose of liquid codeine phosphate [5]. Plasma and concurrent OF samples were collected for 24 h and the codeine S/P ratios computed. Following the oral administration, the plasma codeine concentrations peaked between 30 min and 2 h at concentrations ranging from 19 to 74 ng/mL (mean = 46 ng/mL). Contamination of the oral cavity with codeine from the administration was evident in specimens collected for at least the first 1–2 h after administration. Codeine concentrations in the 15 min OF specimens ranged from 690 to over 15,000 ng/mL. S/P ratios in specimens collected at 15 and 30 min ranged from 75 to 2580. At 1 h, the mean S/P ratios were still elevated, but after 2 h, contamination was not a factor in most subjects and the S/P remained constant with a mean ratio of 3.7 (Table 1). However, because of oral contamination, a poor correlation was

observed between OF and plasma codeine concentrations when the specimens collected in the first hour after drug administration were included in the evaluation ($r = 0.036$).

3. The effects of and pH and stimulation

Salivary/OF pH in healthy individuals is usually between 6.2 and 7.4. For basic drugs, as the pH decreases, a greater portion of drug will be ionized and conceptually the OF–drug concentration should increase. The theoretical S/P ratio for a particular drug can be estimated by the following mathematical models:

$$\text{acidic drugs} - \frac{S}{P} = \frac{1 + 10^{(pH_s - pK_a)} \times f_p}{1 + 10^{(pH_p - pK_a)} \times f_s}$$

$$\text{basic drugs} - \frac{S}{P} = \frac{1 + 10^{(pK_a - pH_s)} \times f_p}{1 + 10^{(pK_a - pH_p)} \times f_s}$$

where S is the concentration of drug in saliva, P the concentration of drug in plasma, pK_a the pK_a of drug, pH_s the pH of saliva, pH_p the pH of plasma, f_p the free (unbound) fraction of drug in plasma and f_s is the free (unbound) fraction of drug in saliva [6].

In these equations, plasma pH is assumed to be constant at 7.4 and protein binding of the drug is assumed to be negligible in the saliva. Therefore, a value of 1 is used for f_s [12]. From the equations, one would predict that small changes in saliva pH should result in profound changes in the S/P ratio.

In the codeine clinical study discussed above, non-stimulated OF was collected by having the subjects spit into inert polyethylene tubes and the pH of each OF specimen was recorded [5]. The observed S/P ratios at pH 6.0 were not as high as predicted by the equation (except within the first hour of drug administration when oral contamination was still present). For codeine (pK_a 8.2), the theoretical S/P at pH 6.0 is 20. However, at pH 7.0 it was only 2.1. The observed mean S/P was 4.7 at pH 6.0, 3.4 at pH at 7.0 and 1.8 at pH 8.0. In addition, there was a large inter- and intra-subject variability observed for the S/P ratios. However, the decrease

Table 1
Plasma and oral fluid concentrations of Codeine and S/P ratio

Time (h)	Plasma (ng/mL; mean)	Oral fluid (ng/mL; mean)	S/P ratio (mean)
0	0	0	0
0.25	12	4129	345
0.5	38	1172	31
1	37	480	13
2	34	154	4.5
4	18	60	3.3
8	5	19	3.8
10	4	11	2.8
12	2	7	3.5
Mean (2–12)			3.7

From O’Neal et al. [28] and NIST, 2004, S/P = oral fluid/plasma ratio.

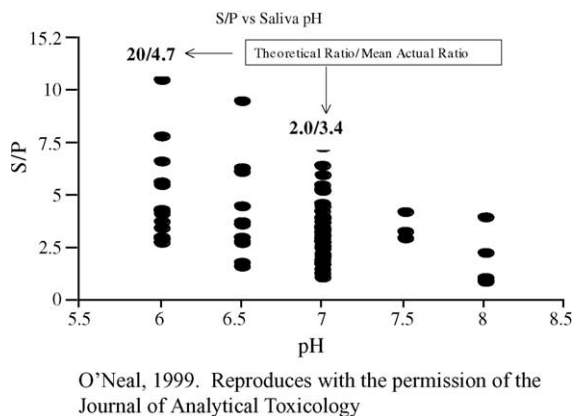


Fig. 1. Saliva/plasma ratio and pH.

was not as dramatic as predicted by the model (see Fig. 1). Kato et al. reported similar results for the distributions of cocaine and its metabolites in OF [27]. They concluded that the S/P ratios were highly dependent on pH, but also affected by the manner in which the OF was collected.

In the Kato study, the authors reported that concentrations of cocaine, benzoylecgonine and ecgonine methylester were substantially higher in non-stimulated than in stimulated OF. In their study, OF was stimulated by sour candy and the ratio of the cocaine concentrations in non-stimulated/stimulated OF was 5.2:1 (3.0–9.5). The ratios for benzoylecgonine and ecgonine methylester were at least 5.5:1. Clearly, stimulation of OF had a dramatic effect on the S/P ratio. Based on results from the first clinical study, the Kato study, the apparently ambiguous affect of pH and the variety of OF collection methods, a second clinical study was designed to assess the effect of collection technique on OF codeine concentrations [28]. In the study control group (non-stimulated), OF was collected by having the subjects spit into inert polyethylene tubes ($n = 22$). Stimulated OF was produced by having the subjects place either a lemon drop in their mouth ($n = 5$) or by chewing sugarless gum ($n = 5$) prior to OF collection. In addition, two different commercial collection devices were used to collect OF ($n = 5$ /device).

The time course of codeine elimination from OF for subjects using each of the collections techniques is shown in Fig. 2. With the exception of the 8-h time point, codeine concentrations in specimens collected by spitting were consistently higher than those detected in specimens collected by any of the other methods. The mean control concentrations averaged, 3.6 times higher than concentrations in the OF specimens collected by acidic stimulation; and 1.3–2.0 times higher than concentrations in specimens collected by the other methods [28]. The mean area under the concentration curve (AUC) was also significantly greater in the control subjects than in the stimulated acidic and non-acidic collections.

From these data, it follows that there were differences in the length of time that codeine could be detected. The

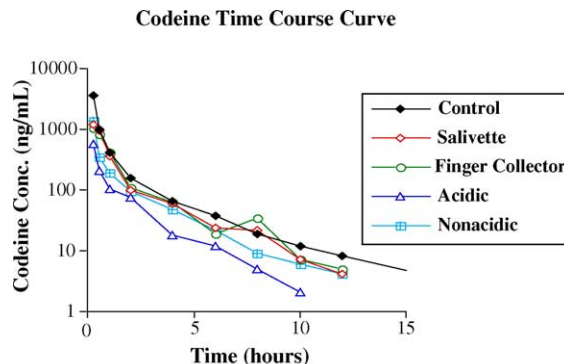


Fig. 2. Codeine time course using various collection techniques.

duration of detection time was related to collection method. In the control group, codeine was detected in all of the 12-h specimens and over 2/3 of the 24-h specimens (LOD = 1 ng/mL). With the Salivette™ and Finger Collector devices, all specimens collected at 12 h contained codeine. However, at 24 h after administration, 40% and 20% of the Salivette™ and Finger Collector samples, respectively, contained detectable codeine. Non-acidic OF stimulation yielded only 60% positive specimens at 12 h and 40% at 24 h. Using the acidic OF stimulation method, only 20% of the 12 h and none of the 24 h specimens contained detectable codeine. The authors cautioned that although they attempted to collect non-stimulated OF, the act of spitting is usually a sufficient stimulus to produce some stimulation. A mechanical stimulus, such as chewing sugarless gum, may stimulate flows in the range of 1–3 mL/min, citric acid stimulation may produce flows of 5–10 mL/min and collections using absorbent materials also produce some degree of stimulation. They further concluded that acidic stimulation had the greatest effect on the codeine concentrations. It is known that stimulation affects OF composition and resulting bicarbonate concentration [16,28]. This in turn effects OF pH and may reduce the concentration of basic drugs in the OF. The authors concluded that in their study, stimulation was a more important factor than pH, thus helping to explain why S/P ratios may not always follow predictive models.

4. Effects of collection devices

In vitro drug-recovery studies have suggested differences in drug absorption, or recovery, from commercial OF collection devices [28]. The percent recovery of codeine and morphine was 46.7% and 39.1% less than control, respectively, from one device that was evaluated [28]. It was also reported that the amount of OF recovered from the devices for use by the laboratory varied between 50% and 90% [28]. Therefore, a study was designed to evaluate the in vitro collection volume; volume recovered and drug recovery

Table 2
Oral fluid volumes collected from various devices

Parameter	Salivette	Intercept	Finger collector	ORALscreen	Hooded collector
Volume collected (mL)	1.86	0.82	1.62	1.76	1.69
Volume recovered (mL)	1.48	0.64	1.24	0.58	0.30
% Recovered	83	78	77	33	18

Results presented as mean ($n = 10$). Volume estimated from weight (NIST, 2004).

from a series of commercially available OF collection devices [4]. The devices evaluated were the Salivette™ (Salivette) Intercept® (Intercept), Finger Collector (Finger Collector), ORALscreen® (ORALscreen) and Hooded Collector® (Hooded Collector) [4].

To determine the volume collected by each device, culture tubes were placed on a laboratory balance and 2 g of OF was added to each tube. An OF collection device was placed in the culture tube and allowed to remain for the collection time recommended by the manufacturer. If no collection time was specified, devices were left in the tubes for 5 min. The devices ($n = 10$) were removed and the weight of each culture tube and residual OF was recorded. The difference between the initial and final weights of the culture tubes was used to calculate the amount of OF absorbed by each device.

In a related set of experiments, the volume of OF recovered from each device was assessed ($n = 10$). It was assumed that 1 g of OF = 1 mL of OF. Table 2 shows that on an average, the Salivette absorbed 1.86 g (mL) of OF and 1.48 g (mL) 83% was recovered. The Intercept was designed to collect approximately 1 mL of OF and the studies demonstrated that an average of 0.82 mL was collected and 0.64 mL recovered. On an average, the Finger Collector absorbed 1.62 mL of oral fluid, of which 77% was recovered. The ORALscreen absorbed an average of 1.76 mL of OF and approximately one-third of that volume was recovered. The Hooded Collector absorbed an average of 1.68 mL of OF and only 18.2% of that volume was recovered from the device.

A third set of in vitro experiments was designed to determine the recovery of drugs of abuse and their metabolites from the collection devices. In these experiments, pools of drug-free OF were fortified at the drug/metabolite at concentrations shown in Table 3. The fortified pools were then aliquoted into 2 mL volumes and ‘collected’ by the devices ($n = 5$). (Recall that device collection volumes and volume recovered varied by device.) Additionally, 2 mL portions of the pool were aliquoted as controls that were not collected by the device, but were extracted and analyzed as if they were ‘neat specimens’. Quantitation of the drugs/metabolites was performed by LC/MS using a multi-point calibration curve for each analyte. Recovery was calculated by dividing the mean concentration from each device by the mean concentration of the control and reported as percent recovery. The data in Table 3 assumes that each device collected 2 mL of OF. Therefore, the study design simulates an authentic collection because, in actual donor collections, the laboratory would not know the volume of OF collected by the device. Given the similarity in chemical structure of

amphetamine and methamphetamine, one would predict similar recoveries of the two drugs from the devices as demonstrated in the table. Recoveries exceeded 50% from the Salivette and the Finger Collector for both drugs at the tested concentrations. Recoveries at the same concentrations from Intercept and the ORALscreen were 16–36%. Recoveries from the Hood Collector for were all approximately 25% and only the recovery of amphetamine at 100 ng/mL exceeded 30%. Given the similarity in chemical structure between codeine and morphine, one would predict similar recoveries of these drugs as well. Recoveries approached 50% from the Finger Collector for both drugs and were in the range of 37–46% from the Salivette and Hooded Collector. Recoveries from the Intercept and ORALscreen for morphine and codeine were only slightly less at 33–39%. The recovery of morphine and codeine (33–50%) and cocaine and benzoylecgonine (BZE) (69–97%) from the devices tended to be higher and more consistent than the other drugs shown in the table. The table also shows that the recovery of PCP approached, or exceeded, 50% from only one device. Recoveries from the Intercept and Finger Collector were similar and PCP recoveries from the ORALscreen and the Hooded Collector were poor (<25%). Similarly, recoveries of carboxy-THC approached 50 only with the Salivette and the Intercept. All other devices demonstrated almost no recovery. These data suggested that lipophylicity might play role in recovering drugs from the devices [4].

Limited or unknown collection volume, unknown OF-recovery volume and unknown drug recovery from the collection device or method may create a number of challenges for the laboratory. More sophisticated screening and confirmation technologies may be needed to achieve the desired sensitivity. The limited volume may preclude screening or confirmation of multiple drugs. The limited volume might also preclude retesting by the laboratory, agency or donor. The variable collection and recovery volumes between devices may cause quantitative differences if the laboratory assumes a consistent collection volume, volume recovered from the device and drug recovered from the device. Importantly, it may confound interpretation and estimation of blood–drug concentration given an OF–drug concentration.

5. Additional considerations: drug stability in collected of and specimen validity

Many of the devices discussed were designed for “off-site” collection of the OF and subsequent testing in the

Table 3
Drug recoveries from various collection devices

Drug/concentration (ng/mL)	Salivette (%)	Intercept (%)	Finger Collector (%)	ORALscreen (%)	Hooded Collector
Amphetamine					
10	54	31	53	36	24
25	57	35	57	31	25
100	56	34	54	16	34
Methamphetamine					
10	55	33	55	36	25
25	58	36	59	32	26
100	59	36	58	33	28
Codeine					
25	46	36	47	34	46
50	40	35	49	37	37
200	39	33	47	34	40
Morphine					
25	46	36	49	36	46
50	39	34	50	39	37
200	38	33	48	36	42
Cocaine					
5	92	96	65	72	69
10	92	97	62	68	69
100	91	95	61	68	62
Benzoylcegonine					
5	92	89	93	95	93
10	98	91	94	97	97
100	93	85	91	97	87
Phencyclidine					
5	54	26	23	14	9
10	49	34	29	17	12
50	56	37	31	23	17
Carboxy-THC					
2.5	12	47	0	0	0
5	33	52	0	2	2
25	47	53	<1	2	2

Results presented as mean% ($n = 5$) of control and rounded to whole number (NIST, 2004).

laboratory. Therefore, following collection, the specimen may be subjected to various handling, transportation and storage conditions and the stability of the drug(s) under these conditions will be a factor in drug detection and quantitation. For this reason, we evaluated the stability of delta-9-tetrahydrocannabinol (THC) in a commonly used OF collection device procedure. The Intercept[®] device was evaluated in these experiments. It is designed to collect OF on an absorbent pad and the pad is then immersed in a buffer. The buffered-OF is transported to the laboratory for analysis. A pool of OF was fortified with THC, portions of the pool were collected ($n = 3$ /storage condition) and the buffered OF stored at -20 , 4 and 21 °C in the collection vial. The remainder of the fortified-pool was aliquoted into 1 mL control portions and stored frozen. At each time point, the entire volume of the collected and stored samples was extracted along with the controls ($n = 3$). The samples were analyzed by negative ion chemical ionization GC/MS at 2, 3

and 6 weeks after collection. The accompanying (Table 4) shows the experimental results. In samples stored at -20 °C, the THC concentrations decreased to 72% from 87% of the control value. A decrease of 20% is likely within the expected variability of the experiment given that the devices do not collect 1 mL of sample (discussed above) and normal analytical variability. At 4 °C, the concentration decreased to

Table 4
Stability of THC when collected with the Intercept

Duration (weeks)	Temperature (°C)		
	-20	$+4$	$+21$
2	87.0%	54.7%	61.2%
3	72.0%	61.7%	15.0%
6	79.4%	13.2%	13.6%

Data as % of control.

Table 5
Oral fluid IgG concentrations in normal volunteers and following rinsing of the mouth

	IgG in randomly selected specimens	IgG following rinsing	IgG following second rinsing
	<i>n</i> = 100	(50 mL) (<i>n</i> = 6)	(Additional 50 mL) (<i>n</i> = 6)
Mean (µg/mL)	2.98	2.33	1.99
S.D. (µg/mL)	0.58	0.77	0.57
Decrease from initial concentration (%)	na	12.70	25.50

Initial concentration = (2.67 µg/mL).

54.7% of control in 2 weeks and remained at approximately that level at 3 weeks, but deteriorated to 13% by 6 weeks. At room temperature (21 °C), the concentration was 61.2% at 2 weeks and decreased to 13.6% of control by 6 weeks.

The forensic literature has demonstrated the need to collect representative samples to ensure accurate analysis and interpretation [29,30]. Similarly, for OF to be accepted as a viable specimen for drug testing, we must be able to ensure that a 'valid' and 'representative' specimen has been collected. This implies that there are objective methods to ensure that the specimen integrity such as the chemical markers often used for urine specimens [31]. It has been variously suggested that an OF specimen was valid if it contained ≥ 0.1 , 0.5 or 1.0 µg/mL of IgG [29]. Table 5 shows the mean IgG concentrations from *n* = 100 OF specimens that were randomly selected from those collected by spitting in the clinical studies [5,28]. The mean concentration was approximately 3 µg/mL and the standard deviation 0.58 µg/mL. Two standard deviations below the mean = 1.82 µg/mL—far in excess of the 0.5 µg/mL standard often employed. Only subjects with IgG concentrations 4.28 or 3.48, respectively, standard deviations below the mean would have failed the proposed 0.5, or 1.0 µg/mL validity criteria. Although this ensures a safe margin for the donor, are 0.5 or 1.0 µg/mL effective tests of specimen validity? Table 5 also shows the potential effect of *in vivo* dilution on OF-IgG concentrations. Six subjects were asked to provide an OF specimen by spitting. Each subject then rinsed their mouth with 50 mL of tap water and provided a second specimen. The subjects then performed an additional 50 mL rinse and provided a third specimen. All specimens were then tested for IgG. The IgG concentrations decreased as a function of rinsing. However, even with 2×50 mL rinses and dilution of the OF, the mean IgG concentration was still 2.61 standard deviations above the 0.5 µg/mL specimen validity criterion and approximately $2 \times$ the 1.0 µg/mL criterion. Thus, questioning the utility of the IgG concentration as an indicator of OF specimen validity.

6. Conclusions

Compared to collecting a blood or urine specimen, collecting an OF specimen is relatively easy and much less invasive. However, collecting a valid and representative OF

specimen takes insight into the collection process and its related effects. Oral contamination has been demonstrated with cocaine, THC, heroin and codeine. However, it is clear that contamination from other drugs taken orally, insufflated or smoked will potentially cause falsely elevated drug concentrations in the OF. These artificially elevated concentrations may make drugs more readily detected, but they adversely affect S/P and S/B ratios, which precludes reliable interpretation of the test results. In our studies, oral contamination appeared to be a much less influential factor by 2 h post-administration.

Data were presented using several common OF collection methods such as acidic and non-acidic stimulation, device collection and non-stimulated collection by spitting. However, even spitting or use of commercial collection devices produces some degree of stimulation. Acidic stimulation (which stimulates OF production at a faster rate than the above methods). Studies showed that stimulation reduced saliva codeine concentrations and that the effect pH was not as pronounced as predicted by mathematical models.

There has been a steady stream of new OF collection devices introduced onto the commercial market. Many of the devices are subsequently withdrawn from the market, or modified. Therefore, the devices discussed here may not be representative of the current products available. However, the data presented are exemplary of the variables that should be considered when collecting OF using commercial products. Stimulation, pH, the volume of OF collected and recovered as well and drug recovery and stability should be determined from the device. Drug recovery is often difficult to estimate *in vivo* because the volume of collection and volume of OF recovered from the device may not be known. In addition, some collection protocols require that the collection device be placed in a dilution buffer for subsequent handling again masking the actual volume of OF collected.

Ensuring that a representative specimen has been collected is fundamental in forensic investigations and clinical settings. Clearly, more study is needed to ensure that IgG concentration is an acceptable marker for specimen validity. Perhaps alternate chemical markers should be identified that are easily tested, representative and independent of the clinical condition of the donor.

In many studies, the authors have neglected to address the effects of the collection procedure on their results. All future studies investigating OF as a matrix for drug testing should be preceded by a thorough investigation of the potential effects of the proposed collection procedures on the results and conclusions. They should include rationale for the collection procedure chosen and a discussion of its effects on OF–drug concentrations, results and conclusions. For each drug of investigated, the kinetics of the drug in stimulated and non-stimulated in OF needs to be described. For day-to-day applications, collection procedures should be characterized and optimized to reduce the potential for erroneous results from inadvertent effects. Data interpretation needs to address the potential for oral contamination, stimulation, volume collected and recovered from the collector, drug recovery and drug stability.

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