

Oral Fluid – A Suitable Matrix for Proficiency Testing?

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Advantages of oral fluid as a drug testing matrix

Interest in using oral fluid as the matrix for undertaking screening for drug use has expanded significantly over recent years. There are some good reasons in support of this expansion. The ability to supervise collection without causing embarrassment significantly reduces the chances of the sample being tampered with by the subject. Oral fluid testing is particularly well adapted to roadside testing, providing a sample for immediate analysis. When combined with, for example, the new Cozart® DDS system that boasts a turn around time of just ninety seconds for two classes of drug and five minutes for six separate classes¹, it can provide data sufficient to initiate law enforcement procedures that will include confirmatory drug analyses. The drug levels found in oral fluid relate to blood levels and correlate well with^{16, 12, 14}, though they don't necessarily indicate actual extent of impairment. The use of oral fluid in roadside testing has been trialled by various governments in recent years. DRUID (Driving under the Influence of Drugs, Alcohol and Medicines) and ROSITA (Roadside Testing Assessment) programs have conducted field trials of various collection devices and review a large amount of data on their web-sites (<http://www.rosita.org> and <http://www.druid-project.eu>).

Disadvantages of oral fluid - pH

Oral fluid is not without problems as a matrix for drug analysis. Collection of blood and urine samples is, as a rule, not affected by the method used. Blood collection does not have to be stimulated, a simple needle prick will do. Urine will always come to those who wait. Stimulated collection of saliva actively changes the composition of the oral fluid collected and affects the pH. As a result, the distribution of drugs across the blood / oral fluid interface is altered^{17, 3}. Indeed even physiological variability of oral fluid pH can have a considerable influence on these ratios. For example, when codeine was administered to a subject, the levels in oral fluid collected after stimulation with a lemon drop in the mouth decreased by an average of 3.6 times compared to the non-stimulated control sample collected by having the subjects spit into inert polyethylene tubes².

Problems with collection devices for oral fluid

The majority of collection devices take the form of an inert absorbent pad which is placed into the mouth between the cheek and jaw (Table 1). These collect unstimulated saliva. The Orasure Intercept® device contains salts in the pad which exert osmotic pressure to draw additional fluids into the pad from the interstitial spaces of the cheek. Once saturated, the pad is inserted into a sample vial containing a preservative solution. The vial is then sealed and transported to a laboratory for testing. A key factor with all such devices is how much oral fluid is absorbed during the collection process. If an assumed value is used which proves to be incorrect, all quantities of drug measured in the sample (irrespective of method) are thrown into doubt, making comparison to a given threshold impossible. There are currently two approaches to solving the volume problem. An indicator in the stem of the device that changes colour when a sufficient volume of oral fluid is taken up and a dye-dilution approach, which allows measurement of the volume

collected. Of the devices listed in Table 1, only the Greiner Bio One¹⁵ utilises the dye-dilution approach. All of the other devices that assess volume rely on an indicator.

The material chosen for the construction of the pad is important. Care must be taken to use materials that will not irreversibly adsorb the drugs being sought. The cannabinoids are particularly vulnerable due to their tendency to adhere to different surfaces and materials^{3,4}. The choice of extraction buffer is therefore equally important.

A study comparing various collection devices found the general trend for recoveries was cocaine and benzoylecgonine greater than codeine, morphine, methamphetamine, and amphetamine greater than cannabinoids¹¹.

The buffers/preservatives used vary from device to device and they can cause problems with hyphenated MS techniques used to confirm drug levels. There have been complaints of ion suppression and/or enhancement in liquid chromatography-tandem mass spectrometry instruments and long-term contamination of columns and ion source in gas chromatography – mass spectrometry instruments⁷.

Problems with Point of Collection Testing (POCT) devices

Devices that perform the drug testing at the roadside utilise lateral flow immuno-chromatography (the test sample flows along a solid substrate via capillary action). Depending on the type of assay used, a positive result will be indicated by either the presence or absence of a coloured band in a set position in a viewing window of the device. Reading these devices in poor light/weather conditions can be problematic and electronic readers are becoming more prevalent. These devices include a control line to monitor correct movement of fluid through the device.

Sample Transport

Any positive result produced by a point of collection device immunoassay must be confirmed by a technique capable of unambiguously identifying individual compounds, typically performed in a laboratory some distance away. The transport delay introduces the possibility of sample degradation that will cause inaccuracies. Stability studies of drugs in oral fluid plus preserving buffer have been conducted for two of the kits currently available. ORALVEQ¹⁸, an EQA scheme for oral fluid, organised by the Institut Municipal d'Investigacio Medica, Barcelona, Spain and the Department of Therapeutic Research and Medicines Evaluation of the Istituto Superiore di Sanita, Rome, Italy prepared two different samples using the Cozart® drug detection system and Intercept® oral fluid collection devices. In a sample including spiked 6-monoacetyl morphine (6-MAM) and cocaine, the study⁵ found that, for the Cozart® and Intercept® devices, respectively, 11.8 % and 8.8% of the 6-MAM had degraded to morphine and 40.8 % and 26.2% cocaine had hydrolysed to benzoylecgonine. In a separate study of drug stability in spiked oral fluid (carried out by the same institutions) it was found that the addition of citrate buffer (pH 4) and sodium azide (0.1 %) prevented this type of degradation for up to 7 days at 25°C and 37°C, and up to 2 months at 4°C and -20°C⁶.

The role of External Quality Assessment (EQA)

There is a clear need for EQA of drug detection and measurement in oral fluid as a result of the diversity of technologies in use. This need has grown in recent years with routine testing becoming ever more prevalent at the roadside, during pre-employment screening or post-incident at work. Indeed, the framework of international quality standards for analytical laboratories requires involvement in such a scheme⁸ with assessment of

laboratory performance through EQA being an important element of a complete quality system^{9, 13, 20}.

One such scheme was commissioned by Altrix Healthcare (subsequently merged into the Concateno group) and run by Cardiff Bioanalytical Services Ltd¹⁰. This scheme was offered to laboratories in the Altrix group using the Intercept® oral fluid collection device (Orasure Technologies, Inc., Bethlehem, PA, U.S.A.) followed by analysis using an immunoassay technique from the same organisation. The scheme uncovered a lack of sensitivity as the major source of error where the immunoassays failed to achieve their specified cut-offs.

The variety of available collection devices (see Table 1) poses a problem when it comes to designing a more general EQA scheme for oral fluid analyses. The drugs found in a sample are not purely dependent on what drugs are in the subject's system. When an assay fails to detect a drug that is present, i.e. returns a false negative result, is that the failure of the assay or of the collection device? This issue can be addressed by EQA. In the first of two UKNEQAS (United Kingdom External Quality Assessment Service) pilot surveys performed by Cardiff Bioanalytical Services in an attempt to gauge the needs of would-be participants in an oral fluid EQA scheme, the issue of recovery of drugs from collection devices was investigated. Comparison of results for non-extracted samples with those from samples extracted by the laboratory using Cozart oral swab, Greiner Bio-One, Intercept, OraLab6, Quantisal, Salivette and Statsure collection devices showed no significant loss of performance in detection rate nor in quantitative measurements for delta-9-tetrahydrocannabinol (130% recovery), cocaine metabolite (103%), 6-MAM (112%) and morphine (87%). There thus appeared to be no great issue with current collection devices (the over recovery of delta-9-tetrahydrocannabinol was probably an artefact of the losses incurred during laboratory preparation of the more dilute non-extracted sample).

A second issue that is well addressed by EQA schemes is analytical performance. This is gauged as true positive or true negative results versus a defined cut-off. To date, no internationally recognised set of cut-offs have been defined. SAMSHA (Substance Abuse and Mental Health Services Administration) has draft suggestions out for consultation¹³ and the DRUID/ROSITA programs worked to their own defined levels. The SAMSHA guidelines are applicable to work place drug testing and some regard these cut-offs a little high for drug driving. The UKNEQAS pilot surveys involving 21 laboratories in the clinical, workplace testing, forensic, research and horse racing sectors have identified the following median values to be in use.

Relative to these median values, the main source of false negative reports in the UKNEQAS surveys derived from under performance by immunoassay products. Four screening laboratories reported buprenorphine as 'not found' in positive samples. The number of false negative reports was amfetamine 22%, benzoylcegonine 10%, nordiazepam 11%, dihydrocodeine 19% and buprenorphine 67%. A further 38% of laboratories missed dihydrocodeine as it fell outside the fixed range of analytes on which they reported.

The third area of strength of EQA surveys is their ability to investigate and illustrate problems in data interpretation and hence provide educational support. There is still unfortunately a need to continually reinforce the mantra that immunoassay positive results need confirmation. This objective will underpin the oral fluid EQA scheme being launched by UKNEQAS in June 2009. The need can be demonstrated by incorrect interpretation of positive group test results produced by common over the counter and

prescribed medications. The cross reactivity of codeine with opiate assays is a good instance which has been interpreted as heroin abuse.

Matthew Gist (heathcontrol@btinternet.com), Deputy Laboratory Manager for Cardiff Bioanalytical Services Ltd., Cardiff, Wales, UK.

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20. Table data drawn from information available on the kit manufacturer's websites (accessed: April 2009)

Table 1: Oral Fluid / Saliva Collection Devices²⁰

Collection Device	Company	Method of collection	Control of saliva volume	Buffer / diluent	Stimulated Collection	POCT capability
Cozart® oral swab	Cozart, Abingdon, Oxfordshire. UK	Absorbent swab	Yes	Yes	No	Yes, with Rapiscan or DDS device.
Intercept® device	Orasure, Bethlehem, PA. USA	Salt impregnated pad	No	Yes	Possibly, Osmotic pressure claimed but citric acid present on pad	No
Drugwipe	SecureTec, Brunnthal, Germany	Absorbent pad	Yes	No	No	Yes
Greiner Bio-One	Greiner Bio-One GmbH, Kremsmünster, Austria	Saliva extraction solution (mouth rinse)	Yes	Yes	No	No
Saliva Twist Device Drug Test	Surescreen Diagnostics, Derby. UK	Sponge swab	No	Yes	No	Yes
Oralab 6	Varian, Palo Alto, CA. USA	Absorbent pad	Yes	No	No	No
Oraline	Sun Biomedical Laboratories, Blackwood, NJ. USA	Collection cup	Yes	No	No	Yes
OralStat	American Biomedica Corporation, Kinderhook, NY. USA	Sponge swab	No	Yes	No	Yes
Oratect	Branan Medical Corporation, Irvine, CA. USA	Absorbent pad	Yes	No	No	Yes
VerOFy	Oasis Diagnostics, Vancouver, WA. USA	Absorbent pad	Yes	No	No	Yes
SalivaScreen	Ulti med products GmbH, Ahrensburg, Germany	Absorbent pad.	Yes	Yes	Yes, acidic available & mechanical	Yes
Smartclip Multidrug	EnviteC-Wismar GmbH, Wismar, Germany	Absorbent pad	No	Yes	Yes, mechanical	Yes
Quantisal	Immunalysis, Pomona, CA. USA	Absorbent pad	Yes	Yes	No	No
Salivette	Sarstedt, Nümbrecht, Germany	Cotton wool or polyester swab	No	No	Yes, acidic available & mechanical	No

Table 2: Thresholds to be adopted by the UKNEQAS for Drugs in Oral Fluid

Screening tests	µg/L	Single analytes	µg/L
Amfetamine group	35	Amfetamine	20
		Methyl-amfetamine	15
		MDMA / MDA / MDEA	15
Barbiturate group	50	Specific barbiturate	5
Cannabinoid group	4	Delta-9-THC	1
Cocaine metabolites	20	Cocaine	5
		Benzoylecgonine	6
Benzodiazepine group	15	Specific benzodiazepine	3
Methadone or metabolites	25	Methadone	9
		EDDP	12
Propoxyphene or metabolites	22.5	Propoxyphene or metabolite	35
Opiate group	40	Morphine	12.5
		6-monoacetylmorphine	4
		Codeine	15
		Dihydrocodeine	10
Buprenorphine or metabolites	1	Buprenorphine or metabolites	1
Phencyclidine	6	Phencyclidine	7.5
LSD or metabolites	0.6	LSD or metabolites	1