Review Article

Drug Testing in Oral Fluid

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Abstract

Over the last decade there have been considerable developments in the use of oral fluid (saliva) for drug testing. Oral fluid can provide a quick and non-invasive specimen for drug testing. However, its collection may be thwarted by lack of available fluid due to a range of physiological factors, including drug use itself. Food and techniques designed to stimulate production of oral fluid can also affect the concentration of drugs. Current applications are mainly focused on drugs of abuse testing in employees at workplaces where drug use has safety implications, in drivers of vehicles at the roadside and in other situations where drug impairment is suspected. Testing has included alcohol (ethanol) and a range of clinical tests eg antibodies to HIV, therapeutic drugs and steroids. Its main application has been for testing for drugs of abuse such as the amphetamines, cocaine and metabolites, opioids such as morphine, methadone and heroin, and for cannabis. Oral fluid concentrations of basic drugs such as the amphetamines, cocaine and some opioids are similar or higher than those in plasma. Tetrahydrocannabinol (THC), the major species present from cannabis use, displays similar concentrations in oral fluid compared to blood in the elimination phase. However, there is significant local absorption of the drug in the oral cavity which increases the concentrations for a period after use of drug. Depot effects occur for other drugs introduced into the body that allow local absorption, such as smoking of tobacco (nicotine), cocaine, amphetamines, or use of sub-lingual buprenorphine. Screening techniques are usually an adaptation of those used in other specimens, with an emphasis on the parent drug since this is usually the dominant species present in oral fluid. Confirmatory techniques are largely based on mass spectrometry (MS) with an emphasis on Liquid Chromatography-Mass Spectrometry (LC-MS), due to low sample volumes and the low detection limits required. Drug testing outside laboratory environments has become widespread and provides presumptive results within minutes of collection of specimens. This review focuses on the developments, particularly over the last 10 years, and outlines the roles and applications of testing for drugs in oral fluid, describes the difficulties associated with this form of testing and illustrates applications of oral fluid testing for specific drugs.

Introduction

Drug testing has undergone major advances, particularly over the last 10 years. The use of alternative specimens to blood or urine for establishing exposure to drugs has become a significant direction in clinical and forensic toxicology.[1] These alternative specimens include hair, sweat and oral fluid. Oral fluid has been seen as a non-invasive alternative to blood but also as an alternative to urine when substitution or adulteration is suspected. While these attributes are real, oral fluid cannot be seen as a substitute for blood or urine drug testing. Each specimen has its own distinct advantages and disadvantages.

The introduction of LC-MS as a routine laboratory technique has enabled the benefits of High Performance Liquid Chromatography (HPLC) separation techniques to be linked to the high sensitivity and specificity of MS.[2-4] This has assisted in the development of drug testing in oral fluid due to the relatively small sample volumes that are usually collected. The last decade has also seen a significant development in the understanding of the target drugs and their pharmacokinetics in oral fluid. This has applied particularly to the abused drugs and what concentrations need to be targeted, and also how these concentrations may or may not relate to blood concentrations and the likely drug effects on the individual. In addition, the use of initial screening cartridges or devices providing an electronic readout has developed and is now widely used. In particular, kits are now designed for on-site drug detection without the need for sophisticated laboratory screening equipment. These are able to provide a preliminary drug result within minutes.

A number of reviews and major papers currently exist for various aspects of drugs and drug testing in oral fluid. These include its use as a diagnostic tool,[5] workplace applications,[6] applications in drugs in driving,[7] legal issues associated with drug testing in oral fluid,[8] and detection times and pharmacokinetics of selected drugs.[9,10]

This review outlines the roles and applications of testing for drugs in oral fluid, describes the relative advantages and disadvantages of this form of testing and illustrates applications of oral fluid testing for specific drugs.

Scope of Review

This paper reviews the developments and applications of drug testing in oral fluid particularly over the last 10 years. Published peer-reviewed literature and other selected references in the English language in humans as sourced by Medline and Science Direct (since 1995) are reviewed for clinical and forensic applications of drug testing in oral fluid. Publications before this time are included if pivotal or later papers were not available. The term oral fluid refers to saliva and other secretions in the oral cavity. The focus is on testing for drugs of abuse such as the amphetamines, benzodiazepines, cannabis, cocaine and opioids, but other applications will be discussed in the context of abused drugs.

Source of Oral Fluid

Oral fluid (saliva) is excreted primarily by three glands: the parotid, submaxillary and sublingual and by other smaller glands. Oral fluid has low protein content (0.3%) and can vary in flow rate from zero to several mL per minute depending on influences from various factors, including emotional state and hunger. Dry mouth syndrome is relatively common and can be caused by the anxiety of the collection procedure, or even by lack of proper hydration of the individual. Dry mouths require much longer collection times; often several minutes to collect 1 mL. On some occasions this may force the collection of an alternative specimen if collection is too slow, ie blood (unpublished data). Aps and Martens provide an excellent review of physiological and pharmacological issues involved in the production of oral fluid.[11]

Collection Techniques and Adulterants

Expectoration (or spitting) provides neat oral fluid, but this is relatively viscous and can be difficult to work with in the laboratory. It may also be contaminated with food and other debris from the mouth and will therefore require centrifugation. More often than not, the volume will be less than 1 mL requiring the use of sensitive detection techniques. Some of the commercial collectors available use some form of proprietary diluent to mix with the collected oral fluid (Table 1). In this situation, typically the absorbent pad/foam

that is used to collect the oral fluid is added to a diluent. Following mixing, the solution is used for drug analysis. Other devices involve squeezing absorbed oral fluid from a pad or foam onto the drug-detection device. The collection time is typically one to three minutes, however this can vary as discussed earlier. A number of the devices have some form of indicator to show that sufficient oral fluid has been collected.

The DrugWipe® only involves swiping a collection pad on the tongue or skin, a process that takes only seconds (Table 1). However, there is no oral fluid for any confirmatory assay if the result is positive.

Oral fluid production is stimulated by use of agents such as citric acid candy, chewing gum or other agents. This will inevitably change the pH and concentration of drug in the oral fluid. This has been shown to lower concentrations of codeine by about two- to six-fold,[12,13] two- to four-fold for methamphetamine,[14] and about five-fold for cocaine.[15] It is likely that similar changes will occur for other drugs.

A number of drugs are known to affect the secretion of oral fluid.[11] 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).

Consequently, there is significant intra- and inter-subject variation in relation to drug concentrations depending on the technique used, the physiology of the person and the influence of factors affecting drug concentration in oral fluid.

Since the collection of oral fluid specimen can be viewed by a second person without infringing privacy it does not suffer from the same issues regarding possible adulteration or substitution as for urine.[13] While this can be a distinct advantage, it must be recognised that methods can be employed to potentially affect the collection of oral fluid or the concentration of drugs in oral fluid. The prior administration of drugs and a range of physiological factors covered earlier can affect drug concentration. Foodstuffs, various beverages and various toothpastes did not affect the concentration of drugs of abuse using the Oratect® device 30 min after exposure.[16] The use of commercial adulterants or other products capable of acting as adulterants, such as Clear Choice®, Fizzy Flush™ Spit and Clean®/™ mouth wash and Cool Mint Listerine® also had no substantial effect after 30 min. In an early study using a small number of volunteers, the consumption of beer immediately smoking a marijuana joint appeared to lower concentrations

Table 1. Selection of collection devices reported in literature.

Name of collector	Method of operation	Reference
DrugWipe®	Swipe only (tongue or skin)	62, 66, 67, 93, 94
Cozart® collector	Absorbent foam pad plus diluent	75, 76, 93, 95, 96
Dräger DrugTest®	Absorbent foam pad with diluent	97
Intercept®	Absorbent foam pad with diluent	77, 83, 98-100
OralScreen®	Absorbent foam pad only, drops applied to device	101
OralLab®	Absorbent foam pad, collector squeezed to apply oral fluid into test cartridge	93
OraLine®	Direct application to oral cavity, or use of other collectors	102
OraTect®	Absorbent directly connected to device	16, 94
Quantisal™	Absorbent foam pad plus diluent	21
SalivaScreen®	Absorbent foam pad, drops applied to device	93
Salivette®	Cotton wool swab which is then filtered and centrifuged	12, 19, 66, 103
Toxiquick®	Absorbent bud, oral fluid squeezed into syringe and applied to device	104

of THC in oral fluid at 1 h post dose.[17] It is likely that a short rinsing effect is seen with these agents and others, including water, for a short period after use. Since the oral fluid in the mouth is rapidly turned over, a wait of several minutes should allow re-equilibration of drug in the surrounding tissues.

The rinsing effect with drugs is in some way similar to the contamination of breath alcohol by recently consumed alcohol in that a wait of 15-20 min allows any mouth alcohol to be removed by normal physiological processes.[18] However, more research is needed to investigate this phenomenon in oral fluid for the various collection techniques.

Recovery of Drugs from Collectors

There is no one type of collection device that is clearly superior based on design or ease of use. However, recovery studies conducted on some devices suggest that desorption of drugs may limit the usability of some collection materials. For example, the Salivette® has poor recovery for THC but is reasonable for codeine,[12,19] whereas the Cozart® collector has good recovery for THC,[20] and methamphetamine (unpublished data). The Quantisal® collection device has a good recovery for THC,[21] although another study found lower recoveries for THC.[20]

Clearly more information is required for all drugs likely to be measured in oral fluid, for each collection device. Indeed a device should not be used until recovery and stability studies have been performed and show adequate performance. However, it should be emphasised that products are continually being developed and hence published results on an earlier design may not bear any resemblance to more recent designs.

Applications of Oral Fluid Drug Testing

The use of oral fluid to detect drugs has potentially wide applications. To date its main application has been to provide a non-invasive specimen for testing of possible drug-affected drivers.[22] It has also been used for workplace testing, particularly following a safety incident, to check for possible drug use.[23,24] Other applications include testing of persons in prisons and other correctional institutions, the monitoring of drug use by drug courts, or testing of detainees suspected of a crime who may be under the influence of a drug.

Oral fluid should not be seen as a specimen that replaces the use of other specimens. As discussed later the pharmacokinetic characteristics of drugs are more closely aligned to blood concentrations than, for example, urine or hair. Urine should still be seen as the specimen of choice if evidence of prior exposure to drugs of abuse is sought (eg routine workplace screening without cause and drug screening of prisoners). Hair will still be much more useful if a longer time frame of exposure to drugs is sought. However, if evidence of recent use (or abstinence) of drugs is sought then either blood or oral fluid are preferred specimens.

Oral fluid has the advantage over blood in that it can be obtained non-invasively in a situation where adulteration or substitution is difficult. A review of the advantages and disadvantages of specimens is available.[25]

Pharmacokinetics

A recent review of the pharmacokinetics of some drugs in oral fluid has been published.[10] As distinct from urine the dominant species in oral fluid is the parent drug.[10] Hence, initial screens and confirmatory techniques target the parent drug. For example, there is almost no carboxy metabolite of THC present in oral fluid. However, due to the rapid bioconversion of cocaine, benzoylecgonine and ecgonine methyl ester they are also detectable in oral fluid.[26,27] Moreover, anhydroecgonine methyl ester is also detected in oral fluid after smoking cocaine.[27]

As a general rule there is some similarity between an oral fluid concentration and a blood/plasma concentration. In the case of most drugs the oral fluid concentration can be estimated from the pH of oral fluid and blood, the protein binding of the drug and its pKa.[13,28] For acidic drugs the equilibrium favours blood, hence oral fluid concentrations are less than for blood, while for basic drugs higher oral fluid concentrations occur. The average concentration ratio is shown in Table 2. In the absorptive phase there are often higher concentrations in the oral fluid due to local absorption in the mucous membranes of the buccal cavity. This local absorption effect is probably highest for THC due to its higher fat solubility and ease of penetration through membranes and the very low partitioning from blood to oral fluid. However, as discussed later in this section this effect is also seen for other drugs.

The most commonly detected drug toxicologically, alcohol (ethanol), has been subject to much research in terms of its presence in oral fluid. The oral fluid to plasma concentration is similar to that predicted based on the water content of the two fluids and averages just over unity and has been used to assess alcohol exposure.[25,29-31]

Subjective intoxication and the increase in heart rate in volunteers taking cannabis correlated well with oral fluid THC concentrations.[32] Oral fluid concentrations also correlated well with plasma concentrations.[33]

The administration of 30 mg doses of codeine phosphate showed a good correlation of plasma and oral fluid concentrations particularly after 2 h following the initial contamination of the oral cavity.[34] The individual oral fluid to plasma concentration ratios varied substantially and was partly due to the pH of the oral fluid. Some concordance of the physiologic and subjective effects of codeine and oral fluid concentration occurred following single oral codeine doses to volunteers.[35]

Orally administered morphine shows a delay in the appearance in oral fluid compared to its presence in plasma suggesting some rate limiting movement in oral fluid possibly due to its relatively low lipid solubility.[25] 6-Acetylmorphine and morphine are also present in detectable amounts in oral fluid after use of heroin.[27]

Buprenorphine is widely used for the treatment of opioid dependency and is available (amongst other formulations) as a sublingual tablet.[36,37] The local absorption of drug in the mucous membranes of the oral cavity produces a depot-like effect for some hours after administration of drug. In the terminal phase of elimination oral fluid concentrations were similar to plasma.[38] Nevertheless, the data suggested that oral fluid could be used to monitor the use of this opioid. A similar depot effect occurs with nicotine. Research suggests that measurement of the major metabolite cotinine is more useful than nicotine to determine exposure to this drug.[39,40]

Table 2. Average oral fluid to blood concentration ratios for selected drugs.

Drug (type)	Average oral fluid to blood concentration ratio	Reference
Alcohol (ethanol)	1.07	29
Barbiturates	0.3	105-107
Buprenorphine	1	38
Codeine (basic)	4	34, 35
Methamphetamine (basic)	2	14
MDMA (basic)	7	108
Cocaine (basic)	3	26
Diazepam (acidic)	0.01-0.02	42, 43
Methadone (basic)	1.6	109
Morphine (basic)	0.8	25, 27
Δ9-Tetrahydrocannabinol (neutral)	1.2	33

Type refers to physiochemical property of drug, ie acidity, basicity or neutrality. The average ratios are indicative figures derived from pharmacokinetic studies and will change depending on a number of factors, including pH of oral fluid, protein binding and degree of contamination of the membranes in the oral cavity by recently consumed drug.

Slow equilibration between plasma and oral fluid has also been observed for diazepam.[41] Since diazepam and other benzodiazepines are highly protein bound and are weakly acidic they have low oral fluid concentrations. Mean oral fluid to plasma ratios for diazepam are about 0.01-0.02.[42,43] Little data is available for other benzodiazepines, however, they are likely to behave similarly.[44,45] Stability problems for the nitrobenzodiazepines (clonazepam, flunitrazepam and nitrazepam) have been reported in which conversion to the corresponding 7-amino metabolite also occurs.[45,46] The use of fluoride has been shown to stabilise the drug.[45]

These data show that the pharmacokinetics of drugs in oral fluid is more complex than that of blood. Detection times in this specimen will depend on a range of factors including dose, frequency of use (ie acute versus chronic use) and detection limits of analytical assays. A number of other drugs of forensic interest have been measured in oral fluid. These include hydromorphone,[47] phencyclidine,[48] pholcodine,[49] and sildenafil.[50]

Therapeutic Drug Monitoring (TDM)
TDM of drugs in oral fluid has been studied for at least 30 years although it has been increasingly used in a range of applications. There is some support for its routine application for some anticonvulsants and theophylline.[51,52] Recently applications for carbamazepine,[53] digoxin,[51] topirimate,[54] methadone,[55] disopyramide,[56] and docetaxel and paclitaxel have been described.[57]

Other clinical applications include testing for HIV-antibodies,[58,59] and a number of steroids including cortisol and dehydroepiandrosterone,[60] and 17-hydroxy progesterone.[61] An often mooted advantage for oral fluid over serum is the ease of collection and is, of course, less invasive than venepuncture. However, care will be needed to avoid changes in concentration in oral fluid caused by a variety of factors discussed elsewhere in this review.

Initial Testing Techniques
Initial testing of oral fluid for drugs can either occur in the field (other words for this form of testing include on-site or point-of-care testing) or in the laboratory.

1. Field Testing
A number of devices are available for field use. These include

instrumental devices providing an electronic readout such as the Dräger DrugTest® and Orasure Uplink®, Cozart Rapiscan® and Drugread® hand photometer (Securetec) to hand-held cartridges requiring visual identification such as the DrugWipe® (Securetec AG), iScreen OFD™ (Rapid Detect Inc.), OralScreen® (Avitar Technologies), Oratect® (Branan Medical Corp.), SalivaScreen™ (Craig Medical Inc.) (Table 3). These are optical readers that provide a visual readout of intensity of response of the immunoassay signal.

Unfortunately, there is no consistency in the specifications applied to these devices. For some, cut-off concentrations are used to define their detectability, for others concentrations are given when drugs can be detected. The apparent sensitivity is often not defined in terms of consistency of detection in oral fluid specimens. This means that at the present moment there is no objective way to assess performance of these devices or cartridges.

In the laboratory, terms such as false positive (FP) and false negative (FN) are used. FP refers to a situation when a presumptive initial test result is not confirmed. FN refers to a situation when a confirmation test finds a drug present that was not detected by the initial test. Sensitivity is often used in defining performance of initial testing kits and refers to the relative detectability of the kit or device (of positive cases) in question over a comparison method. On the other hand, specificity refers to the percentage of negative results using the kit or device compared to the total number of negative specimens using a comparison method. The comparison method is usually a Gas Chromatography-Mass Spectrometry (GC-MS) or LC-MS method.

A number of published studies have evaluated devices either using fortified oral fluid with known drug concentrations or real specimens taken from humans exposed to the drug under question.

A number of studies have used the DrugWipe® cartridge. These have found a high rate of FN for volunteers given 60 mg codeine over a 24 h period using a limit of quantitation of 5 ng/mL.[62] The testing for amphetamines, cocaine and opiates in drivers was reasonably reliable when tested against a GC-MS technique, except for some FN in heroin users.[63] Amphetamines and opiates performed better than for benzo-diazepines and THC on the DrugWipe® and Rapiscan®/™ in a drug driving study in Finland.[64] The sensitivity and specificity were all close to 90% for Rapiscan®/™ when testing for cocaine using a confirmation cut-off of 30 ng/mL.[65] In a Belgian study the DrugWipe® performed best for amphetamines and cocaine.[63] The reliability of DrugWipe® was assessed on drivers detained at special roadblocks in

Belgium who failed a sobriety assessment but were below the legal limit of alcohol (0.05%). Oral fluid concentrations were closely related to plasma concentrations and gave positive predictive values and sensitivities exceeding 90% when evaluated against plasma legal cut-offs. The DrugWipe® device through a tongue swipe was not recommended since accuracy of more than 90% was only obtained for amphetamine and MDMA.[66] The use of Drugread® hand-held photometer with the DrugWipe® cartridge has been used to assess the detection of MDMA in oral fluid following a 100 mg single dose in volunteers. Detection times ranged up to 10 h post-dose although it was most reliable to 6 h. The detection limit was about 450 ng/mL.[67] The pharmacological effects of the drug were most significant up to about 6 h.

A recent study in Victoria, Australia in which over 13,000 motorists were randomly screened for presence of methamphetamines (methamphetamine and MDMA) and THC as part of a campaign to reduce drug affected driving found a positive rate of 1:40 compared to about 1:100 for alcohol. This testing was based on an initial tongue wipe using DrugWipe®, and if positive, repeat testing on the Rapiscan®/™ following collection of oral fluid with the Cozart® collector (unpublished data). The overall FP rate using both devices was very low (one cannabis and four methamphetamines). Individually the devices gave more FP for methamphetamines (DrugWipe®) and THC (Rapiscan®/™).

As for urine, immunoassay tests of a drug class will not detect all members of the drug class equally. For example, the required sensitivities of the initial test for the amphetamine, opiate and benzodiazepine classes will be different for the various drugs since the concentration of drugs in these classes are quite different to that of blood. For example, amphetamines have higher concentrations in oral fluid compared to blood and benzodiazepines have concentrations only a fraction of those in blood. Hence, it is important that the selection of on-site testing devices has the appropriate sensitivity (and indeed other performance characteristics) for the intended applications.

2. Laboratory Testing
There are a number of commercial kits based on ELISA technology available for laboratory screening of oral fluids.

These generally work satisfactorily for amphetamines,[68,69] buprenorphine,[70] cocaine,[65,71-73] methadone,[74] and other opioids,[72,75,76] and provide a reliable means to screen oral fluid. Cannabis can be more difficult particularly if the immunoassay has little cross-reactivity to THC. Nevertheless enzyme immunoassay has been successfully used for this drug.[77] The same applies for benzodiazepines despite their low concentrations in oral fluid.[78]

The power of MS has been used as a screening system for a range of drugs.[79] Other general drug screening systems using GC-MS,[80,81] or LC-MS have been published that allow multiple drugs to be detected and quantified in oral fluid.[81-84] The choice of method is more to do with availability of instrumentation and costs since both forms of MS show sufficient sensitivity for most forms of drug detection in oral fluid.

Confirmatory Analytical Techniques
Confirmatory techniques for drugs in oral fluid are for the most part adaptations of their counterparts in blood or plasma/serum. Given the larger water component and lower protein content of oral fluid compared to blood, recovery of drugs is not usually a limiting factor. The smaller sample volume and often lower concentrations in oral fluid require the most adjustments to analytical techniques.

The required detection or quantification limit for drugs in oral fluid depend very much on the application and type of screening test employed. For example, in workplace applications the Substance Abuse & Mental Health Services Administration in the USA (SAMHSA) has recommended confirmation cut-offs for THC, cocaine, morphine and the amphetamines of 4, 8, 40, and 50 ng/mL, respectively.[22] In contrast, the European Union roadside assessment testing study (ROSITA) for impaired drivers have recommended somewhat different cut-offs,[22] as has the Australian Draft Standard for the collection, detection and quantification of drugs of abuse in oral fluid.[85] (Table 3)

The variable target minimum concentrations probably reflect the relative embryonic stage of drug testing compared to urine drugs of abuse testing. It is possible that some international agreement may exist in the future regarding minimum detectable concentrations (or cut-offs). However, this is unlikely since there are still significant differences in cut-offs in urine between countries after over 30 years of testing. Moreover, inadvertent exposure may limit the concentrations that can be used to prove deliberate use. In the case of cannabis, a study has found THC concentrations for a short period following high passive exposure in an unventilated room of up to 26 ng/mL.[86] Ingestion of poppy seeds in food can cause a positive test result for morphine and exceed the 40 ng/mL SAMHSA cut-off for about one hour following consumption.[87]

Nevertheless, numerous papers exist that provide validated methods for the detection of notable drugs in oral fluid. A summary of these is shown in Table 4. Predominantly, the preferred technique is MS due to its high sensitivity and specificity. Consequently the focus is on the use of this technique.

MS can be in the form of GC-MS or LC-MS including tandem mass spectrometry (MS-MS) applications for both forms of chromatography. Detection limits are within or less than those mentioned in Table 3 and use volumes of oral fluid from 0.1-0.5 mL. The majority of methods use LC-MS as distinct from GC-MS to cater for the lower sample volumes and required low detection limits, although a number of GC-MS techniques have been reported with adequate sensitivity. A review of the pros and cons of LC-MS methods in oral fluid drug detection has been published.[88]

Whatever technique is used it is important that the detection limits applied to confirmation testing is the same, but preferably lower, than the initial testing threshold concentration. This avoids not being able to confirm an initial on-site positive result because of insufficient sensitivity and to cater for presence of some metabolites that cross-react with antibodies used in immunoassays.

Table 3. Recommended minimum detectable concentrations of drugs in oral fluid.

Drug	SAMHSA cut-offs (ng/mL)	ROSITA cut-offs (ng/mL)	Standards Australia proposed target concentrations (ng/mL)
6-AM	4	-	10
Morphine	40	-	25
Cocaine	8	5-10	25
THC	2	1.9	10
Methamphetamine/MDMA/amphetamine	50	70-90	25

6-AM = 6-Acetylmorphine, MDMA = methylenedioxymethamphetamine, THC = Δ^9-tetrahydrocannabinol.

Table 4. Selected mass spectrometric methods used to quantify some common drugs in oral fluid.

Drug	Method Type	LOQ (volume of specimen) ng/mL	Reference
Amphetamines (AM, MA, MDMA, MDEA etc)	GC-MS	20 (100 µL)	110
	LC-MS	2 (50-200 µL)	82, 83, 111
Benzodiazepines	LC-MS	500 µL (0.1-0.2 ng/mL)	45, 100, 112
Cocaine*	LC-MS	2 (200-250 µL)	82, 83
	LC-MS^2 (APCI)	1 (200 µL)	84
THC	LC-MS	2 (500 µL)	19
Morphine/6-AM	LC-MS	2 (200-250 µL)	82, 83
	LC-MS^2 (APCI)	1 (200 µL)	84
Methadone	LC-MS^2 (APCI)	1 (200 µL)	84
	GC-MS		74

6-AM = 6-acetylmorphine, AM = amphetamine, APCI = atmospheric pressure chemical ionization, MS^2 = MS/MS, MA = methamphetamine, MDMA = methylenedioxymethamphetamine, MDEA = methylenedioxyethylamphetamine, LOQ = limit of quantification – note some methods have used limit of detection in validation, * most methods also measure benzoylecgonine, ecgonine methyl ester and other metabolites of cocaine.

Quality Issues

It is now expected that laboratories in many parts of the world, including the author's own laboratory, are subject to some form of certification or accreditation process. Most commonly laboratories conform, or should conform to the ISO/IEC 17025 standard.[89,90] This means that laboratories testing batches of specimens would also employ blank samples, samples with known concentrations (calibrators) and quality controls to ensure the results of each batch of specimens meet appropriate laboratory performance criteria. Only results from those batches where performance criteria are satisfactorily met are therefore accepted. All other results are rejected and the analysis repeated. Additionally the methods used must be fully validated and comply with International harmonised guidelines.[91]

In addition, most laboratories take part in some form of proficiency test to independently assess their ability to detect drugs. One program in oral fluid has been reported.[92]

In essence this means that there are checks and balances for using laboratory-based test kits including their calibrations and monitoring their performance. When cassettes by themselves or cassettes placed in readers are used in the testing location

(workplace, street, etc) there is a need to ensure a level of quality assurance takes place to ensure that the devices are used as recommended by the manufacturer and sufficient quality issues have been addressed to ensure optimum and consistent performance. This includes the training of staff, the running of suitable quality controls and participation in external proficiency tests.[85]

The principles of good laboratory practice do need to be also considered for on-site testing. In practice this may be more difficult given that the environmental conditions and location are much less controlled than a laboratory. Nevertheless, it is imperative that the collection and testing process is as controlled as is reasonably feasible and the staff performing the collection of specimens and the testing are properly trained, otherwise it is likely that initial on-site results will be less reliable. This may produce a higher rate of FP and FN.

Conclusions

The last decade has seen a revolution in the development of alternative specimens for drug analysis. The use of oral fluid has been found to offer significant promise when detection of relatively recent use of drugs is sought in a non-invasive manner. Technological advances do allow on-site detection of

drugs, but there are technical issues in relation to collection of oral fluid and in the variability of drug concentrations (of different drug types) in this fluid. More research is needed to further the detection of drugs present in this fluid which should allow improved reliability of detection of drugs. Similarly, future technological developments of on-site devices should allow more sensitive and reliable detection of a number of drugs.

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