

Analytical Evaluation of a New Oral Fluid Sample Drugs of Abuse Diagnostic System

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Background

Substance abuse and addictive behaviour have negative consequences both for the individual and for those around him. The main reason for conducting substance screening is to detect and identify any substance abuse. Contrary to widespread opinion, the majority of drug addicts do not fit the classic picture of a junkie or drunk. If the facts and figures related to the prevalence of substance abuse in the population as a whole are examined and viewed in the context of the different occupations and branches of industry in which professional drivers, for example, who have been found to be abusing substances such as alcohol, prescription medication and/or illegal drugs are active, the conclusion is that substance abuse is a problem which now affects all public-sector and privately-run companies and organizations [1]. Occasional drug users pursue the same goals and have the same ambitions as the rest of society, and in no way believe that their performance or motivation would be impaired by their “habit” – quite the contrary. Such users are to be found in all strata of society and, potentially, in any position.

For on-site testing, e.g. roadside testing, the desire to perform a drug test has been hampered by the problems of collecting an adequate test specimen. As a potential alternative to urine screening, oral fluids can be tested to reveal the presence of pharmacologically active drugs in an individual at the time of testing. Significant correlation has been found between oral fluid concentrations of drugs of abuse and behavioural and physiological effects. Results indicated that oral fluid screening can provide valuable diagnostic information in various situations, including testing at the roadside [2, 3, and 4].

Objectives

Oral fluid drug screening is suitable as a diagnostic method of obtaining at least “quick” preliminary information, while supporting workflow and operational process organization during on-site testing, e.g. at the roadside, in the workplace, or in criminal justice and medical applications [5, 6].

The new Dräger DrugTest 5000 concept comprises a rapid on-site immunoassay (IA), intended for use with an opto-electronic analyser for the qualitative detection of substance abuse such as cocaine metabolites, opiates, amphetamines, methamphetamine, benzodiazepines and, specifically, Δ^9 THC in oral fluid samples.

The “analyser” is a device for detecting drugs in oral fluid samples collected with the Dräger DrugTest 5000 test kit. The measurement is based upon an optical evaluation of the immunochemical test strips contained in the test kit. Test results are displayed immediately after finishing the analysis and can be stored in the analyser, printed with the Dräger Mobile Printer or transferred to a PC via the integrated USB interface. The system is suited to stationary and mobile use.

The Dräger DrugTest 5000 test kit combines a sampling system, immunochemical assays and a test cassette in one “multitask unit”, minimizing user interaction and increasing overall system performance. The assays are based on the immunoassay principle of competitive inhibition and are designed for the following cut-off concentrations in oral fluid samples:

Dräger DrugTest® 5000 Cut-off Concentrations

	Drug	Calibrator	ng/mL
COC	Cocaine	Cocaine	20
OPI	Opiates	Morphine	20
BENZO	Benzodiazepines	Diazepam	15
THC	Delta-9-Tetrahydrocannabinol	Delta-9-THC	25
AMP	Amphetamine	D-Amphetamine	50
MAMP	Methamphetamine	D-Methamphetamine	35

Method

The test kit is designed to be used for samples collected with the oral fluid collector. No special treatment of the specimen is required. Oral fluid is collected by direct absorption into the porous collector, which forms an integral part of the test cassette. After sampling is completed, the analysis is initiated by placing both the test cassette and the buffer cartridge into the analyser. Thanks to the autonomous function of the analyser, the specimen is transferred into the test cassette, thereby initiating the test development.

To evaluate test kit performance, two samples were collected consecutively from each individual tested. One sample was collected and analysed using the system described in this paper; the second was collected using a separate device (“DCD 5000”). After collection, the samples were prepared for storage and transport and sent to a laboratory for confirmation analysis.

On-site drug test

The DrugTest analyser is ready for immediate use, requiring no assembly or installation before it is started up and placed onto a plane, solid and horizontal base. Optional accessories such as an external keyboard, printer or barcode scanner can be connected if required.

The analyser is switched on by pressing the “OK” button for approx. three seconds until an acoustic signal sounds and the start screen is displayed. After successful completion of the automatic self-check, the analyser displays readiness for use.

The oral fluid sample is collected using the Dräger DrugTest 5000 test kit.

For ten minutes prior to sampling, the donor should refrain from any intake of substances through the mouth or nose.

The protective cap of the test cassette from the oral fluid collector is removed and the test cassette handed to the donor (Figure 1).

The donor is requested to place the collector in his mouth between cheek and gum and to swab it from side to side, wiping the tissue inside the mouth (Figure 2).

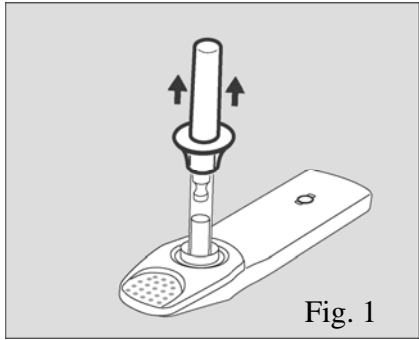


Fig. 1

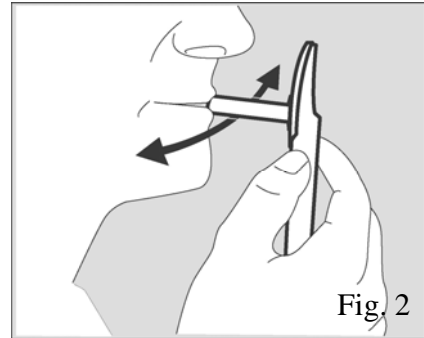


Fig. 2

This sampling process should be carefully observed by the operator. During collection of the sample, the sample adequacy indicator turns blue, indicating that a sufficient sample has been collected (Figure 3).

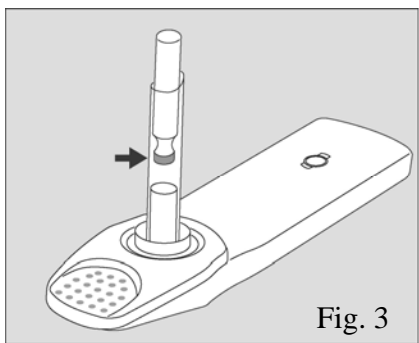


Fig. 3

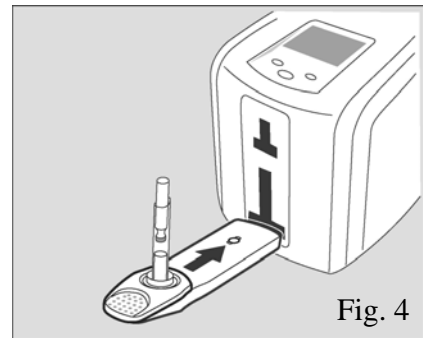


Fig. 4

Once sample adequacy has been indicated, the operator takes the test cassette back from the donor, opens the flap at the front of the analyser and inserts the cassette into the lower compartment until it engages audibly (Figure 4).

The kit cartridge is then “clicked” into the upper compartment of the analyser until it engages audibly (Figure 5).

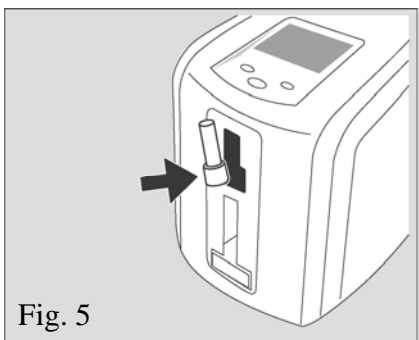


Fig. 5

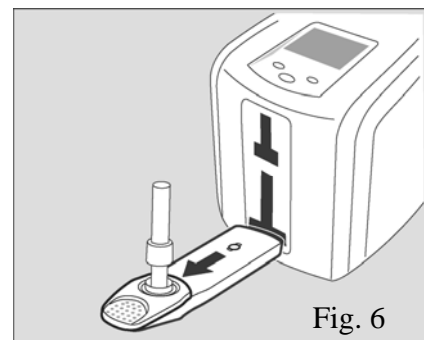


Fig. 6

The flap of the analyser is then closed, and the process starts and runs autonomously and automatically. The screen guides the operator through all subsequent timing and operational steps. A status bar on the display shows the progress of the evaluation process.

The evaluation results are displayed about five minutes after the start of the process. To ensure a high degree of $\Delta 9$ THC sensitivity, an additional incubation step is required for this parameter, and the result is displayed about ten minutes after the start.

Confirmation analysis

Verification of on-site results was obtained by laboratory analysis of a second sample from each individual tested.

After collection of an individual's sample using the system described herein, a second sample was collected using the DCD5000. This collector resembles the porous collector of the DDT5000 but does not have the immunoassay component, and collects approx. 380 μ l of oral fluid.

The second collector was immediately centrifuged at 1600 RCF using an Eppendorf MiniSpin centrifuge. The then dry collector was loaded with 450 μ l of methanol, allowed to incubate for five minutes, and centrifuged again. Both eluates were merged and stored at 4-8 °C for transportation.

At the laboratory, samples were analysed by means of GC/MS (Agilent GC 6890 / MSD 5973N) using deuterated drug-analogues as internal standards. Samples were split and underwent different extraction procedures depending on target compounds: liquid-liquid extraction with n-butyl chloride after addition of 0.1 N of NaOH for amphetamine-type stimulants, solid-phase extraction with Clean-Up[®] C18 cartridges (United Chemical Technologies, Inc) after addition of diluted acetic acid for $\Delta 9$ THC, and solid-phase extraction with Clean Screen[®] DAU cartridges (United Chemical Technologies, Inc) after addition of a pH 6 buffer solution for cocaine metabolites and opiates. Prior to analysis, the extracted dry residues were either acetylated with acetic anhydride pyridine mixture (amphetamine group) or silylated using MSTFA (other drugs). Verification of benzodiazepine* results was obtained using a commercially-available Benzo-ELISA-kit (target compound: Oxazepam).

Results and conclusions

Collection parameters (sampling time and sample quantity) were evaluated by collecting 117 individual samples from patients in drug treatment centres.

This first evaluation showed a median sampling time of 64 seconds and a median sample volume of 318 mg of oral fluid (CV: 16%).

The POCT-assay sensitivity, specificity and accuracy were defined by analysing and evaluating oral fluid specimens collected with the new device from up to 503 individual patients.

The following table summarizes the analytical performance as compared to the GCMS data and the BNZO verification:

Individuals screened	POCT - IA	Prevalence [%]	Cut-off [ng/mL]	Sensitivity [%]	Specificity [%]	Accuracy [%]
503	COC	4.5	20	86	99	98
441	OPI	13	40	90	98	97
341	Δ 9THC	20	25	76	99	93
155	AMP	0	50	n/a	99	99
155	METAMP	0	25	n/a	99	99
194	*BENZO	30	15	74	98	97

The results achieved exceed the target values for, for example, collection precision, sampling time, and assay sensitivity, specificity and accuracy, as set by state-of-the-art oral fluid DOA screening devices. The 74% sensitivity achieved for BENZO is directly related to the broad spectrum of this drug consumed in the screened population; the cross-reactivity of the evaluated POCT-test and the commercial ELISA varied. There is no prevalence of AMP and METAMP in the screened population.

The combination of sampling-device and test-development cartridge (test kit) enhances ease of use by indicating sample adequacy, preventing operation errors and substantially increasing the overall precision of the system measurement, thereby addressing one of the major failings so far reported in such diagnostic tools. Further sample confirmation capability and sample batch vs. continuous measurement process will guarantee a high degree of flexibility in use.

The analyser concept – a “lab for field use” – functions fully autonomously as regards power supply, intrinsic quality control, sample processing and process-driving steps, timing, temperature control (homeostatic testing conditions), data processing and storage. The “encasing” of essential process steps guarantees measurement capability even under extreme environmental conditions, taking into account that this is a mobile measurement system – suitable for operation wherever and whenever.

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