Over the last decade there have been major developments in the use of oral fluid for drug testing, especially for rapid tests, but our involvement with oral fluid point-of-care started back in 1993 with AIDS testing, because oral fluid is rich in antibodies for all manner of medical conditions such as cancer, hepatitis and HIV.

Current applications in the drug of abuse arena focus on testing in the workplace, where drug use has safety implications; for example haulage drivers, fork-lift truck operators and plant workers who are under the influence are a major safety risk. Testing of oral fluid is valid in other situations too such as prisons, or whenever you are looking to detect drug impairment.

Oral fluid concentrations of basic drugs such as amphetamines, cocaine and some opioids are similar or higher than those in plasma, so are easily detected in oral fluid—but acidic drugs are problematic. Currently, the antibodies used in most commercially available rapid tests are optimised for drug metabolites, not the raw drugs usually found in oral fluid. For instance, they can only detect the metabolite of Tetrahydrocannabinol (Δ⁹ THC-COOH) from cannabis use, but the metabolite cannot be found in oral fluid. Some test manufacturers have used inventive ways to solve the seemingly impossible task of detecting parent cannabis in oral fluid.

When illicit drugs are taken it is usually only the parent drug that is psychoactive, but as we have seen previously, these drugs become bound to proteins and are metabolised into non-active conjugates. Since oral fluid usually contains high proportions of the active drug, oral fluid is an ideal medium in which to study the level of intoxication. Only blood itself offers similar opportunities. This is not surprising since oral fluid is a partition of blood plasma and therefore mimics it.

**IS IT ORAL FLUID, OR SALIVA?**

Oral fluid and saliva are not the same thing. Saliva comes from numerous glands lining the mouth, while oral fluid mainly consists of saliva but contains other fluids such as sputum from the lungs, throat, and nasal drainage. Drugs permeate the body, irrespective of whether they are ingested, snorted or swallowed; therefore any oral fluid can be used for drug testing, not just saliva. So it is correct term is oral fluid.

Because oral fluid is a partition of blood serum, we can say that if drug is present in the oral fluid it’s in the blood; and of course if it’s in the blood, it’s supplying the brain too, so the person is impaired by the drug. This view is becoming more widely acknowledged by the drug testing fraternity, possibly helped by the acceptance of this idea in the study of alcohol.

That’s why many companies are turning to oral testing instead of urine testing in ‘for cause’ cases, and it makes sense. Legislation passed in March 2015 enable Police to conduct road side screening for a range of drugs of abuse and medication that can impair the ability to drive safely. The same principles are used in the test products available to you, and because oral fluid has been chosen for road side testing, it is likely to become even more popular.

**PRODUCTION OF ORAL FLUID AND ITS COMPOSITION**

Oral fluid is a combination of gingival crevicular fluid, which has a composition similar to serum, and fluid produced by the salivary glands, of which the parotid, submandibular and sublingual are the three major sources.

The components of oral fluid are water, proteins, electrolytes, organic molecules secreted from salivary glands, blood, microbes, epithelial lining cells, extrinsic factors and some additional fluids. Oral fluid is an alkaline, viscous secretion, with a pH of 6.4-7.1 and a water content of 99.5%, a solid content of 0.5% (40% inorganic constituents / 60% organic constituents) and organic constituents of mucin, enzymes, amylase, lysozyme, albumin, and globulin. Other ingredients include urea, uric acid, cholesterol, vitamins, minerals, electrolytes, buffers, enzymes and enzyme inhibitors, growth factors and cytokines, immunoglobulins, phosphor-lipids, mucins and other glycoproteins. Mucin is a glycoprotein giving viscosity to saliva and mucous.

Proteins in saliva such as lactoferrin, lysozyme peroxidase, defensins and histatins, destroy or inhibit the growth of micro-organisms in the oral cavity. Saliva also contains glucose, potassium thiocyanate and cyanate which possibly come from ingested cyanides present in certain fruits, in tobacco smoke and from breaking down protein material. Apoerythein, a protein fraction that protects vitamin B12 from digestive destruction is...
also present in oral fluid; also containing solids consisting of epithelial cells, salivary corpuscles, food debris, bacteria, fungi and protozoa.

Salivary composition depends on many factors such as, diet, age, time of day, health etc. Oral fluid can be weakly alkaline to weakly acid, the pH ranging approximately 6.0-7.9 with optimum pH of 6.6. Lower pH values (acidic) occur more frequently among susceptible individuals and dental erosion is often accompanied by greatly increased total salivary acidity. Oral fluid has a specific gravity of 1.007.

The amount of oral fluid secreted by an adult in 24 hours varies between about 600 ml and 1500 ml per day. In the absence of obvious external stimuli, the rate of salivary secretion in adults is between 0.1 ml & 0.25 ml per minute and values <0.1 ml/min should be considered abnormal, and probably the result of changes to the parasympathetic system by drugs of abuse.

The stimulated flow rate varies between 1-2 ml/min and values <0.5 ml/min should be considered abnormal. Oral fluid is routinely categorised as resting (unstimulated) or stimulated. The resting saliva reflects the basal flow rate and it is present in the mouth, for about 14 hours of the day. Stimulate is also protective and is present in our mouths for up to two hours of the day.

A number of drugs are known to affect the secretion of oral fluid. Most commonly these are amphetamines, including the designer forms such as ecstasy (MDMA), and cannabis. Other drugs include the sedating antihistamines, antipsychotic drugs, anticholinergic drugs and a number of antidepressants. There are less commonly used drugs that increase flow and these include clonidine, pilocarpine and beta-2 stimulants (salbutamol, terbutaline etc).

Oral fluid is an ideal medium for many diagnostic procedures because it is rich in cytokines, antibodies, hormones and disease markers. Oral fluid is sometimes preferred to urine because collection is non-invasive, and only requires basic training. Problematic sample collection from seriously ill people, children, or hemaphobics can be made much easier by using oral fluid as the test medium.

One major advantage of oral fluid tests is that the collection can easily be observed, and there is no known method of adulteration. Probably the greatest benefit from the toxicologists viewpoint is that oral fluid can be substituted for plasma in the areas of pharmacokinetic studies and drug monitoring because it replicates the properties of plasma, but is easily collected.

**ORAL FLUID STIMULATION**

Salivary secretion is a reflex response controlled by both parasympathetic and sympathetic secretomotor nerves, and it can be influenced by several stimuli, as anyone exposed to the smell of pickled onions will testify. So anyone taking medication (or drugs of abuse) which influences either the central nervous system or the peripheral nervous system will have an altered salivary composition and salivary volume. The circadian rhythm determines both the volume of saliva that is secreted and the salivary electrolyte concentrations. Dietary influences and the patient's age also have an impact on composition and volume of saliva. The latter implies a wider variation in composition both inter and intra-individually. Bearing all this in mind, each sample taken when will be subtly different.

There have been many studies investigating whether drug concentrations in oral fluid are affected by stimulation, and in general, more saliva will dilute the concentration of drug metabolite. However this is not significant since common doses of drugs give peak oral fluid concentrations that exceed 0.1μg/ml and often more than 1μg/ml, while drug tests set to 10 to 50 ng/ml are between two and twenty times more sensitive.

Because of this ratio, unstimulate saliva will always represent best practice, with stimulate following closely behind. Some tests contain a buffer to increase the volume of the sample. However, judging the precise volume of oral fluid is usually difficult, and dilution can cause a false negative; there have even been collectors which feature an indicator to estimate fluid volume. Many authorities still prefer the use of neat oral fluid as it is the most accurate. Unstimulate saliva can be more viscous and of smaller volume, and stimulate saliva is more plentiful and runs a test quicker. Both are usually preferred to swab and buffer systems.

The argument is somewhat academic because our work on collection systems show that the action of introducing a collector into the mouth encourages oral fluid stimulation. The tongue senses the object, and the saliva ducts begin production to prepare the ‘food’ for digestion. Donors report a general dislike of spitting, and favour sponge collectors, and spitting samples tend to include more debris and bubbles.

**DOES ORAL FLUID HAVE ANY DISADVANTAGES?**

Yes. We can see that oral fluid is not the perfect medium for highly accurate determinations. Forensic toxicologists work to published and established practices and some are still reluctant to use oral fluid as a drug determinant fluid because its validity for some drugs is still questionable, especially if blood or urine is readily available. In support of oral fluid, it is much safer and far easier than blood collection, which has slight risk of infection, especially in non clinical conditions.

The biggest problem with oral fluid is collecting sufficient volume, especially from drug users who may have a dry mouth syndrome from drug use, hepatitis or a compromised immune system. TISPOL, the European Police Network, evaluated the leading oral test devices for use in roadside testing and their biggest problem was the oral fluid collection. They developed the view that an inability to provide a sample may be a good indicator of drug use in the same way that the inability to blow into a breathalyser can be a reason to suspect inebriation.

Sponge collectors must be used with care as some drugs, especially cannabis, are retained by the sponge, reducing the concentration of drug. Microporous polyurethane sponges represent a good compromise and will yield around 90% of what they absorb when they are squeezed.

**SALIVA TO PLASMA RATIOS**

The scientist's interest in oral fluid as a means of measuring impairment have led to mathematical models to predict the amount of drug found in oral fluid for a given plasma concentration; the saliva/plasma ratio or S/P. This ratio can be calculated as follows:
**Plasma pH and Drug Binding**

Plasma pH is assumed to be a constant at 7.4 and drug protein binding in the oral cavity is assumed to be zero, so $f_s=1$. This equation shows that lowering the oral fluid pH will reduce the amount of drug present in oral fluid when in equilibrium. However, using citric acid as a salivary stimulant will not have a significant effect on drug concentrations because the oral fluid is already available in the salivary glands at the original S/P ratio.

\[
S/P = \frac{1 + 10^{(pH_{s} - pK_a) \times f_p}}{1 + 10^{(pH_{p} - pK_a) \times f_s}}
\]

Where:
- $S$ = concentration of drug in saliva (oral fluid)
- $P$ = concentration of drug in plasma
- $pK_a = pK_a$ of the drug
- $pH_s = pH$ of the saliva
- $pH_p = pH$ of the plasma
- $f_p = free$ (unbound) fraction of the drug in plasma
- $f_s = free$ (unbound) fraction of the drug in saliva

**Rapid Tests for Oral Fluid**

In view of the link with oral fluid to actual impairment, screening onsite is ideal because the results are known in a few minutes, so decisions can be made straight away. Oral fluid tests began as more sensitive versions of the existing urine tests, but as the interest in application grew, they have been refined and developed into tests in their own right. Indeed, these tests are so good that many companies also have readers which will interrogate a visual result for the intensity of the test lines, enabling a semi-quantitative reading.

Tests come in different formats and the customer has a choice of collectors and devices. This choice should be made on ease of use, clarity of test lines, accuracy (especially to drugs like cannabis and benzodiazepines which are the most challenging) and price. Any reputable provider will also offer comprehensive and informative training, and guide you through the process of confirmation, should you have any positive results.

**Laboratory Screening of Oral Fluid**

When oral fluid samples are dealt with in a laboratory the sample may initially be screened using automated immunoassay microplates. These give a quantitative result which is used to decide whether the sample is positive or negative for the suite of drugs applied. Positive samples then go on for confirmation. It is important to appreciate that the laboratory will only be able to screen for those drugs its instrument is calibrated for.

Some laboratories will bypass this system if the sample has already been proven positive for a drug group after conducting a rapid test. This keeps costs to a minimum and improves turn around times; and of course you’ll only pay the lab for the samples that have proved positive when initially screened, which will hopefully be minimal.

**Laboratory Confirmations for Oral Fluid**

Many laboratories use conventional GC/MS systems for detecting drugs in oral fluid but recent advances in LC/MS/MS make liquid chromatography a far better system. Unlike gas chromatography, oral fluid injected into a liquid chromatograph does not have to be chemically treated first (this is called derivatisation) and in fact no pre-treatment is needed at all except for filtering through a serum separator, and dosing with an internal standard. Quantities used are very small, usually 20 microlitres (0.02ml) so if 100 to 200 microlitres of oral fluid are available it will be enough.

LC/MS/MS has another very big benefit when more than one drug is detected. In GC/MS, it is necessary to return to the sample again for every drug that has been detected, derivatise each one separately, and then inject and analyse each of those samples. This takes a long time and very often there is not enough sample to allow this to be done. But with LC/MS/MS there is no derivatising and one injection will produce confirmation data for all of the drugs in the sample. This makes sure there is always enough sample to complete the analysis. The latest instruments will also fragment the drugs even further, giving an absolute fingerprint of that drug as well as identifying its concentration. This information allows you to ask the right questions of your chosen laboratory.

Neat oral fluid from bufferless systems are easily dealt with, but the swab-with-buffer systems claim to collect a certain ratio of buffer to oral fluid, such as for example four to one. The lab deals with these systems by multiplying the result by that ratio, remembering of course to add a modified amount of the internal standard to compensate for that dilution factor.

**NEQAS, the external quality assessment scheme for laboratories**

NEQAS, the external quality assessment scheme for laboratories does not yet include oral testing in their programme but a number of UK laboratories now hold UKAS accreditation for oral testing or else have systems based on these procedures.

**How is Oral Fluid Stored?**

Once the samples have been collected, it is important that they...
are properly stored unless analysis is to be performed immediately. Our experience is that oral fluid is stable for 48 hours, after which it will start to degrade due to bacterial action. When refrigerated, the sample will survive for one week, but any cannabis present may start to bind to the plastic container and eventually could give a false negative. Longer term storage should be carried out in a freezer at minus 20 degrees Celsius.

In forensic analysis for serological purposes, it is common practice to place the oral fluid sample and container in boiling water prior to freezing, unless there is a possibility that the sample could contain volatile material. This is not recommended when the sample is for drug analysis.

**ORAL TEST APPLICATIONS FOR SPECIFIC DRUGS**

**CANNABINOIDS**
Attempts to measure cannabis in oral fluid date from the early 1970s. Surprisingly, consumption of food and drink does little to remove evidence of taking cannabis—both smoking or eating it, and oral tests can detect prior cannabis use in a window of <4 hours to 14 hours.

Early rapid testing kits found cannabis detection a challenge. It has been widely reported that parent cannabis stays in the oral cavity but the primary metabolite, Δ⁹-THC carboxylic acid cannot pass from serum back into the oral fluid, so for this drug the S/P ratio is irrelevant. Rapid tests and laboratory microplates which are based only on detection of the primary metabolite cannot therefore detect cannabis use from oral fluid.

Oral fluid contains a number of cannabinoid compounds that have only been detected in minute amounts in urine, if at all. SureScreen’s early tests had excellent affinity for cannabidiol, present in quantity in the oral cavity after smoking. This gave our earlier tests the lead over the competition. Today, our cannabis tests use clever chemistry to detect parent cannabis, as trials in the TISPOL roadside screening programme have shown. They also correlate well with impairment trials using pupillometry as a comparison methodology.

Laboratory LC/MS/MS/MS should look for the parent drug tetrahydrocannabinol (THC). Today’s cannabis, bred from *Cannabis sativa* and *Cannabis indica*, can be potent. Our work has shown that THC levels in skunk can be above 20%, four times stronger than that material we were testing in the late 1960s.

**COCAINE**
Snorted cocaine can be found in oral fluid virtually instantly, but even injected cocaine and other injected drugs become available extremely quickly, because the blood does a circuit of the body around every minute, even for someone at rest.

The concentration of cocaine in oral fluid is higher than that in plasma because it has a S/P ratio of between 1 and 2.

Oral fluid cocaine levels and mental state are closely related. The half life of cocaine is about 60 minutes but that of the metabolite benzoylecgonine (BE) is 7.5 hours. Laboratory analysis of the oral fluid for BE will extend the capability of cocaine detection in oral fluid. This can also be useful when someone has high pH because this reduces the cocaine S/P ratio, while that of BE is barely affected. The graph on page 3 showing this effect also includes a drug, Valproate, which exhibits a reverse effect on pH.

**CANNABINOIDS**

**Drug Class**

**Concentrations of Drugs in Saliva**

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Analyte</th>
<th>Conc. (ng/mL)</th>
<th>Window of Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannabinoids</td>
<td>Δ⁹-THC</td>
<td>5-300</td>
<td>0-10 hrs-12 hrs</td>
</tr>
<tr>
<td></td>
<td>Cocaine</td>
<td>0-200</td>
<td>0-10 hrs-12 hrs</td>
</tr>
<tr>
<td></td>
<td>Phencyclidine</td>
<td>0-200</td>
<td>0-10 hrs-12 hrs</td>
</tr>
<tr>
<td></td>
<td>Opioids</td>
<td>0-200</td>
<td>0-10 hrs-12 hrs</td>
</tr>
<tr>
<td></td>
<td>Barbiturates</td>
<td>100-8000</td>
<td>12-50 hrs</td>
</tr>
<tr>
<td></td>
<td>Methaqualone</td>
<td>20-300</td>
<td>3-24 hrs</td>
</tr>
<tr>
<td></td>
<td>Diazepines</td>
<td>2-1000</td>
<td>&lt;5-50 hrs</td>
</tr>
<tr>
<td></td>
<td>Amphetamines</td>
<td>20-40</td>
<td>60 hrs</td>
</tr>
</tbody>
</table>

**OPiates**
All of the parent opioids are detectable in oral fluid, including the synthetic forms such as methadone, pholcodine and oxycodone.

Concentrations in oral fluid are variable and while most have a S/P ratio greater than 1, morphine, the final metabolite, has a lower S/P ratio and so levels are depressed.

**BENZODIAZEPINES**
Like cannabis, benzodiazepines have poor solubility and partition out in the body in liquids, so they are characterised by very low S/P ratios. Diazepam, the primary metabolite of many benzos, and the common target for immunoassay drug tests, has an S/P of only 0.03. Oral drug tests therefore have to be extremely sensitive if they are to have any chance of detecting benzodiazepines, but can detect the drug for some time after consumption. SureScreen’s oral test has a cut off of 8 ng/ml (nominally 10 ng/mL) and in trials gave positive results for 15 hours to 1mg Alprazolam (Xanax) taken orally. Until recently, this impressive level of detection would have been impossible in a routine rapid drug test.

**AMPHETAMINES**
Researchers in the effects of amphetamines and methamphetamines prefer oral fluid because the excretion of these drugs in urine is highly dependent on urine pH and oral fluid contains more reliable quantities of these drugs, with a positive S/P ratio.

**OTHER DRUGS**
Oral fluid is becoming the preferred choice for testing of impairment, as the products have evolved to a stage where they are reliable and accurate enough to be used in on site screening. It is suitable for many other drugs provided their S/P ratio allows sufficient drug to be present in the oral fluid, but drugs like buprenorphine, which are taken in very small doses still represent a considerable challenge. However the range of oral drug tests is increasing all the time and the range includes nicotine, hormones, cytokines and disease markers.