

Rapid Competitive Immunochromatographic Assay for the Detection of Tetrahydrocannabinol in Human Urine

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Abstract

Background: Cannabis is among the earliest mind-altering drugs known to man and has been around for at least 4000 years. Today it is world's most commonly used illicit drug, with more than 300 million regular users. The aim of the study is detection of tetrahydrocannabinol (THC) in urine using immunochromatographic assay- a qualitative method.

Materials and method: Retrospective study was done based on the report of urine tests conducted in the Dept. of Forensic Medicine in one year from July 2022 to June 2023. Consent from the individual brought for examination by the police.

Results and Conclusion: Total 118 cases were studied; all were males among which 93 were positive and 25 negative. It is a preliminary screening test for cannabis substance abuse which will help the Police for further investigation.

Key words: Cannabis, tetrahydrocannabinol (THC), immunochromatographic assay, qualitative method.

Introduction

Cannabis is among the earliest mind-altering drugs known to man and has been around for at least 4000 years. Today it is world's most commonly used illicit drug, with more than 300 million regular users.

In term of popularity ratings, it stands 4th among psychoactive drugs (after caffeine, nicotine and alcohol)¹.

Cannabis preparations are derived from Indian hemp plant (*Cannabis sativa*) which is hardy,

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aromatic annual herb that grows wild under most climatic conditions. The main active principle is delta-9 tetrahydrocannabinol (THC). Apart from THC, *Cannabis sativa* contains a number of other cannabinoids, including cannabidiol, cannabinol, cannabidolic acid, cannabicyclol and cannabigerol. So far more than 60 of these cannabinoids have been identified¹.

Toxic effects arise mainly from the abuse of various cannabis preparations for their mind-altering properties.

1. Marijuana- refers to any part of the plant or its extract that is used to induce psychotomimetic or therapeutic effects.
2. Ganja- resinous mass composed of leaves and bracts, in India (where the term actually originated), it is used to refer to crushed leaves and inflorescences of female plants.
3. Bhang- consists of dried mature leaves and flower stems that are ground with water and mixed with milk or fruit juice.
4. Hashish (charas) - preparations made out of dried resin collected from flower tops.
5. Sinsemilla- refers to seedless (unpollinated female) plant
6. Marijuana 'blunts'- cheap cigars sliced open, packed with cannabis and resealed.

Mode of Action:

- It appears that cannabinoids exert many of their actions by influencing several neurotransmitter systems and their modulators. These include GABA, dopamine, acetylcholine, histamine, serotonin, noradrenaline and prostaglandins.
- Cannabinoid receptor location and density in animal models has correlated well with clinical effects in humans. The highest density of receptors occurs in the basal ganglia and molecular layer of cerebellum, which correlates with its interference in motor coordination.
- Intermediate levels of binding were found in the hippocampus, dentate gyrus and layers I and IV of cortex, consistent with effects on short term memory and cognition.
- Low receptor density is noted in the brainstem areas controlling cardiovascular

and respiratory functions, which correlates with cannabinoids known lack of lethality.

- After binding to receptors, cannabinoids also produce effects through second messenger systems including inhibition of adenylylase and calcium channels and also probably by enhancing potassium channel activity.¹

Cannabis drug testing describes various drug test methodologies for the use of cannabis in medicine, sport and law. Cannabis use is highly detectable and can be detected by urine analysis, hair analysis, as well as saliva tests for days or weeks.

Purpose of the test:

- Medical screening- although its not common to screen hospitalized patients for drug use, cannabis testing may be used to assess patients in specialty medical settings, such as psychiatric care and substance use treatment programmes.
- Employment testing- workplaces may require that applicants be tested for use of cannabis and other drugs. Testing is required by law in some workplaces.
- Military testing- random drug test are required by the department of defence for members of the military. Drug tests can also be ordered when a commander believes service member using drugs or after safety issue or accident.
- Athletic testing- drug testing may also be required for professional athletes. The anti doping agency prohibits cannabinoids for competing athletes regardless of the legality of cannabis in the location of competition.
- Legal and forensic testing- testing for cannabis and other drugs may be conducted in court case or investigation.²

Biological timeline- most cannabinoids are lipophilic (fat soluble compounds that easily store in fat, thus yielding a long elimination half life relative to other recreational drugs. Metabolites of cannabis are usually detectable in urine drug tests from 3days up to 10 days according to Redwood laboratories; heavy users can produce positive tests for 30days or longer after ceasing cannabis use^{3,4}. The length of time may vary to some degree according to metabolism, quantity and frequency of use.

Marijuana use can be detected up to 3-5days after exposure for infrequent users, 1-15days for heavy users, and 1-30days for chronic users and/or users with high body fat^{5,6}. The main metabolite excreted in the urine is 11-nor-9-carboxy-THC, also known as THC-COOH. Most cannabis drug tests yield a positive result when the concentration of THC-COOH in urine exceeds 50ng/ml⁷. Urine testing is an immunoassay based test on the principle of competitive binding.

Of all the matrices, urine is the most commonly used for adolescent drug testing and is the most thoroughly studied^{8,9}.

However, for an adolescent patient, its collection is somewhat invasive since it requires either a sophisticated collection protocol which is not readily available in medical offices or direct observation (*e.g.*, by a clinician or a parent) to prevent tampering^{10,11}.

Currently, the most commonly used urine drug testing approach involves automated immunoassay either alone as a point-of-care test or as an initial screen for a 2-step testing procedure^{10,12}. Results from IA are qualitative (*i.e.*, a drug or its metabolite is denoted either present or absent, without the quantity reported). In the 2-step approach, a screening IA is followed by confirmatory gas chromatography-mass spectrometry (GC-MS).

Aims and Objectives:

- Detection of tetrahydrocannabinol (THC) in urine using immunochromatographic assay- a qualitative method.
- To reduce the incidence of addiction of Cannabis abuse by helping the investigating agency.
- Utilising this diagnostic test to assess patients in specialty medical settings, such as psychiatric care and substance use treatment programmes.

Materials and Method

Retrospective study was done based on the records of urine tests conducted in the Dept. of Forensic Medicine in one year from July 2022 to June 2023. Permission obtained from the Head of the Department for access to reports and study was conducted for two months.

Methodology

INSIGHT-THC device is a rapid, qualitative, immunochromatographic assay for the detection of tetrahydrocannabinol(THC) in human urine. This test is used to screen the tetrahydrocannabinol intoxication.

Principle:

INSIGHT THC is based on the principle of agglutinating sera on membrane and utilizes the technique of competitive immunochromatography. The conjugate pad is impregnated with two components- Anti tetrahydrocannabinol antibody monoclonal conjugated to colloidal gold and rabbit IgG conjugated to colloidal gold.

As the test specimen flows through the membrane assembly of the device, the Anti tetrahydrocannabinol antibody monoclonal colloidal gold conjugate complexes with Tetrahydrocannabinol present in the test specimen and travels on the membrane due to capillary action along with the rabbit IgG colloidal gold conjugate. This complex moves further on the membrane to test region (T) where it is not immobilized by Tetrahydrocannabinol conjugated to BSA coated on the membrane, forming no band. The absence of this band in the test region (T) indicated a positive result.

The rabbit IgG colloidal gold conjugate and unbound complex if any move further on the membrane and are subsequently immobilized by the goat anti rabbit IgG antibodies coated on the membrane at the control region (C) forming a pink colored band. This control band acts as a procedural control and serves to validate the test results.

CASE DETAILS:

The accused with history of Ganja consumption were brought by the Police to Department of Forensic Medicine and Toxicology KRIMS Karwar. Total 118 cases are examined from July 2022 till June 2023, after receiving requisition from the Police.

Specimen collection: no special preparation is necessary. The urine was collected when the accused was brought for examination to the Dept of Forensic Medicine, KRIMS Karwar in a sterile plastic container. Consent from the individual brought for examination by the police was taken and 5ml of urine was collected in a sterile container.

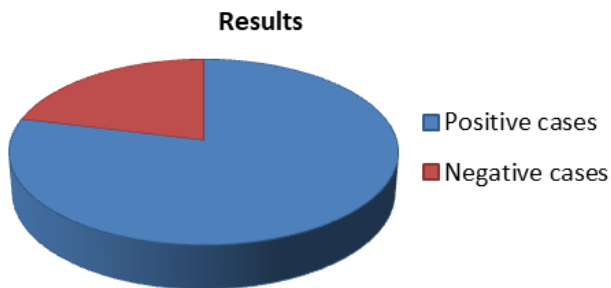
By holding the sample dropper vertically, two drops of test specimen urine is dispensed into the specimen port (S) of INSIGHT-THC device. Stop watch is started and the results are read after 5 minutes. One pink coloured band appears at the control region (C) which indicates that the specimen contains detectable amount of tetrahydrocannabinol.

Testing procedure:

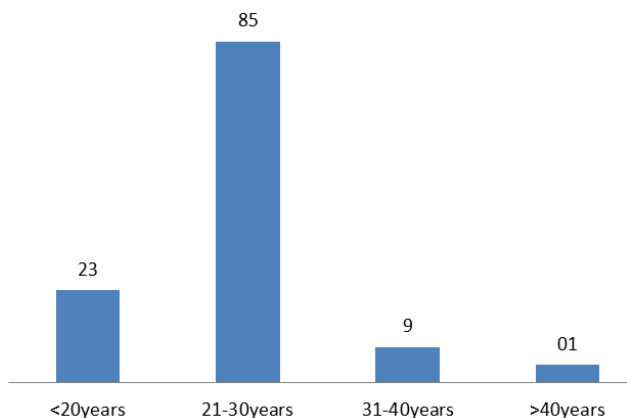
- The INSIGHT-THC kit was kept at room temperature and opened from the foil pouch.
- The device was labelled with marker bearing the name of the accused to be examined.
- The testing device was placed on a flat horizontal surface.
- Holding the sample dropper, two drops of the test specimen was dispensed into the specimen port (S) on the device and stop watch started. The results were read at the end of 5minutes.

INTERPRETATION OF RESULTS:

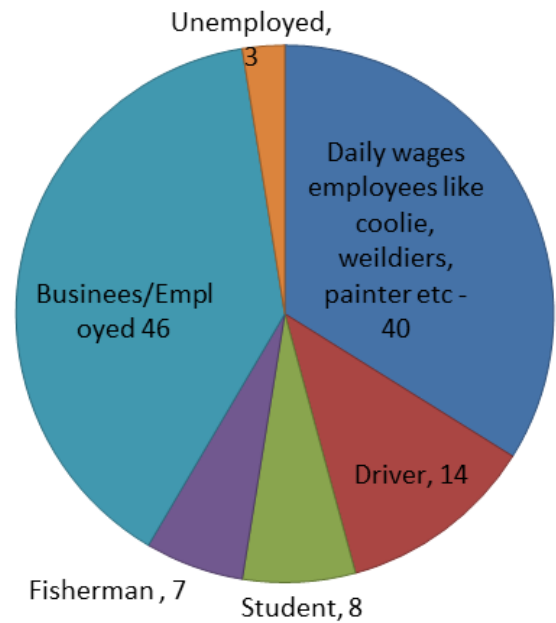
- Total number of cases examined 118. (all males)
- Positive: 93
- Negative: 25



Graph 01: Showing total results of the study



Graph 02: Age wise distribution of the cases:



Graph 03: Occupation wise distribution of the cases:



Figure 01: Insight THC Device

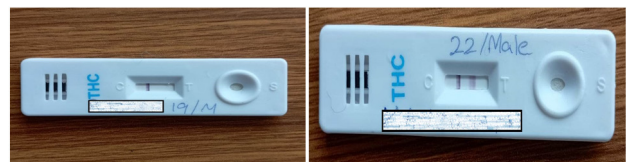


Figure 02: Positive Result

Figure 03: Negative Result

Discussion

Rosendo LM, et al¹³ study proposes a rapid procedure for simultaneous quantification of delta-9-tetrahydrocannabinol (THC), 11-hydroxy-delta9-tetrahydrocannabinol (11-OH-THC), 11-nor-9-carboxy-delta9-tetrahydrocannabinol(THC-COOH), cannabidiol (CBD), and cannabinol (CBN) in urine

samples. The limits of quantification and detection were between 1 and 10ng/ml using 0.25ml of sample. The findings of our study detect THC levels equal or greater than 50ng/ml using 0.1ml of sample (2drops of urine sample).

Rosendo LM, et al¹³ study used micro extraction by packed sorbent (MEPS) to pre-concentrate the analytes, which were detected by gas chromatography-mass spectrometry, and our study is based principle of competitive immunochromatography.

B Wei, L Wang, BC Blount¹⁴ have validated a multifunctional method using ultrahigh performance liquid chromatography coupled with tandem mass spectrometry for analysis of delta-9-tetrahydrocannabinol (THC), cannabidiol and cannabinol and two major metabolites of THC, 11-nor-9-carboxy-THC and 11-hydroxy-THC in active users and particularly in people exposed to secondhand marijuana smoke (SHMS). This method used positive electro spray ionization (ESI) mode to reach the sensitivity needed to detect trace SHMS exposure with limits of detection (LOD) ranging from 0.002 to 0.008ng/ml and 0.005 to 0.017ng/ml for free (unconjugated forms) and total (unconjugated plus conjugated forms) measurements respectively. This study is sensitivity rates higher than our present study.

Conclusion

The assay is designed for use with human urine only.

Sensitivity- INSIGHT-THC detects THC at concentrations equal to or greater than 50ng/ml.

Specificity- the following structurally related compounds produced positive results when tested at levels equal to or greater than the concentrations listed below:

Compound	Concentration (ng/ml)
11-nor- 8-THC-9-cacoxylic acid	50
11-nor- 9-THC-9-cacoxylic acid	50
11-hydroxy-9-tetrahydrocannabinol	1000
8-tetrahydrocannabinol	7500
9-tetrahydrocannabinol	10000
Cannabinol	10000

The cut off levels for distinguishing positive from negative specimens is not universal and depends on the laboratory, testing device or the agency conducting the test commonly either 20ng/ml, 50ng/ml or 100ng/ml.

There is a possibility that technical/or procedural errors as well as other substances or factors may interfere with the test and cause false results.

Testing of cannabis especially in urine detects evidence of use, not current intoxication or addiction.

The length of time following drug use for which positive result may occur is dependent upon several factors, including the frequency and amount of drug, metabolic rate, excretion rate, drug half life, the user's age, weight, activity and diet.

Drug testing can be stressful process. Positive drug test result requires additional confirmation testing conducted in laboratory. Possibility of positive cannabis test result due to passive or secondhand exposure to cannabis smoke. Research suggest that testing positive after second hand exposure to cannabis smoke is unlikely as metabolite levels in the body aren't sufficient to be detected in most drug tests.

Urine test report is qualitative, meaning that it may only show a positive or negative result and not additional information about the type and level of specific cannabinoids. Hence it is a preliminary screening test for cannabis substance abuse which will help the Police for further investigation.

Conflict of Interest: None

Ethics approval and consent to participate: Approved by Institutional Ethics Committee of Karwar Institute of Medical Sciences, Karwar. Reference no: IEC/KRIMS/O/20/2023-24; dated: 6th October 2023.

Consent for publication: The article does not disclose any personal data of the subjects and consent to publish the article is obtained from Institution Ethics Committee.

Availability of data and materials: The datasets analysed during the current study are not publicly available due to confidentiality reasons as these are Medico legal documents. But are available from the

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