Journal of Substance Abuse Treatment 48 (2015) 132–136

Contents lists available at ScienceDirect

Journal of Substance Abuse Treatment

Clinical trial of a new technique for drugs of abuse testing: A new possible sampling technique☆

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ARTICLE INFO

Article history:
Received 31 March 2014
Received in revised form 7 August 2014
Accepted 16 September 2014

Keywords:
Drug testing
Exhaled breath
Illicit drugs
Self-report
Cannabis

ABSTRACT

Exhaled breath has recently been proposed as a matrix for drug testing. This study aims to further explore, develop and validate exhaled breath as a safe and effective non-invasive method for drug testing in a clinical setting. Self-reported drug use was recorded and drug testing was performed by mass spectrometry and immunochemical methods using breath, plasma and urine samples from 45 individuals voluntarily seeking treatment for recreational drug use. Cannabis was the most prevalent drug detected by any method. Urine sampling detected most cases. The exhaled breath technique was less sensitive (73%) than plasma analysis for detection of cannabis uses but captures a more recent drug intake than both plasma and urine. Exhaled breath was the preferred specimen to donate according to interview data of the participants. Testing illicit drugs with the exhaled breath sampling technique is a sufficient, non-invasive and safe alternative and complement to plasma and/or urine sampling.

1. Introduction

In the treatment of patients with dependency disorders or misuse of illicit drugs, the systematic monitoring of drug use by laboratory testing is a common practice. The American Society of Addiction Medicine notes that drug testing can be a component of the plan of care during treatment for a substance-related disorder (Public policy statement on drug testing as a component of addiction treatment and monitoring programs and in other clinical settings, 2003). Lennox, Dennis, Ives, and White, (2006) have demonstrated the potential of combining urine drug testing with self-reporting measures in order to obtain the best information for monitoring and predicting drug use. In a recently published review and meta-analysis covering 29 studies, it was argued that timeline follow-back (TLFB) has good validity in identifying illegal drug consumption (Hjorthoj, Hjorthoj, & Nordentoft, 2012).

Combining biological markers with self-reporting can also be justified by the possibility that individuals may both exaggerate and minimize their problems. A well-known concept in psychological treatment research is the "hello–goodbye pattern" which means that people embarking on treatment are in certain cases more inclined to exaggerate their problem in order to justify a need for help. At the end of the treatment it is more likely that problems will be minimized as more support is not desired (Hathaway, 1948).

It is, however, a well-known fact that the risk of under-reporting increases if individuals suspect that a correct response will lead to negative ramifications (Midanik, 1982). Similarly, it is a common clinical occurrence that people occasionally try to manipulate biological samples as a positive test may lead to unwanted consequences.

Urine drug testing is the predominant form of drug testing in the treatment of dependency disorders or misuse of illicit drugs. However, urine sampling as a procedure is sometimes time-consuming for health care staff and may be perceived as distressing by the patients. There is also a well-known occurrence of adulteration during the sampling process, such as providing someone else's urine or water loading. Also, a positive urine analysis cannot, with certainty, be said to reflect a more recent consumption. This applies, for example, when the consumption of cannabis is in question since following repeated cannabis use, tetrahydrocannabinol (THC) is known to accumulate in the body leading to a slow elimination of the metabolite tetrahydrocannabinol carboxylic acid (THCCOOH) in urine. This leads to a situation where it is difficult to confirm discontinued use or detect relapse in cannabis use using urine drug testing.

In recent times, the possibility of drug testing using exhaled breath has been demonstrated and has created an option for a much different sampling procedure as compared to urine. It has been previously established that non-volatile substances are part of normal human breath. Human breath contains aerosol particles, including both lipids and peptides of endogenous origin that are formed from the respiratory tract lining fluid during normal breathing (Almstrand et al., 2009, 2010; Papineni & Rosenthal, 1997). The sampling device has developed into a more user-friendly device since the first detection of amphetamine in exhaled breath (Beck, Leine, Palmskog, & Franck, 2010; Beck, Sandqvist, Dubbelboer, & Franck, 2011; Beck, Sandqvist, Eriksen, Franck, & Palmskog, 2010, 2011; Beck, Sandqvist, & Franck, 2011; Beck, Stephanson, Sandqvist, & Franck, 2013; Beck et al., 2011). These promising findings triggered the present

☆ Conflict of interest: OB holds patents related to the sampling device.
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http://dx.doi.org/10.1016/j.jsat.2014.09.003
0740-5472/

study to further explore exhaled breath as a possible matrix for abused drug testing.

The present study explored the potential of exhaled breath analysis as a feasible sampling technique in a population of young adults seeking treatment for drug abuse at an open psychiatric care facility. We compared exhaled breath with data from plasma and urine analysis and from self-reports using TLFB and also compared how complementary biological tests are to self-reports regarding recent intake.

2. Materials and methods

2.1. Participants

Adolescents and young adults seeking treatment for drug abuse or drug related social problems participated in the study. The patients were recruited from two psychiatric outpatient units within "Maria Ungdom" in Stockholm Center for Dependency Disorders (Beroendecentrum Stockholm). These outpatient units treat adolescents aged up to 18 years and young adults aged 18–26, with a pattern of primarily recreational drug use. During a period of 3 months from January to April 2013, 45 patients (41 males, age range 16–31) voluntarily seeking medical treatment and/or counseling for drug abuse were included in the study. Patients were allowed to provide repeated samples with a minimum of at least 7 days between sampling. Four patients provided two samples each resulting in a total of 49 study samples. All subjects or in the case of under-aged participants, parents or guardians gave informed consent for participation.

2.2. Clinical procedures

During the study period, all patients visiting the two psychiatric outpatient units with a confirmed or suspected drug intake within the last 7 days were asked by their attending healthcare professional (physician, nurse or therapist) to attend the study. The participants were then interviewed by a research nurse not affiliated to the clinic who assessed drug intake (cannabis, central stimulants, opiates and/or narcotic drugs) within the last 7 days. After this interview (based on a modified version of TLFB with 1 week windows), breath, plasma and monitored urine samples were collected. Patients then anonymously filled out a questionnaire on how they had perceived each sampling technique according to a general experience and possibilities to manipulate the test (do not agree, agree, and fully agree). Each participant who attended the study was informed of their right to retract from the study at any chosen time and was, regardless of complete participation, reimbursed with a food coupon worth 100 SEK. Ethical approval was obtained from the Stockholm Regional Ethics Review Board (no. 2008/1347-31).

2.3. Biological samples

The sampling of breath and blood was performed only as part of the study and results were not used in the clinical work. The patients were not informed of the results.

A sampling procedure for breath using a commercial sampling device was employed (SensAbues AB, Huddinge, Sweden). Micro-particles present in the exhaled breath were selectively collected by letting the exhaled breath during normal breathing pass through a mouth-piece constructed to only allow micro-particles to pass through. The micro-particles passing the mouth-piece were collected on a polymer filter inside the device (Beck et al., 2013). The sampling procedure was standardized by filling of a plastic bag and collecting about 20 L of exhaled breath (time required was approximately 2–3 minutes). Following sampling, the device was sealed with plugs and stored at −20 °C (maximal storage time was 1 month).

Capillary blood was collected by finger-prick and EDTA-plasma was prepared by centrifugation and stored in plastic test-tubes at −80 °C. Urine was collected according to a clinical standard supervised procedure, where the research nurse supervised the process through a one-way transparent mirror. Urine samples were stored in plastic test-tubes at −80 °C. Breath and urine samples were collected in all cases, but plasma sampling failed in three cases.

2.4. Chemical analysis

Following storage, the breath collection devices were analysed according to a previously published procedure using liquid chromatography-tandem mass spectrometry (LC-MS/MS) in selected reaction monitoring mode (Beck et al., 2013). In brief, the devices were put on top of glass test-tubes and analytes were eluted from the filter with methanol. Following evaporation to dryness, the extract was reconstituted and subjected to mass spectrometric investigation. The following analytes were monitored: amphetamine, methamphetamine, THC, morphine, 6-acetylmorphine, cocaine, benzoylecgonine, diazepam, oxazepam, methadone, tramadol and buprenorphine.

The analyses of plasma were done with ultra-performance liquid chromatography-mass spectrometry methods following previously published procedures (Beck et al., 2013). Urine samples were screened by CEDIA immunoassay reagents and positive findings confirmed with ultra-performance liquid chromatography-mass spectrometry methods (Beck et al., 2013). In addition, urine creatinine concentrations were routinely measured. The use of the creatinine concentration and cannabis/creatinine ratio is a standard way to compensate urine drug concentration values for the variable urine dilution seen within and between individuals.

The LC-MS/MS system consisted of a Thermo Fisher Scientific TSQ Vantage triple quadropole mass spectrometer connected to a Dionex Ultima 3000 UHPLC. The liquid chromatography system is composed of an Ultimate 2000 SRD degasser, Ultimate 3000 RS binary solvent pump system, column oven and Ultimate 3000 RS autosampler. The softwares used were Chromeleon Xpress v. 3, TraceFinder Clinical Research v. 2.1 and Thermo TSQ Tune Master v. 2.3.0. Identifications were based on correct relative retention time (±0.5%) and product ion ratio (±20%).

3. Results

3.1. Analytical findings

The dominant analytical finding was residua of cannabis use (Table 1). Morphine was detected only in a breath sample from one case. Cocaine and benzoylecgonine were detected in five cases in breath and four cases in plasma, while benzoylecgonine was detected in three cases in urine. In the latter three cases, cocaine and benzoylecgonine were detected also in breath and plasma. Tramadol was detected in two cases in breath but only in one of these also in plasma and urine. Oxazepam was detected in three cases in plasma and urine but not in breath. Finally, amphetamine was detected only in plasma in three cases.

Among the 49 study samples, 35 analytical findings of the THC metabolite tetrahydrocannabinol carboxylic acid (THCCOOH) were made in urine. Of these 35 THCCOOH positive urine samples, 11 were also positive for THC in the exhaled breath samples, while 15 were positive for THC in plasma. In the 20 cases with negative plasma samples, a significantly lower THCCOOH/creatinine urine value was observed (median: 1.5, 95% CI; 0.83–3.52) as compared to the 15 cases with THC detected in plasma (median: 26.95, 95% CI; 7.24–33.4) (Fig. 1). The non-parametric Mann–Whitney test for independent samples was used to statistically verify the difference between the two groups ($p = 0.0001$).

THC was detected in plasma in all cases with a positive THC finding in breath. In four of the cases with negative breath samples THC were detected in plasma indicating a shorter detection time window in exhaled breath as compared with plasma. Fig. 2 illustrates that a

Table 1
Summary of analytical results.

	Breath		Plasma		Urine	
	Detected samples - number	Concentration - range, pg/filter	Detected samples - number	Concentration - range, ng/ml	Detected samples - number	Concentration - range, ng/ml
Cannabis	11	3.7–1170	14	0.29–8.64	35	10–1180
Opiates	1	24	–	–	–	–
Tramadol	2	12–53	1	0.51	1	20500 O-DM-tramadol 8600
Cocaine	5	12–455	4	1.0–4.2	–	
Benzoylecgonine	5	5–55	4	0.7–50	3	60–380
Oxazepam	–	–	3	38–60	3	1100–6400
Amphetamine	–	–	3	0.1–0.2	–	

positive breath sample corresponds well with presence of THC in plasma and that a negative breath sample is similarly associated with a low or no THC plasma concentration. The non-parametric Mann–Whitney test for independent samples was used to statistically verify the difference between the two groups ($p = 0.0001$).

3.2. Interview data

In total, 42 responses were obtained regarding the anonymous questionnaires. The majority of the patients ($n = 32$) had, prior to this study, been exposed to multiple sampling situations. Six patients had been subjected to drug testing during the last year and four patients had not.

As shown in Fig. 3, drug testing procedures in general were well tolerated in this population. An overall observation was that approximately two thirds of the participants were generally positive to the sampling process, regardless of sampling technique. Breath samples were considered preferred over plasma and urine sampling in the majority of cases (72%). Most reported (85%) that drug testing does not lead to one becoming more interested in continuing to use drugs, and the majority (59%) considered drug testing of assistance to stay drug free. However, nearly half (44%) emphasized that drug testing is an unnecessary control for their abstinence.

When asked which sampling procedure was preferred, 72% answered breath, 15% capillary blood and 13% urine. In the questionnaire, patients were also asked to express in words why they preferred one sampling procedure over another. In two cases where exhaled breath was not preferred the patients claimed to suffer from asthma or poor endurance, thus finding the breathing procedure difficult per se.

3.3. Comparison of drug testing and self-report

Urine analyses in individuals reporting cannabis use at least once a week within the last week, showed a specificity of 0.71 and a sensitivity of 0.95 as compared with self-report. The breath and plasma samples showed lower sensitivity (0.48 resp 0.67) but a specificity and a positive predictive value of 1.0. In eight of 26 cases where patients had reported no drug intake within the last 7 days, the breath as well as the plasma samples were negative but the urine samples were positive. Five of these eight cases had THCCOOH/creatinine values between 1.3 and 3.5 indicating either a more recent intake than reported or, since both plasma and breath samples were negative, a "carry-over" effect of accumulated THC from previous heavy and/or prolonged intake. Despite no self-reported intake of cocaine among the participants, five positive analytic findings of cocaine in exhaled breath were made. Of these five cases only three were detectable in urine.

Table 2 shows that both urine and plasma toxicology provide excellent sensitivity and negative predictive values compared to self-reported recent cannabis use (i.e. last 1–2 days). However, urine toxicology might, according to our results, also risk producing "false" positive samples due to longer detection time (specificity 0.55). The exhaled breath technique with a specificity of 0.91 and plasma with a specificity of 0.86, provided a more accurate correlation in relation to self-reported recent intake compared to urine analysis.

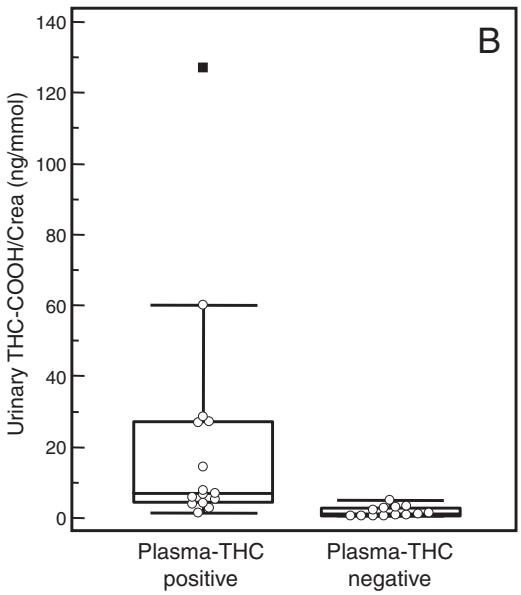

Fig. 1. The THCA/creatinine ratio (ng/mmol) in cases with and without detected THC in plasma. The two groups were significantly different according to Mann–Whitney test ($p = 0.0001$).

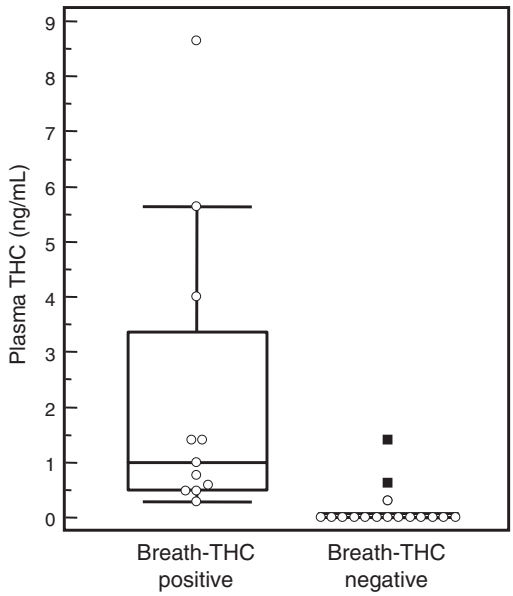

Fig. 2. The concentration of THC in plasma in cases with and without detected THC in breath. The two groups were significantly different according to Mann–Whitney test ($p = 0.0001$).

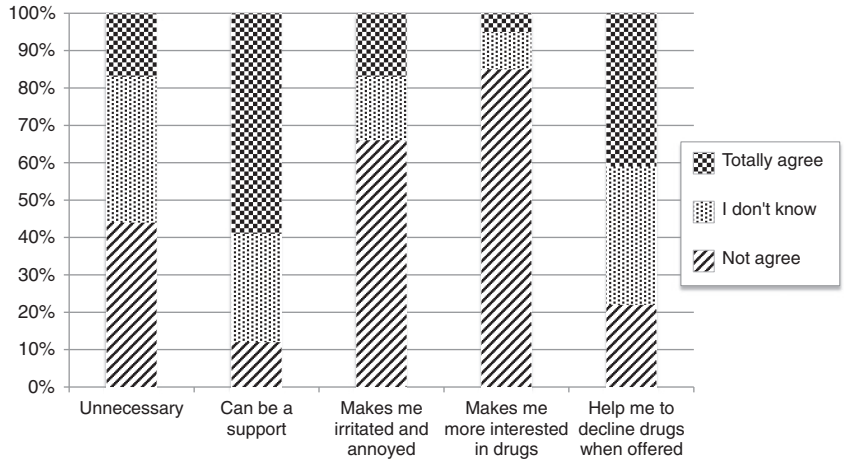

Fig. 3. Patients ($n = 41$) were given the opportunity to reflect on the following statements. Drug testing is; unnecessary control for my abstinence, may be of assistance to me through of abstinence; makes me annoyed and upset; important to me so that I can demonstrate abstinence for others; leads me to become more interested in continuing and helps me to say no to peers to continue using.

3.4. Additional observation

One male participant, despite providing a negative urine sample and self-report, had breath and plasma samples strongly positive for cocaine, indicating a high cocaine intake within the last 24 hours. One hypothesis is manipulation of the urine sample since the plasma and breath sampling procedure by definition do not allow manipulation.

4. Discussion

A main finding is that breath drug testing is well tolerated among patients and detects clinically relevant drug intake. In the studied patient group cannabis use was most common. All samples positive for THC in exhaled breath were also positive in blood, demonstrating congruence between exhaled breath and plasma analytical findings. Our results show that a positive breath sample reflects a more recent drug intake (i.e. within 24 hours) than urine testing. Urine test results had the best overall correlation with self-reports and also the best overall detection rate. However, when assessing data from patients with self-reported recent drug intake (1–2 days), 27 positive analytic findings of THC were made in urine in relation to only 12 positive self-reports (specificity 0.55). In contrast, 7 out of 10 patients with THC positive samples in exhaled breath had also reported recent THC intake (specificity 0.91). The explanation for this is most probably a longer detection time in urine. The exhaled breath technique is as accurate as plasma and a most feasible alternative when a recent drug intake needs to be investigated.

Interestingly enough, five individuals did report a recent drug intake while providing negative urine samples. This might be due to recall bias or a phenomenon consistent with Midanik's hypothesis (Midanik, 1982) that patients sometimes exaggerate their drug intake in order to justify a need for therapeutic interventions.

A narrower window of detection time is valuable for several reasons and has several practical clinical implications. It is of importance in the therapeutic process for the alliance between patient and healthcare professional in, for example, the methadone substitution programs and for ADHD patients with a history of substance use disorders. In these patients, where central stimulants are prescribed, any recent illicit drug intake must first be excluded. It might also be of use for addiction treatment or other in-patient care facilities to be able to test clients for illicit drug intake before readmission following a weekend's leave of absence. The breath test method could also provide a more effective alternative to urine THC testing in an emergency room setting, when time of ingestion is critical in trying to establish the cause of acutely intoxicated behavior. The long detection time of THC in urine makes it hard to exclude cannabinoids as cause of the observed symptoms and the breath technique would eliminate the need to rely on patient self-reporting regarding the time frame of THC ingestion, thereby avoiding a potentially trust-undermining physician–patient interaction.

The importance and potential of using the combined information of laboratory based testing and self-reported information were pointed out by the work of Lennox et al. (2006). It is important to carefully select relevant information and understand how best to synthesize and utilize it in an effective way. It was observed in this study that different

Table 2
Self-reported recently intake of cannabis (1–2 days) compared with laboratory analyzes measuring sensitivity, specificity, and the positive and negative predictive values.

		Self-report intake		Estimate (95% CI)			
		Yes	No	Sensitivity	Specificity	PPV	NPV
Urine ($n = 45$)				1.00 (0.74–1.00)	0.55 (0.38–0.72)	0.44 (0.26–0.63)	1.00 (0.81–1.00)
	Pos	12	15				
	Neg	0	18				
	Tot	12	33				
Exhalation ($n = 45$)				0.58 (0.28–0.85)	0.91 (0.76–0.98)	0.70 (0.35–0.93)	0.86 (0.70–0.95)
	Pos	7	3				
	Neg	5	30				
	Tot	12	33				
Plasma ($n = 39$)[a]				0.73 (0.39–0.94)	0.86 (0.73–0.99)	0.67 (0.35–0.90)	0.89 (0.71–0.98)
	Pos	8	4				
	Neg	3	24				
	Tot	11	28				

[a] Six patients missing.

information can be obtained, not only by comparing laboratory data and self-report but also by comparing various laboratory markers. Further work is therefore warranted as to how apply drug testing by collecting exhaled breath, a far more convenient sampling technique, can be best applied in clinical treatment settings.

4.1. Strengths and limitations

This study has a number of key strengths. First, this is to our knowledge, the first study of its kind exploring exhaled breath as a matrix for testing drugs of abuse in a real world clinical setting. Second, since the trial was conducted in a controlled clinical setting, studying a population of primarily recreational drug users and/or individuals in an early phase of addiction, we maintained sufficient control over our study participants. Third, the sampling procedure was performed by an experienced research nurse not affiliated with the clinic or involved in treatment of the study subjects and specifically trained for these sampling procedures. Thus, the patients did not risk possible negative consequences related to the analytic findings or self-reports. Finally, the study compared three different matrices for drug testing and used methods offering reliable analytical results.

Our study may also be viewed in the context of some limitations. Since the questionnaire was anonymous we were unable to relate these questionnaire responses to outcomes on self-reported consumption and analytical results from the laboratory. Also, all patients were offered collection of all three samples, urine, blood and breath in the same sequence. If subjects had been randomly assigned to the different sampling methods, it cannot be excluded that the outcome, general experience and possibilities to manipulate the test, would have been different. The blood and breath testing results were not used in the therapeutic work and it is not known how patients would react to the feedback of those results. Also, the fact that exhaled breath was considered preferred over plasma and urine sampling in the majority of cases must be interpreted with some caution. The novelty of the sampling technique might influence how patients perceive and report experiences from the test situation.

In conclusion, testing illicit drugs with the exhaled breath sampling technique seems to be a promising, non-invasive and safe alternative and complement to plasma and urine sampling. Our results suggest that the exhaled breath technique is well tolerated and captures a more recent drug intake than both plasma and urine, making it a potential candidate for drug testing in a clinical setting as well as in a forensic environment. As for future implications, a situation where the exhaled breath technique provides more immediate results is an attractive possibility. Future clinical studies should continue to compare breath testing with other matrices, involve more patients, have better control over the sampling process and apply the results in clinical work. In addition, the potential benefit to the patient–staff relationship of avoiding supervised urine collection should be addressed.

Acknowledgments

We thank Inger Engman-Borg, Fatima Tajik and Eva-Charlotta Clasing for excellent clinical assistance. This work was supported by a grant from the Stockholm County Council.

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