

## **A Brief Introduction to Oral Fluid Drug Testing**

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### **Introduction**

The first studies reporting the detection of drugs of abuse in oral fluid (saliva) were published more than 20 years ago. Oral fluid has been widely used as a specimen in pharmacokinetic studies, therapeutic drug monitoring and for the detection of illicit drugs. Laboratories specializing in the risk assessment business have used oral fluid to test for HIV, cotinine and cocaine metabolite for more than five years. LabOne has performed tens of thousands of tests on such specimens. This brief will review methods of collection for oral fluid, the benefits of collection using the Epitope device compared to urine collection, and the detection of a number of drugs of abuse, particularly marijuana (or hashish). This drug presented the biggest challenge and we have developed data showing some of the advantages of oral fluid testing. We also examined other drugs, including heroin and cocaine, and showed that oral fluid was at least comparable to urine for detecting these drugs. In fact it was more efficient at detecting the 6-monoacetylmorphine (MAM) metabolite of heroin than urine in a population of known users.

### **Collection of Oral Fluid**

A number of procedures have been described for collecting non-stimulated mixed saliva. There are a number of limitations to all of these, including that often more froth is collected than fluid. For this reason a number of workers have suggested that stimulating salivary flow collects a more representative specimen. Mechanical stimulation can be achieved by having the individual chew on a piece of Teflon. Alternatively chemical stimulation will result from the sucking of citric acid crystals. In all these situations the donor is still required to "spit" into the collection tube in order to collect a specimen suitable for testing.

A number of devices have been introduced to facilitate the collection of oral fluid. These include the Intercept® system developed by Epitope. The collection pad is placed between the cheek and gum. The specimen collected is a mixture of gingival crevicular fluid and saliva, and in this article it is referred to as oral fluid. In fact, the specimen collected is more akin to an ultrafiltrate of plasma than common saliva, and is often referred to as mucosal transudate.

Collection of oral fluid is an extremely straightforward process and a specimen can be collected in five minutes. LabOne recommends that the donor collects the specimen and that the "collector" merely witnesses the collection. The three simple steps in the process are:

- a) inserting the pad between the cheek and gum for between two and five minutes
- b) transferring the pad to a specimen vial for shipment to LabOne
- c) sealing the vial with a bar-coded seal, completing the chain of custody and packaging the specimen for shipment to LabOne.

### **Ability to Overcome Adulteration, Substitution and Dilution**

Over the past few years increasing attention has been paid to adulterated, substituted and dilute specimens. This attention resulted in HHS issuing two guidance documents - PD 35 and 37 - to define an adulterated specimen, a substituted specimen, and to reaffirm what is a dilute specimen. LabOne has for many years been one of the leaders in detecting attempts to "beat the system", but with the advent of a new generation of chemicals designed specifically to destroy the carboxylic acid metabolite of delta-9-tetrahydrocannabinol (THCA) it is becoming increasingly difficult to do so. Oral fluid presents a new method of achieving this goal - the collection is a witnessed one and there is very little, if any, concern regarding its invasiveness.

As a measure of the extent of the problem with adulterated, substituted and dilute specimens, the table shows LabOne's experiences for the last few months:

Period	Nitrite %	Other Adulterant %	Substituted %	Dilute %
October, 1999	0.11	0.02	0.06	4.06
November, 1999	0.10	0.02	0.05	4.38
December, 1999	0.09	0.02	0.04	3.66
January, 2000	0.10	0.01	0.04	4.31
February 2000	0.10	0.02	0.05	4.31

The number of adulterated and substituted specimens is almost 0.2% of the total non-DOT testing population - we have no reason to believe that we are detecting all the adulterants. For example, Stealth appears to have the ability to destroy THCA in a few hours and then destroy itself. There will undoubtedly be more of these.

We are certainly looking at broadening our abilities by incorporating a "general oxidant" screen into LabOne's processes. This may only be the panacea for a few months, after all the positive rate for nitrite positives at LabOne has dropped by 50% over the last six to nine months. Urine testing laboratories are constantly playing catch up in this field - a different approach needs to be taken.

There is also a widespread belief that drug users are "beating the system" by diluting themselves. There is some justification for that. In an earlier study approximately 10,000 urine specimens were examined, of these approximately 7% were considered dilute based on a creatinine reading of less than 20 mg/dl. When these 700 or so specimens were examined by immunoassay and GCMS (if necessary) to the limits of detection (LOD) the positive rate increased 6 to 7 fold. It should also be noted that there was a similar, although not as dramatic, increase in the number of positives in a control population using these LODs. Here the positive rate increased 4 to 5 fold. Obviously a specimen that is not affected by dilution has some advantages - again oral fluid fits the bill.

We do perform an integrity check on oral fluid specimens by measuring the amount of human IgG in the specimen. We have reviewed a large number of specimens tested for risk assessment purposes, and of the 18,000 only 3 had an IgG reading less than 500 mg/dl (the cut-off used to determine integrity) - and these three were found to be either canine or feline IgG. These collections were certainly not witnessed.

In summary alternative specimens to urine have major advantages in overcoming the ability of drug users to "beat the system". Because of the ease of collection oral fluid is a perfect specimen.

#### **Pharmacokinetics of Smoked (or Snorted) Drugs: General Comments**

A number of the common drugs of abuse are either smoked or snorted. These include marijuana, cocaine, and on occasion heroin and the amphetamines. When these routes of administration are used there are three (or more) distinct phases in the pharmacokinetic profile: absorption, distribution (and redistribution), and metabolism and excretion.

Cocaine and marijuana are the most frequently detected drugs of abuse in workplace drug testing programs. LabOne's confirmed positive rates for these were 1.38 and 3.64% respectively in the non-regulated sector during 1999. By comparison the positive rates for opiates and amphetamines were 0.53 and 0.73% respectively. Because cocaine and marijuana are the most prevalent LabOne has focused on detection of these in oral fluid, and on a comparison of their detection rates in this specimen with that in urine. But first some general comments regarding their pharmacokinetic profiles:

**Absorption:** for both cocaine and marijuana after smoking (in addition for cocaine after snorting) this phase is rapid. Peak delta-9-tetrahydrocannabinol (THC) concentrations have been reported to occur within 5 minutes of the start of smoking or during smoking. Similar absorption would be expected for cocaine.

**Distribution:** marijuana (more correctly THC) has a complex distribution phase. Initially it is bound to red blood cells and then redistributes to fatty tissue, where it is likely bound to fatty acids over time. There is evidence that very small amounts of THC can remain in lipid depots for significant periods. Cocaine is a far simpler drug and after distribution into the central nervous system it essentially disappears from plasma as a result of metabolism.

**Metabolism and Excretion:** the primary urinary metabolite of THC is THCA and its conjugates. Detection periods for this metabolite are variable and depend upon the dose and the type of user. There are distinct differences between "light" and "heavy" users and these are discussed below. Some early studies indicated that urine specimens were positive for several weeks in "heavy" users after they stopped smoking. These positives were detected by enzyme immunoassay and no confirmations were performed. It is difficult to compare these data with the techniques used today, the immunoassays are much more specific for THCA and all results are confirmed by GCMS.

Cocaine is metabolized by plasma and hepatic esterases to benzoylecgonine and ecognine methyl ester. The former is the metabolite that is detected in urine and again, detection periods vary depending upon dose and pattern of use.

#### **Analysis of Oral Fluid**

LabOne has developed defensible testing methods for oral fluid. The approach used is one that is identical to that used for testing urine specimens - an initial immunoassay screen using an FDA approved procedure and if the specimen is presumptive positive confirmation by a gas chromatography mass spectrometric procedure. In the case of oral fluid we use ELISA for the initial test and GCMSMS for the confirmation procedure. These are both techniques that have a long history of applications in clinical medicine and pharmaceutical research. It should be noted that LabOne has used GCMSMS for testing urine specimens for 6-monoacetylmorphine (MAM) for some time and that the National Laboratory Certification Program (the SAMHSA Certification process) has sanctioned this application. It should also be noted that LabOne uses identical chain of custody, quality control and data review procedures for testing oral fluid specimens as it does for urine.

LabOne and STC (the manufacturer of the immunoassay kits) have spent considerable time in determining the optimal cut-offs for the analysis of oral fluid specimens. As was the case in the original selection of cut-offs for the urine drug testing programs these choices were a compromise between the abilities of available technology and knowledge of the pharmacokinetics of the drugs of abuse in oral fluid. On an historical basis the urine cutoffs were also established based on the experiences of the US Military, there have been no large-scale drug testing programs that have used oral fluid. Certainly the risk assessment companies have used oral fluid for cocaine screening for a number of years and we have included their data in this brief (see below). Tabulated below are the cutoffs currently being used for oral fluid testing compared to those used in the majority of urine drug testing programs:

#### **Initial Testing Cutoffs:**

<b>DRUG CLASS</b>	<b>URINE (ng/ml)</b>	<b>ORAL FLUID (ng/ml)</b>
<b>Amphetamines</b>	1000	40
<b>Cocaine</b>	300	5
<b>Marijuana</b>	50	1

<b>Opiates</b>	2000	10
<b>PCP</b>	25	1

Per ml. of preservative fluid

**Confirmation Cutoffs:**

<b>DRUG CLASS</b>	<b>URINE (ng/ml)</b>	<b>ORAL FLUID (ng/ml)</b>
<b>Amphetamines</b>	500	40
<b>Methamphetamine</b>	500	40
<b>Benzoyllecgonine</b>	150	2
<b>Marijuana</b>	15*	0.5**
<b>Codeine</b>	2000	10
<b>Morphine</b>	2000	10
<b>PCP</b>	25	0.5

Per ml. of preservative fluid

\* as THCA

\*\* as THC

There are several comments to be made regarding these tables:

a) these cutoffs are per ml of preservative fluid. There is an approximate four fold dilution of oral fluid after it is collected based on the volume of preservative in the collection vial and adsorption onto the collection pad. Cutoffs in saliva would be approximately four fold greater.

b) there are, as yet, no cutoffs approved by the Department of Health and Human Services (SAMHSA) for oral fluid. The Drug Testing Advisory Board is examining the use of alternative specimens, including oral fluid, and the expectation is that they will issue a notice of proposed rule making in late 2000.

c) the amphetamines screening immunoassay is directed to d-methamphetamine.

d) both the marijuana immunoassay and confirmation procedures are designed to detect THC (for a further discussion see below)

e) d- and l-methamphetamine separation will be performed on all positive methamphetamine specimens.

f) MAM analysis will be performed on all positive morphine specimens.

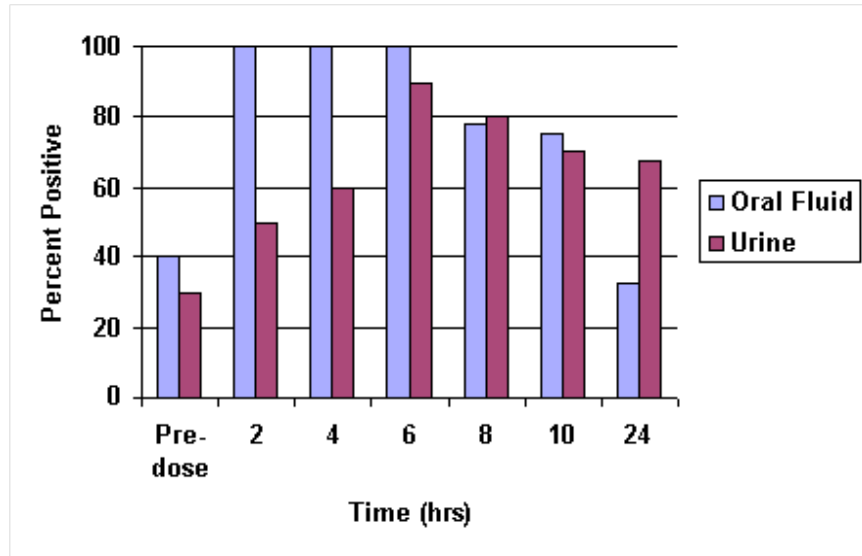
**Marijuana in Oral Fluid**

The primary plasma metabolite of THC is THCA and we hoped to be able to detect this in mucosal transudate. Unfortunately we were unable to do so, even though the sensitivity of the GCMSMS assay was 0.1 ng/ml. Although we have been unable to determine the reason for this failure, we believe that it is due to THCA being strongly bound to plasma proteins. If this were not the case it should diffuse from the plasma into the mucosal cavity.

On the other hand, we have been able to detect parent THC in oral fluid specimens. Other workers in the early 1980s made the same finding. It is likely that the THC detected is residual from smoking. It is likely adsorbed into the mucosal tissue, although we cannot rule out some

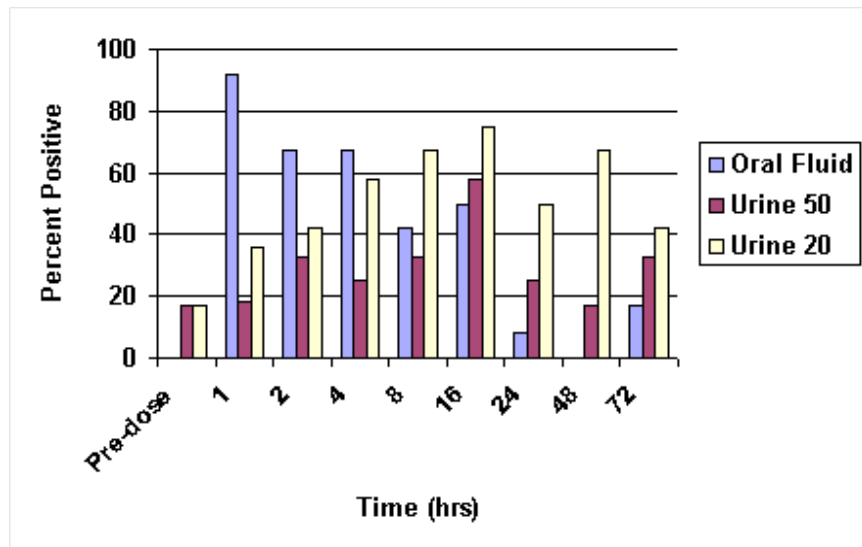
diffusion back from the plasma. Interestingly enough the decay curve after a single dose of THC (in marijuana or hashish) is similar to the plasma decay curve.

LabOne and STC have completed a number of studies in which experienced hashish users were asked to smoke one cigarette. These studies were performed in Amsterdam. In the first study (10 participants) oral fluid and urine specimens were examined by GCMS using cutoffs of 1 ng/ml for THC and 15 ng/ml for THCA respectively.



These data suggest that in the immediate time period following smoking oral fluid is more efficient at detecting THC use than urine, and that over the initial 24 period the specimens become more equal in doing so. It is interesting to note that at no time over the 24 hour period after smoking were all the urine specimens positive.

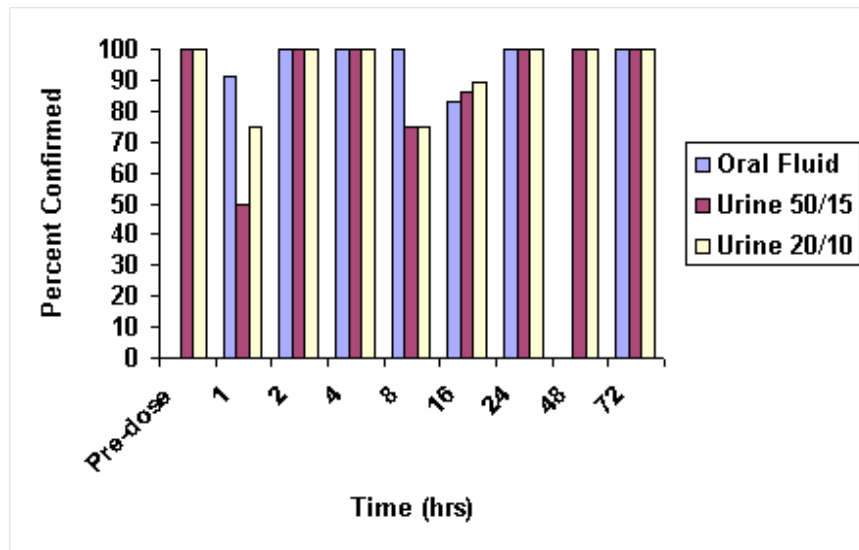
However, these data are not "real life" in that they do not account for the initial immunoassay testing. A further study was therefore carried out using 9 subjects and the immunoassay cutoffs indicated earlier in this brief.



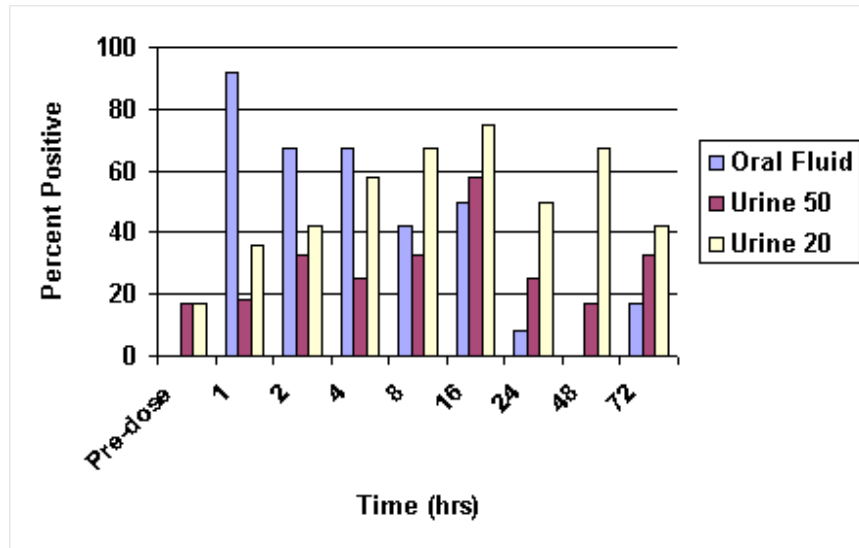
There are several comments to be made regarding these data:

- a) the immunoassay in urine data for a 20 ng/ml cutoff are interpolated from the 50 ng/ml calibrator
- b) there was evidence of reuse between 48 and 72 hours in two of the 12 subjects. This was clearly evident in the oral fluid data
- c) as with the GCMS data presented above there is strong evidence that oral fluid is more efficient in detecting recent use than urine.

Plotted below are the confirmation rates for both specimens - for the urine 50 ng/ml immunoassay cutoff a GCMS cutoff of 15 ng/ml was used and for the 20 ng/ml a 10 ng/ml. Oral fluid specimens were confirmed using a 0.5 ng/ml cutoff. The majority of specimens that screened positive at the cutoffs indicated.



Plotted below are the percentage of positives in each population based on the immunoassay and confirmation results:



There are several comments to be made regarding these final data:

- a) there is strong evidence that oral fluid is more efficient in detecting recent use than urine at either of the urine cutoffs, but especially at the combination of 50 and 15 ng/ml.
- b) after 48 to 72 hours oral fluid is less effective at detecting positives than urine, particularly at the 20 and 10 ng/ml cutoffs
- c) on the other hand it appears that oral fluid may be useful for detecting reuse. Two of the twelve subjects had positive oral fluid specimens after 72 hours after testing negative at 48 hours. This reuse was not clear when looking at the urine data.
- d) there is a general belief that urine specimens can test positive for up to 30 days after smoking marijuana (or hashish) and our data clearly show that not to be the case. In fact in this study there were never 100% of the urine specimens positive.

Please note that although attempts were made to include daily and long-term users of marijuana or hashish in the study, such individuals were unwilling to cease use.

What do these data suggest for the application of oral fluid testing to the workplace? Firstly, when the widely used cutoffs of 50 and 15 ng/ml were used to test the urine specimens from this population of known THC users, there were a total of 26 positive specimens (27%) over the 72 hours for the twelve subjects. The corresponding numbers for oral fluid were 39 (41%). Not surprisingly oral fluid was more reliable in catching recent. As a comparison when the less widely used urine cutoffs of 20 and 10 ng/ml were used the number of positives was 51 (54%). Please remember that if an individual was a repeated user of marijuana one would expect certainly oral fluid to be positive, particularly if he/she was a daily user.

The real questions to ask are:

- a) is an employer interested in detecting those employees or applicants who have used marijuana recently or who use it daily, or

b) is an employer interested in detecting those who may have used on one occasion 24 to 48 hours prior to the test, even though there is no evidence of impairment for this period of time after use, and

c) is an employer interested in insuring that employees or applicants do not "beat the test"

A number of employers are certainly more interested in answering questions a) and c) as yes, and if so oral fluid is an ideal specimen.

LabOne in cooperation with a number of groups has performed some pilot studies comparing positive rates in urine and oral fluid. As would be expected from the data presented above these produced some interesting results. Of 912 specimens tested 54 (5.8%) oral fluid and/or urine specimens were positive. Of these 29 (3.2% of the total) were positive in urine only and 17 (1.8% of the total) were positive in oral fluid only. The remaining 8 (0.9%) were positive in urine and oral fluid. These data clearly show that collecting both specimens gives a truer reflection of drug use, but obviously that is impractical. What they do show is the differences in excretion curves mentioned above.

#### **Cocaine metabolite in oral fluid**

The issues here are a lot simpler than they are for THC. One of the two major metabolites of cocaine is benzoylecgonine and this is detectable in oral fluid specimens. In fact LabOne has performed thousands of such analyses over the past five years for the risk assessment companies. We compared the positive rate in this testing environment with a DOT and non-DOT one. As best as we could we matched the demographics of all three populations. The positive rates were 0.35, 0.24 and 0.18% respectively.

When the pilot study data were examined the total number of specimens testing positive in urine and/or oral fluid was 33 (3.6%). Of these 8 (0.88% of the total) were positive in urine only and 7 (0.77% of the total) were positive in oral fluid only. The remaining 18 (1.97% of the total) were positive in urine and oral fluid.

#### **Heroin in metabolite fluid**

In the pilot study there were few opiate positive specimens, and those that were positive in urine were in also positive in oral fluid. However, LabOne has tested a number of matched oral fluid and urine specimens from a group of known heroin users and the results were as follows:

a) of 67 matched specimens tested, 52 matched oral fluid and urine specimens screened positive. Of the 52 oral fluid specimens, 36 confirmed positive, and of the 52 urine specimens, 44 confirmed positive. Seven oral specimens screened positive and the corresponding urines were negative - five of the seven confirmed. Seven urine specimens screened positive and the corresponding oral fluid specimens screened negative - six of the seven confirmed.

b) in summary a total of 41 oral fluid specimens confirmed positive for morphine and 50 urine specimens confirmed positive.

c) All the positive specimens were tested for MAM. Thirty-five of the 41 oral fluids were positive for MAM and 22 of the 50 urine specimens.

d) If MAM is taken as the marker of heroin use, then oral fluid detected 67% of the known heroin users and urine only 42%

#### **Other drugs in oral fluid**

Pilot study specimens were also tested for amphetamines and PCP. There were few positives



and there was agreement between the oral fluid and urine specimens. In addition all the oral fluid specimens were tested for human IgG and no specimen was declared invalid based on the IgG result.

FDA approved immunoassays kits are not currently available for other drugs, including methadone, barbiturates and benzodiazepines. These are expected to be available during 2000. Literature reviews have shown that the detection of methadone and barbiturates should be straightforward, and that the detection of the benzodiazepines is feasible.

### **Summary**

LabOne has developed a testing protocol for assaying Intercept® Oral Fluid specimens for the "NIDA five" and has shown that this procedure is suitable for detecting recent and long-term marijuana (or hashish) use. It is comparable to urine specimens for the remaining four drugs (amphetamines, cocaine metabolite, opiates and PCP). None of the oral fluid specimens in the pilot study were declared invalid based on substitution, adulteration and/or substitution.

Oral fluid collection presents a new method of collecting specimens for workplace drug testing. It overcomes the issues of adulteration, substituted and dilute specimens and allows the employer to detect the common drugs of abuse. Not only is it efficient in this regard it is also a non-invasive procedure for performing collections in the workplace.

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