MEDICOLEGAL ASPECTS OF CANNABIS AND ITS METABOLITES DETECTION WINDOW IN ORAL FLUID AND URINE

BY

Abd El-Aziz A. Ghanem, Amal A. El-Bakary and Mona S. El-Hamady

Forensic Medicine and Clinical Toxicology Department, Mansoura University, Egypt

ABSTRACT

Cannabis is one of the most widely used illicit drugs. Oral fluid substance use testing is increasing and has many advantages over urine testing especially for recent substance use diagnosis. The aim of this study was to establish the possibility of detection of recent cannabis smoking through oral fluid and urine testing and its medicolegal aspects. Forty subjects were asked to provide oral fluid and urine samples after a reported period of abstinence. They provided 167 oral fluid samples during abstinence over 5 min – 30 h and 206 urine samples during extended observed abstinence over 1 - 90 days. Cannabis was detected using One Step cannabinoid urine test. The first negative oral fluid sample was that obtained 22 – 30 h after abstinence while the first negative urine sample was detected after seven days of abstinence. Cannabis was detected in urine samples for up to 70 days. These data suggest that oral fluid can be used to discriminate recent from former cannabis use indicating that the user is under the effect of cannabis.

Keywords: *Cannabis, Oral fluid, Immunoassay, One Step cannabinoid urine test.*

INTRODUCTION

Cannabis is one of the oldest and most commonly abused substances in the world and its use is associated with physical and behavioral toxicity. The plant Cannabis Sativa contains more than 400 chemicals but the cannabinoid δ-9-tetrahydrocannabinol (THC) is the major psychoactive constituent (Fitzgerald et al., 2013). THC is metabolized to 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy-THC (THCCOOH) (Huestis, 2005). Cannabinol, a degradation product of THC oxidation, is approximately 10% as potent as THC (El-Sohly, 2002).

Oral fluid (OF) drug testing in workplace, pain management, drug treatment, and driving under the influence of drugs programs is increasing (Lee et al., 2012). In contrast to urine, the advantages include observable sample collection, difficulty to adulterate, and demonstration of recent drug use. However, many immunoassays for detecting THC in oral fluid do not have high reliability. This is mainly due to THC adsorption to the collection device,

which makes recovery from the device difficult, and the relatively high cutoff (Bosker and Huestis 2009; Verstraet, 2005).

As impairment may last for 8 hours post-inhalation of cannabis (Hollister, 1986), the use of oral fluid as a sample for cannabis detection is suitable in case of arrested drivers, accidents, follow up of abstinence and in work place testing. It is a new tool to improve traffic safety with rapid, easy road-side drug testing of drivers (Bosker and Huestis 2009).

The rapid test is used by Egyptian police in road accidents to diagnose cannabis and other drugs of abuse administration. This study aimed to establish the possibility of detection of recent cannabis smoking through oral fluid and urine testing and discuss its medicolegal aspects.

SUBJECTS & METHODS

Study design and samples collection:

This is a prospective non controlled clinical study for detection of cannabis and its metabolites in oral fluid and urine samples of chronic cannabis users attending clinical toxicology clinic seeking cannabis smoking stoppage. Samples were collected during monitored abstinence period (30 hours for oral fluid and 90 days for urine samples) from the last cannabis consumption.

Forty subjects with self reported recent cannabis (cigarette or Goza) smoking were included in this study after providing informed consent. Inclusion criteria were self-reported cannabis use with a minimum frequency of two or more times/ month during the 3 months prior to study entry and cannabinoid-positive urine sample. Participants' gender and age were recorded.

Oral fluid specimens were collected at 4 h, 6 h, 22 h, 24 h and 30 h after smoking. OF was collected by expectoration into polypropylene tubes. Participants were asked to spit into the tube until at least 3 ml OF were collected or for 5 min, whichever occurred first. Urine samples (10 ml) were collected from each participant at 0 d, 7 d, 15 d, 30 d, 60 d, 70 d and 90 days and examined immediately. Participants were instructed not to consume cannabis during the study period.

Analysis using Accu-Tell One Step THC urine test :

Accu-Tell One Step cannabinoid urine test (AccuBioTech Co. Ltd) is a rapid qualitative competitive binding immunoassay method for determination of THC and its metabolites in human urine. In this research, this method is tested for its application on oral fluid testing. It can detect many chemicals related to cannabis use as shown in the following table:

Compound	11- nor- Δ-8- THC- 9- COOH	11- nor- Δ -9- THC- 9- COOH	Δ -8- THC	Δ -9- THC	Cannabinol	11-OH- Δ -9- THC
Level of positive reaction	50 ng/ml	50 ng/ml	1800 ng/ml	2000 ng/ml	5000 ng/ml	800 ng/ml

According to manufacturer, three drops of the sample were dropped in the sample well of the test card. The test card is left in room temperature for 3 - 8 min. A positive control sample is used. One pink/purple band in the control region and no band in the test region mean that the level of THC is above the limits of sensitivity of the test indicating a positive result. Meanwhile two pink/purple bands; one for the control and the other for the sample; mean that the level of THC is below the sensitivity of the test denoting a negative result (Figure 1).

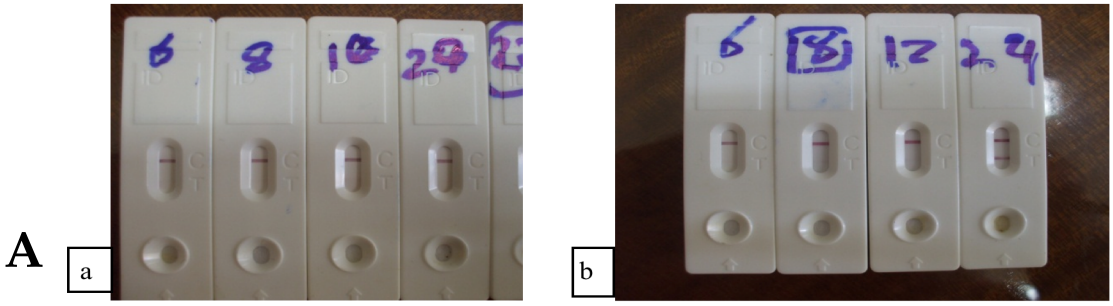

Figure (1) : Examples of test cards of Accu-Tell One Step THC urine test that were applied to oral fluid samples; a negative result (2 red lines) can be seen at 30 hours (a) and at 24 hours (b) after abstinence.

Statistical analysis:

Data were saved as an Excel file. Descriptive statistical analysis was performed using SPSS 17.0 for Windows software.

RESULTS

Forty participants (39 males and one female) aged between 18–40 years had provided 167 OF samples during monitored abstinence over 5 min – 30 h and 206 urine samples during extended observed abstinence over 1 - 90 days. Not all participants presented immediately after smoking, only 22 participants presented within the first 4 hours after abstinence.

Results of oral fluid analysis :

The first provided OF samples were THC positive. The first negative samples were obtained 22 h after abstinence in 3 out of 31 (9.7%) samples. Meanwhile at 24 h, 15 out of 35 (42.9%) samples were negative. All provided samples (19) were negative after 30 h (Figure 2).

Results of urine analysis:

At the beginning of the study, 40 patients shared in this research but only 9 continued to give urine samples to the end of the study that is 90 d. All samples (40 samples i.e. 100%) were THC positive at presentation. By the seventh day, 38 out of 39 (97%) samples were positive but by the fifteenth day, 34 out of 38 samples (89 %) were positive. However, by the thirty day, 25 out of 36 samples (69 %) were positive while only 19 out of 25 (76%) were positive by the sixty day. While, on the seventy day only 8 out of 19 (42%) were positive, none of the nine samples that were provided by ninety day were positive. The first negative urine sample was detected after seven days of abstinence (Figure 3).

DISCUSSION

The present study showed that the first negative oral fluid samples for THC were found at 22 – 30 h after abstinence. An important finding in the current work is that the last positive oral fluid result was detected at 12 -24 h. Similar results were demonstrated by Desrosiers and coworkers (2012) who detected THC in oral fluid samples for 22 h using DrugTest 5000 screening with 5 and 10 μg/L cutoff. However, THC could be detected in oral fluid samples for 48 h of abstinence, while THCCOOH was detected for up to 29 days when measured by 2-dimensional GC-MS (Lee et al., 2011). Lee et al. (2012) proposed that THC confirmation cutoff of ≥1 μg/L offers longer detection times.

Although the present study used a preliminary immunoassay method with a relatively high cutoff that may shorten the detection window, the aim was rapid diagnosis of recent THC use rather than the level. Besides this is the method used by Egyptian policemen for arrested drivers using urine samples as a preliminary test that will not differentiate recent from former use and will not exclude passive exposure.

A legal issue may arise for risk of positive oral fluid tests from passive cannabis smoke inhalation. Practically, this risk is limited to a period of approximately 3 h following exposure (Moore et al., 2011) and can be eliminated by identification of THCCOOH in OF that is not present in cannabis smoke (Dressler, 2000).

The high concentration of cannabis in oral fluids shortly after smoking was attributed to contamination of oral mucosa

(Huestis and Cone, 2004). Many factors can influence the level of THC in oral fluid as the method of sample collection e.g. THC concentrations were higher in samples obtained by spitting than samples collected with Statsure (Houwing et al., 2012). Two main limitations of saliva as a sample for drug analysis are apparent: the amount of matrix collected is smaller when compared to urine and the levels of drugs are higher in urine (Kintz et al., 2000). OF testing does have disadvantages as some drugs may reduce salivation, limiting sample volume (Verstraete, 2005).

For urine samples, THC detection was reduced from 100 % of cases at presentation to 42% of cases after 70 days of abstinence while all available samples were negative by 90 days Similar results were reported for different detection window durations. Goodwin et al. (2008) could detect urinary THCCOOH for 30 d while Ellis et al. (1985) for up to 67 d and Lafolie et al. (1991) for up to 93 d with a 20 ng/mL immunoassay cutoff.

An interesting finding in the present study is that the 1st negative urine sample was at 7 d, meanwhile time was reported to range from 1- 14 days by others (Goodwin et al., 2008). The last positive urine result ranged from 7 -70 days. Similar results were reported by Ellis et al. (1985) who showed that the last positive result ranged from 4–77 days. Cannabis was reported to have a long half-life in humans (67 days) (Huestis et al., 1992). Detection time is dependent on many factors e.g., drug dose, route of administration, state of hydration, rates of metabolism and excretion, cutoff concentrations, specificity and accuracy of the method used. It was concluded that the greater the creatinine corrected initial THCCOOH concentration, the greater the interval until the first negative and last positive specimens and the greater the window of drug detection (Goodwin et al., 2008).

Screening test using an immunoassay is suitable for rapid diagnosis. The positive samples must proceed to confirmatory analysis e.g. liquid chromatography-tandem mass spectrometry especially in legal consequences. Most countries have legislation that covers driving under the influence of alcohol and/or drugs. Some countries including Egypt have introduced zero-tolerance laws, which prohibit the operation of a motor vehicle while an illicit drug or its metabolite is present in the body, whether or not impairment is manifested (Lillsunde and Gunnar, 2005). It is important in legal consequences to exclude passive exposure. Withdrawal of the driving license due to positive urine test for cannabis even if impairment is not present does not seem scientifically sound. Positive urine test may indicate residual drug excretion. Therefore, conventional drug testing with urine cannot accurately

detect usage in the first few hours after use (impairment period), making saliva superior for post-accident testing. As Saugy et al. (2006) suggested, interpretation of the analytical data is very important to distinguish between active consumers of cannabis and those who may have been passively exposed to cannabis smoke especially in legal consequences.

One limitation of the present study was that the exact times and amounts of last cannabis smoking were not fixed. However, this limitation does not change the conclusion that in chronic cannabis smokers, THC can be detected in OF for at least 24 h when measured by One Step THC screening test. Another limitation is that participants left the laboratory after 8 h and were instructed not to smoke cannabis, but they could not be monitored.

CONCLUSION

From the present data, it can be concluded that THC can be detected in oral fluid for 24 hours using One Step THC urine test while it can be detected in urine for up to 70 days using the immunoassay method. These data suggest that oral fluid can be used for detection of recent cannabis use in drug testing for road-side testing in arrested drivers as well as during accidents and drug treatment programs to evaluate abstinence and relapse. So, we recommend the use of saliva- cannabis test instead of urine test for good preliminary differentiation between recent (under the effect of cannabis) and former use, and also reconfirming all positive results with HPLC or GC/MS.

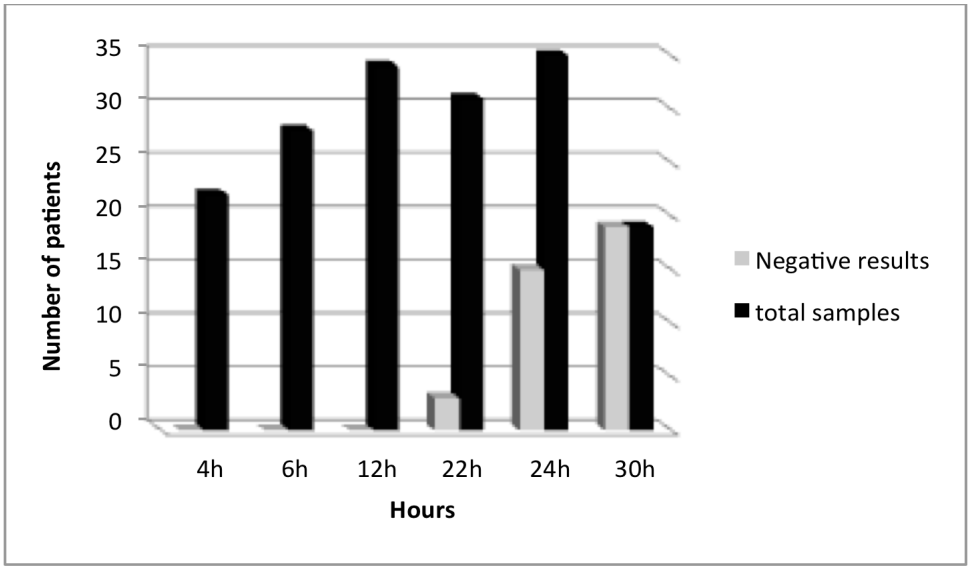

Figure (2) : A diagram showing the frequency of oral fluid samples' negative results over 30 hours of abstinence.

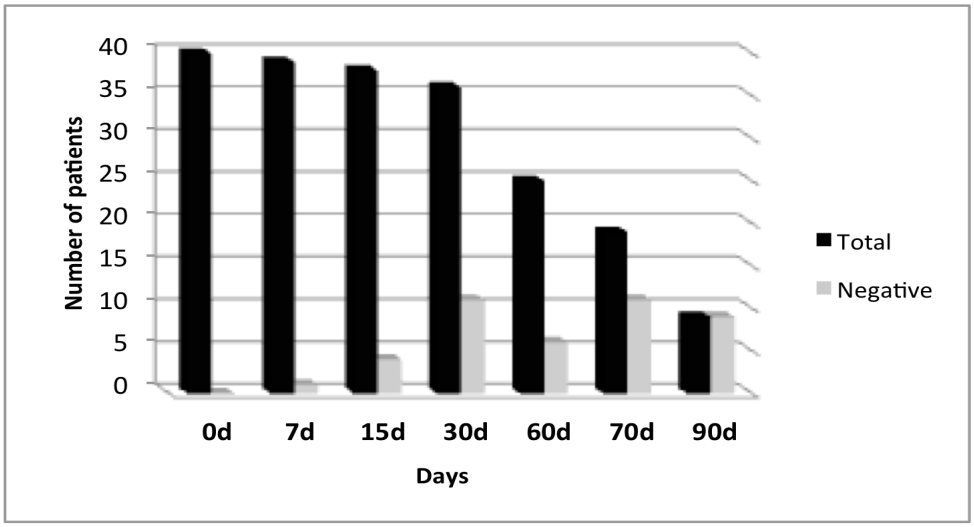

Figure (3) : A diagram showing the frequency of urine samples' negative results over 90 days of abstinence.

REFERENCES

Bosker, W.M. and Huestis, M.A. (2009): "Oral fluid testing for drugs of abuse". Clin. Chem., 55 (11) : 1910 - 1931.

Desrosiers, N.A.; Lee, D.; Schwope, D.M.; et al. (2012): "On-site test for cannabinoids in oral fluid". Clin Chem., 58 (10):1418-1425.

Dressler, U. (2000): "Detection of THCCOOH in hair by MSD-NCI after HPLC clean-up". Forensic Sci. Int., 107: 239–247.

Ellis, G.M.; Mann, M.A.; Judson, B.A.; et al. (1985): "Excretion patterns of cannabinoid metabolites after last use in a group of chronic users". Clin. Pharmacol. Ther., 38: 572–578.

El-Sohly, M.A. (2002): Chemical constituents of cannabis. In: Cannabis and cannabinoids: pharmacology, toxicology, and therapeutic potential. Grotenhermen, F.; Russo, E. (Eds), New York: Haworth Integrative Healing; P.P. 27–36.

Fitzgerald, K.T.; Bronstein, A.C. and Newquist, K.L. (2013): "Marijuana poisoning". Top Companion Anim Med., 28 (1): 8-12.

Goodwin, R.S.; Darwin, W.D.; Chiang, C.N.; et al. (2008): "Urinary elimination of 11-nor-9-carboxy-delta 9-tetrahydrocannabinol in cannabis users during continuously monitored abstinence". J. Anal. Toxicol., 32 (8): 562-569.

Hollister, L.E. (1986): "Health aspects of cannabis". Pharmacological Reviews, 38: 1-20.

Huestis, M.A. (2005): Pharmacokinetics and metabolism of the plant cannabinoids, delta-9-tetrahydrocannabinol, cannabidiol and cannabinol. In: Handbook of Experimental Pharmacology. Pertwee, R.G. (Editor), New York: Springer, P.P. 657–690.

Huestis, M.A. and Cone, E.J. (2004): "Relationship of delta 9-tetrahydrocannabinol concentrations in oral fluid and plasma after controlled administration of smoked cannabis". J. Anal. Toxicol., 28: 394–399.

Huestis, M.A.; Henningfield, J.E. and Cone, E.J. (1992): "Blood cannabinoids. I. Absorption of THC and formation of 11-OH-THC and THCCOOH during and after smoking marijuana". J. Anal. Toxicol., 16 (5): 276-282.

Houwing, S.; Smink, B.E.; Legrand, S.A.; et al. (2012): "Repeatability of oral fluid collection methods for THC measurement". Forensic Sci. Int., 30; 223 (1-3): 266-272.

Kintz, P.; Cirimele, V. and Tudes, B. (2000): "Detection of cannabis in oral fluid (saliva) and forehead wipes (sweat) from impaired drivers". J. Anal. Toxicol., (24): 557- 561.

Lafolie, P.; Beck, O.; Blennow, G.; et al. (1991): "Importance of creatinine analyses of urine when screening for abused drugs". Clin. Chem., 37: 1927–1931.

Lee, D.; Milman, G.; Barnes, A.J.; et al. (2011): "Oral fluid cannabinoids in chronic, daily cannabis smokers during sustained, monitored Abstinence". Clinical Chemistry, 57 (8): 1127-1136.

Lee, D.; Schwope, D.M.; Milman, G.; et al. (2012): "Cannabinoid disposition in oral fluid after controlled smoked cannabis". Clin. Chem., 58 (4): 748-756.

Lillsunde, P. and Gunnar, T. (2005): "Drugs and driving: the Finnish perspective". Bull. Narc., 57 (1-2): 213-229.

Moore, C.; Coulter, C.; Uges, D.; et al. (2011): "Cannabinoids in oral fluid following passive exposure to marijuana smoke". Forensic Sci Int., 212: 227–230.

Saugy, M.; Avois, L.; Saudan, C.; et al. (2006): "Cannabis and sport". British Journal of Sports Medicine, 40: 13-15.

Verstraete, A.G. (2005): "Oral fluid testing for driving under the influence of drugs: history, recent progress and remaining challenges". Forensic Sci. Int., 150 (2-3):143–150.

الوجهة الطبية الشرعية لنافذة الكشف عن القنب و مخلفاته في السائل الفمى و البول

المشتركون فى البحث

أ.د. عبد العزيز أبو الفتوح غانم أ.م.د. أمال عبد السلام البقرى

د. منى صلاح الحمادى

قسم الطب الشرعى والسموم الإكلينيكية – كلية الطب – جامعة المنصورة

يعتبر القنب من أكثر المواد المخدرة والمحظور استخدامها على المستوى العالمى وقد انتشر اختبار الكشف عنه فى السائل الفمى وأثبتت فاعليته وأهميته أكثر من الكشف عنه فى البول وخاصة فى حالات الإستخدام الحديث لهذه المادة. لذلك كان الهدف من هذه الدراسة هو تأكيد إمكانية إكتشاف تدخين الحشيش الحديث من خلال فحص السائل الفمى مقارنة بالبول وأهمية ذلك من الوجهة الطبية الشرعية .

و تم أخذ عينة من السائل الفمى وبول أربعين متطوعا بعد فترات مختلفة من الإمتناع ثم الكشف عن القنب و مخلفاته باستخدام اختبار الكشف السريع وقد تم تجميع ١٦٧ عينة من السائل الفمى خلال فترة امتناع من ٥ دقائق إلى ٣٠ ساعة ، بالإضافة الى ٢٠٦ عينة بول خلال مدة توقف ممتدة تتراوح ما بين يوم واحد و تسعين يوما. وتم الحصول على أول عينة سلبية من السائل الفمى بعد ٢٢ – ٣٠ ساعة من الإمتناع، بينما كانت أول عينة سلبية فى البول بعد سبعة ايام من الإمتناع. وقد استمر اكتشاف مخلفات القنب فى البول لمدة امتدت لسبعين يوما. وهذه النتائج تؤكد أن تحليل السائل الفمى يمكن ان يفرق بين كل من التعاطى الحديث والقديم للحشيش و لذلك نوصي باستخدام كواشف القنب ومخلفاته في السائل الفمى وكذلك تأكيد الحالات الإيجابية باختبارات أخرى.