

Reprint from

RECENT ADVANCES  
IN DOPING ANALYSIS  
(11)

W. Schänzer  
H. Geyer  
A. Gotzmann  
U. Mareck  
(Editors)

Sport und Buch Strauß, Köln, 2003

---

U. MARECK, A. KRESS, U.W. SCHWARZ, H. GEYER, W. SCHÄNZER:  
Feasibility Study on Commercially Available THC Drug Screen Test Devices  
In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (eds.) Recent advances in doping  
analysis (11). Sport und Buch Strauß, Köln, (2003) 109-118

## **Feasibility Study on commercially available THC drug screen test devices**

Institute of Biochemistry, German Sport University Cologne, Germany

### **Abstract**

The analysis of tetrahydrocannabinol in IOC accredited laboratories detecting misuse of cannabis products like marijuana and hashish is performed by GC-MS.

A cut-off limit of 15 ng/ml for the main metabolite 11-nor- $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid has been defined in order to avoid false positive results due to passive smoking [1-9].

THC drug screen devices adjusted to a cut off level of 50 ng/ml carboxy-THC are commercially available. Recently an on site screening method for monitoring marijuana use / misuse at a cut off concentration of 15 ng/ml carboxy-THC urine specimen which corresponds to the cut off limit in sports was developed. The principle of this screening method is based on lateral flow immuno assay technique where monoclonal antibodies are used for the specific binding of carboxy-THC.

The THC drug screen test devices with the IOC cut off level of 15 ng/ml carboxy-THC were investigated and the results compared with the GC-MS analysis [10-11].

A validation including determination of linearity and variability of results was performed.

Additionally various doping control samples were tested: a) carboxy-THC negative urines (partly with high amounts of non THC drugs), b) carboxy-THC positive urines and c) urines containing less than 15 ng/ml carboxy-THC.

The results obtained using the THC drug screen device corresponded in most of the cases with the GC-MS analysis. In 74 THC positive urine samples (concentration of carboxy-THC > 15 ng/ml) four false negative results appeared, whereas in 15 urine samples containing concentrations of carboxy-THC between 2 and 15 ng/ml one false positive result was detected. The evaluation of the test samples containing between 15 and 20 ng/ml carboxy-THC shows 33% incorrect results.

The investigated THC drug screen test devices with the cut off level of 15 ng/ml THC-metabolite are feasible to detect misuse of cannabis products like marijuana or hashish.

## Introduction

According to the IOC rules, the use of cannabinoids (e.g. Marijuana, Hashish) is forbidden and tests for cannabinoids will be conducted at the Olympic Games. The IOC, however, allows each Sport Organisation to decide whether or not to place cannabinoids on their list of forbidden substances.

A cut-off limit of 15 ng/ml for the main metabolite 11-nor- $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid has been defined in order to avoid false positive results due to passive smoking. Positive results due to prohibition of cannabinoids depend on behalf of the single federation [12, 13].

The rules of the different federations regarding the abuse of cannabinoids are still nonuniform but in general a ban is imposed only for competition test samples.

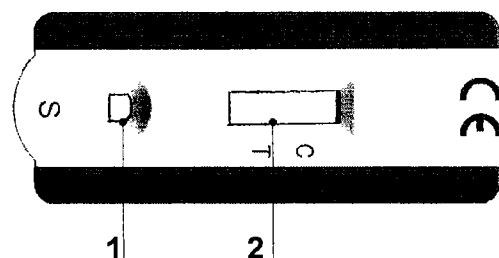
The consumption of cannabis seems to become a social problem. 30% Of European adolescents are familiar with the use of this drug [14-17].

Due to its long half-life time (20-36 hrs) the out of competition consumption of cannabis may lead to longtime elimination of high urinary concentrations of carboxy-THC metabolite resulting probably in positive competition doping control tests [18, 19]. Athletes and their federations may feel encouraged to use this tool preventing the increase of positive doping cases.

Due to legal restrictions the purchase of certified reference material is somewhat difficult for some doping control laboratories. The use of the drug sticks as preliminary screening method allows these laboratories to screen suspicious urine samples before the verification by GC-MS chromatography becomes necessary in the case of positive results.

The performed study should give informations about quality and feasibility of the THC drug screen test devices with the IOC cut off level of 15 ng/ml carboxy-THC.

### Principle of THC drug screen test devices



*Fig 1: THC drug screen test devices*

The principle of this screening method is based on an immuno assay technique where monoclonal antibodies are used for the specific binding of carboxy-THC. The design of the device allows an easy, rapid testing procedure: 3 to 4 drops of specimen are applied to sample well (1). Test results are shown in evaluation window (2) within 5 minutes.

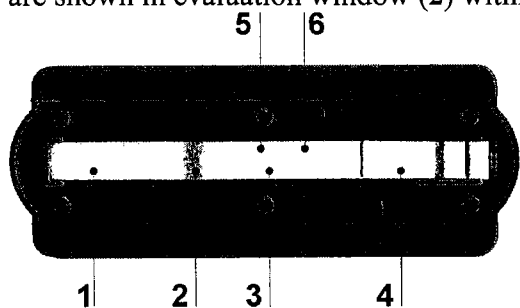


Fig 2: Principle of THC drug screen test devices (immuno assay technique)

The test device contains mouse monoclonal anti-Marijuana antibody coupled particles and Marijuana-protein conjugate. A goat antibody is employed in the control line system. Core part of the test device is an immuno assay strip which has different functional parts. The adsorbent wick (1) ensures a rapid flow of applied specimen. The reagent pad (2) made of glass fibre is impregnated with a defined amount of colloidal gold antibody complex. The impregnation procedure includes a specific treatment of the colloidal gold antibody complex by employing BSA and sucrose. Both additives ensure that the colloidal gold will not aggregate to an insoluble conglomerate when dried. Binding of the colloidal gold antibody conjugate is done on a pretreated nitrocellulose membrane (3) of defined porosity. At the T-line (5) position drug protein conjugates are immobilized to capture unblocked colloidal gold antibody conjugate. At the C-line (6) goat anti-mouse antibodies are immobilized to serve as a procedural control indicating that proper volume of specimen has been added and membrane wicking has occurred. Excess specimen is adsorbed by the porous sink (4).

Principle – drug-free specimen

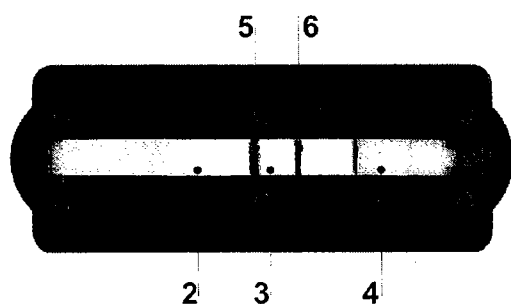


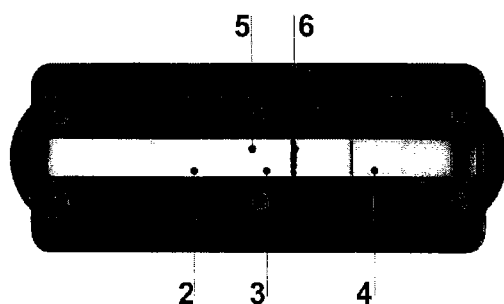
Fig 3: Principle of THC drug screen test devices – drug free specimen

Urine specimen applied to the adsorbent pad dissolves the colloidal gold antibody complex. The dissolved reagents are transported to the capture lines via lateral flow. As the antigen binding site of the reagent complex is not blocked by the drug metabolite the colloidal gold antibody conjugate binds to the capturing molecule at T-line area to form a visible red line.

Excess colloidal gold reagent is bound to the goat antibodies immobilized at C-line area forming a visible red line.

Dissolving of colloidal gold antibody complex in nearly quantitative when specimen is applied (2). Background of the nitrocellulose membrane has cleared up (3). The light reddish color of the porous sink (4) indicates that most of the colloidal gold conjugate has been captured at T- and C-line. The T-line is strong and clearly visible (5). The C-line color may be less intensive compared to that of T-line (6).

#### Principle – positive specimen



*Fig 4: Principle of THC drug screen test devices – positive specimen*

Urine specimen applied to the adsorbent pad dissolves the colloidal gold antibody complex. The dissolved reagents are transported to the capture lines via lateral flow. As the antigen binding site of the reagent complex is completely blocked by THC metabolites binding of the colloidal gold antibody conjugate is disabled at T-line area. There is no red line formed.

The blocked colloidal gold reagent is bound to the goat antibodies immobilized at C-line area forming a visible red line.

The colloidal gold conjugate has been completely dissolved (2). Background of the nitrocellulose membrane has cleared up (3). The light reddish color of the porous sink (4) that most of the colloidal gold conjugate has been captured at C-line. There is no visible line at T-line area (5).

The red line at C-line area indicates that proper volume of specimen has been added and membrane wicking has occurred (6).

**Interpretation of test results**

As the Marijuana screening test has been designed as a competitive immuno assay a negative result is indicated by the presence of color intensive T-line (1) and C-line (2).

In the presence of carboxy-THC (> 15 ng/ml) the T-line (1) will disappear and only the C-line (2) is visible.

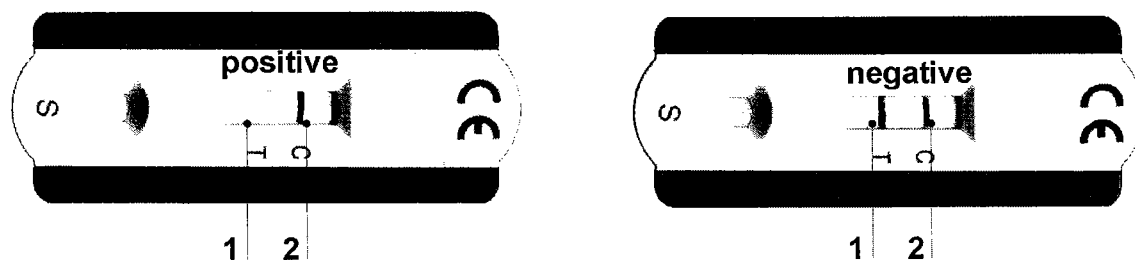


Fig 5: Interpretation of test results

**Positive Test Result:**

1. No T-Line
2. Clear C-Line

**Negative Test Result:**

1. Clear & intensive T-Line
2. Clear C-Line

The THC DrugScreen test device is especially designed for “marijuana” drug screening. The specificity of the monoclonal antibody used has been determined as shown in table1. These data are mandatory for diagnostic medical devices. Minor metabolic substances – not listed in the table – will not interfere as their concentrations are too low to generate a positive signal within a competitive immuno assay.

TEST DEVICE	SUBSTANCE	CAS-No	Cut Off Limit Value [ng/ml]
	11-nor- $\Delta^8$ -THC-9-COOH	[ - ]	10
	11-nor- $\Delta^9$ -THC-9-COOH	[ - ]	15
<b>Marijuana</b>	$\Delta^8$ -THC	[5957-75-5]	4,500
	$\Delta^9$ -THC	[1972-08-3]	4,500
	Cannabinol	[521-35-7]	6,000

Tab 1: Specificity of the monoclonal antibody

## Experimental

### *Sample preparation*

The urine samples were prepared and analysed according to the standard operating procedures for anabolic steroids [10,11] and confirmation of carboxy-THC [12].

### *GC-MS parameters*

GC-MS: HP 6890/HP 5973 (Hewlett Packard)

electron impact: 70 eV

#### Screen 4:

column: HP Ultra I (OV-1), 17m, 0.2mm i.d., 0.11  $\mu$ m film thickness

carrier gas: helium 12 psi, split 1:10

temperature program: 0 min 180°C, + 3°C / min, 0 min 229°C, + 40°C / min,  
2 min 320°C

injection volume: 3  $\mu$ l

#### Confirmation of carboxy-THC:

column: HP Ultra I (OV-1), 17m, 0.2mm i.d., 0.11  $\mu$ m film thickness

carrier gas: helium 12 psi, split 1:10

temperature program: 0 min 200°C, + 15°C / min, 2 min 300°C

injection volume: 3  $\mu$ l

### *Urine samples*

For validation purposes a well characterised, spiked in-house urine was used.

Doping control urine samples (competition and out of competition) from national and international federations containing more than 15 ng/ml, between 2 and 15 ng/ml and less than 0.5 ng/ml carboxy-THC were investigated.

### *Reference standards*

11-nor- $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid in the urinary matrix was identified by comparison of its mass spectrum and retention time with those of a reference standard (Sigma-Aldrich, Steinheim, Germany).

As internal standard for quantitative analysis ( $\pm$ )-11-nor-9-carboxy- $\Delta^9$ -tetrahydrocannabinol-**D**<sub>9</sub> (Cerilliant, Austin, USA) was used.

### *THC drug screen test devices*

THC drug screen test devices with a cut off concentration of 15 ng/ml carboxy-THC urine specimen were provided by ulti med (Ahrensburg, Deutschland).

## Results and Discussion

### *I. Validation*

#### Linearity

For the determination of the linearity a calibration curve with 2, 5, 10, 15, 20 and 25 ng/ml carboxy-THC was established. Additionally a negative and a positive urine were analysed. Each analysis was performed two times. Visually results showed 25 ng/ml carboxy-THC as positive and the carboxy-THC concentrations 2, 5, 10,15 and 20 ng/ml as negative.

At concentrations around cut off limit value of 15 ng/ml carboxy-THC faint T-line colors can be evaluated opto-electronically by means of SureScreen Reader.

#### Variability of results

For the analysis of the variability of results the concentrations 10, 15 and 20 ng/ml carboxy-THC were used. Each analysis was performed 10 times. Visually and opto-electronically results are shown in Tab 1. The detailed evaluation done by the SureScreen Reader leads to less incorrect results.

The THC drug screen test devices are designed to show positive results in the presence of carboxy-THC > 15 ng/ml. As described in the validation protocol the test devices show positive as well as negative results for urines with a carboxy-THC concentration of 15 ng/ml.

Consequently, a reading at 15 ng/ml carboxy-THC is a false positive.

<b>Stick\conc</b>	<b>10 ng/ml</b>	<b>15 ng/ml</b>	<b>20 ng/ml</b>
False positive	2	7	
False negative			2
<b>Opto\conc</b>			
False positive	2	3	
False negative			1

Tab 2: Variability of results

#### Dilution of a positive urine sample

A urine sample containing 52 ng/ml carboxy-THC (according to GC/MS analysis) was used. This urine was diluted several times with blank urine. Resulting are the following urinary concentrations of carboxy-THC: 1:1 (26 ng/ml); 1:2 (17 ng/ml); 1:3 (10 ng/ml); 1:4 (8 ng/ml); 1:5 (6,6 ng/ml). Each analysis was performed twice.



The evaluation show positive results for the samples containing more than 15 ng/ml carboxy-THC (dilutions 1:1 and 1:2); samples containing less than 15 ng/ml carboxy-THC were evaluated negative.

#### *II. Analysis of THC negative urines*

56 urines from a federation using in general high amounts of analgesics were investigated. Each analysis was performed one time. All tested urines were found to be negative for carboxy-THC. Additional 117 doping control urine samples (competition and out of competition) from national and international federations and different sports were investigated. All specimen were tested negative for the presence of carboxy-THC. The results were confirmed by GC/MS.

#### *III. Analysis of THC positive urines*

74 urine samples from 3 previous years were investigated. Although the urines were kept at 4°C and from several papers the instability of cannabis metabolite on storage is well known, re-analysis of the urine samples by GC/MS confirmed the positive THC results (> 15 ng/ml). Analysis with the drug screen test device was performed twice for each urine sample. 4 of the positive samples showed very faint T-lines and were therefore classified as negative when evaluated visually. 3 of them showed increased pH values (> 9).

#### *IV. Analysis of urine samples containing THC concentrations less than 15 ng/ml*

15 urine samples were investigated. Each analysis was performed twice. One urine, containing 10 ng/ml carboxy-THC according to re-analysis by GS-MS, shows a “false” positive result. The sample was tested several times. The “false” positive result was confirmed. The urine sample shows a low specific density ( $\delta = 1,006$ ). Probably an overflow during application may lead to this false positive result.

### **Summary**

- THC drug test device is feasible to detect cannabis misuse in general
- Clear results for significant negative or positive urine samples
- False negative results are mainly connected with urinary pH-values > 9
- No cross reaction from declared drugs leading to false positive results
- One “false” positive result (10 ng/ml carboxy-THC) was not explainable
- Evaluation of the total amount of samples shows 5,7 % incorrect results
- Evaluation of samples containing 10-20 ng/ml carboxy-THC shows 33 % incorrect results
- Opto-electronical evaluation for faint T-lines is recommended

## Conclusion

The THC drug screen test stick is a rapid chromatographic immuno assay for the detection of the THC metabolites 11-nor- $\Delta^8$ -THC-9-COOH and 11-nor- $\Delta^9$ -THC-9-COOH in human urine at a cut-off concentration of 15 ng/ml.

This assay provides only a qualitative, preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography-mass spectrometry (GC-MS) is the preferred confirmatory method.

## Acknowledgements

We thank the Manfred Donike Society e.V. and the Bundesinstitut für Sportwissenschaft, Cologne, for their financial support.

## References

- 1 Perez-Reyes M, DiGuiseppi S, Davis KH. Passive inhalation of marijuana smoke and urinary excretion of cannabinoids. *J.Am.Med.Assoc.* 1983; **249**: 475.
- 2 Perez-Reyes M, DiGuiseppi S, Mason AP, Davis KH. Passive inhalation of marijuana smoke and urinary excretion of cannabinoids. *Clin.Pharmacol.Ther.* 1983; **34**: 36-41.
- 3 Law B, Mason PA, Moffat AC, King LJ, Marks V. Passive inhalation of cannabis smoke. *J.Pharm.Pahrmacol.* 1984; **36**: 578-81.
- 4 Morland J, Bugge A, Skuterud B, Steen A, Wethe GH, Kjeldsen T. Cannabis in blood and urine after passive inhalation of cannabis smoke. *J.Foresnsic Sci.* 1985; **30**: 997-1002.
- 5 Cone EJ, Johnson RE. Contact highs and urinary cannabinoid excretion after passive exposure to marijuana smoke. *Clin-Pharmakol.Ther.* 1986; **40**: 247-56.
- 6 Cone EJ, Johnson RE, Darwin WD, Yousefnajad D, Mell LD, Paul BD, Mitchell J. Passive inhalation of marijuana smoke; urinalysis and room air levels of delta-9-tetrahydrocannabinol. *J.Anal.Toxikol.* 1997; **11**: 89-96.
- 7 Cone EJ. Marijuana effects and urinalysis after passive inhalation and oral ingestion. In: Research Finding on Smoking of Abused Substances. Chiang CN, Hawks RL, Eds. Natl. Inst. Drug Abuse Rs. Monogr. 1990; Ser 99: 88-96.
- 8 Mule SJ, Lomax P, Gross SJ. Active and realistic passive marijuana exposure tested by three immunoassays and GC/MS in urine. *J.Anal.Toxicol.* 1988; **12**: 113-16.
- 9 *Journal of Analytical Toxicology* 1995; **19**: 450-453.
- 10 Donike M, Geyer H, Gotzmann A, Kraft M, Mandel F, Nolteernsting E, Opfermann G, Sigmund G, Schänzer W, Zimmermann J: Dope Analysis. In: Official Proceedings of the International Athletic Foundation World Symposium on Doping in Sport. Bellotti P, Benzi G, Ljungquist A(Hrsg.) IAAF Florence (1988) 53-87.
- 11 Geyer H, Schänzer W, Mareck-Engelke U, Nolteernsting E, Opfermann G: Screening Procedure for Anabolic Steroids – The Control of the Hydrolysis with Deuterated Androsterone Glucuronide and Studies with Direct Hydrolysis.

- In: Schänzer et al (Eds.) Recent advances in doping analysis (5) Sport und Buch Strauß, Köln (1998) 99-101.
- 12 Mareck-Engelke U, Geyer H, Schänzer W: Tetrahydrocannabinol (THC) in dope control. In: Schänzer et al (Eds.) Recent advances in doping analysis (7) Sport und Buch Strauß, Köln (1999) 51-60.
- 13 Mareck-Engelke U, Geyer H, Schänzer W. Cannabismissbrauch im Hochleistungssport. *Deutsche Zeitschrift für Sportmedizin* 2001 Jahrgang 52, 10:280-284.
- 14 „Junge Europäer halten Cannabis für ungefährlich“  
< <http://www.aerztezeitung.de/docs/2002/11/27/215a2003.asp?cat=/medizin/cannabis> >, (Ärzte Zeitung online 16.01.03)
- 15 „Jeder Dritte hat Cannabis probiert“  
< <http://www.aerztezeitung.de/docs/2002/10/24/192a0703.asp?cat=/medizin/cannabis> >, (Ärzte Zeitung online 6.01.03)
- 16 „Cannabiskonsum bei Jugendlichen immer häufiger“  
< <http://www.aerztezeitung.de/docs/2002/10/02/177a0802.asp?cat=/medizin/cannabis> >, (Ärzte Zeitung online 16.01.03)
- 17 „Konsum von Partydrogen in den letzten Jahren deutlich gestiegen“,  
< <http://www.aerztezeitung.de/docs/2002/06/19/112a0501.asp?cat=/medizin/cannabis> >, (Ärzte Zeitung online 16.01.03)
- 18 Moffat AC et al. Clarke's isolation and identification of drugs in pharmaceuticals, body fluids and post mortem material. *The Pharmaceutical Press* (London) 2<sup>nd</sup> ed. (1986) 424.
- 19 Smith-Kielland A, Skuterud B, Morland J. Urinary excretion of 11-nor-9-carboxy-delta9-tetrahydrocannabinol and cannabinoids in frequent and infrequent drug users. *J.Anal.Toxicol* 1999; 23: 323-332.