



TETRA HYDRO CANNABINOL

Rapid Test (THC) strip

Introduction

The THC Rapid Test Dipstick (Urine) is a Rapid chromatographic immunoassay for qualitative detection of Marijuana in human urine at a cut-off concentration of 50 ng/mL which is only utilized for in vitro diagnostics (IVD). It merely provides a preliminary result, therefore, an alternative method with more specificity is recommended to be used to confirm the initial results, in which case, the most preferred method is gas chromatography-mass spectrometry (GC-MS). In cases of drug abuse, especially when the test result is positive, clinical and expert observations are required.

Summary

THC (Δ^9 - Tetrahydrocannabinol) is an active metabolite in marijuana. When taken orally or smoked, it causes euphoria. Marijuana users do harm to their short-term memory and slow down the learning process. They might experience anxiety and distress. Prolonged use of this substance leads to behavioral disorders. The maximum effect of marijuana appears within 20 to 30 minutes and takes 90 to 120 minutes after a smoke. The increased metabolites in the urine appear within a few hours and are detectable for 3 to 10 days after being smoked. The metabolites are excreted through urine in the form of 11-nor- Δ^9 -THC-9COOH. The rapid Marijuana test is a quick screening method which could be performed without laboratory equipment. The test employs monoclonal antibodies for the specific detection of increased levels of marijuana in the urine. When marijuana concentration exceeds 50 ng/mL in the urine, the test result becomes positive.

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Function Method Basis

Rapid Marijuana urine test employs an immunochromatographic method based on competitive binding. During the test, the urine specimen flows upward via capillary action. If the amount of Marijuana in the urine is below 50 ng/mL, it does not bind to the binding sites of the antibodies coated with nanoparticles. Consequently, the antibody conjugated with nanoparticles will be absorbed by the coated Marijuana and will leave a colored line in the test area (T). Should the amount be greater than 50 ng/mL, no colored line will appear in the T area, since all the binding sites of the Marijuana antibodies are occupied. So, if the presence of Marijuana in the urine specimen is positive, no colored line will be formed in the test area (T) due to drug competition, whereas if the urine specimen contains Marijuana amounts lower than the desired level in the test, a colored line will appear in this area. In order to have a procedural control, a colored line always appears in the area of the control line (C), indicating the appropriate volume of the specimen added to the membrane.

Compounds

The test strip contains anti-Marijuana monoclonal antibody particles and a combination of conjugated protein -Marijuana. The secondary antibody prepared in goat species is also employed for the control line.

+ **Caution:** for in vitro diagnostic use only.

Do not use after the expiration date. The test must remain in its package until use. All specimens must be considered infectious and all precautions and safety measures for handling and use must be taken.

Storage

The package should be stored at room temperature or in refrigerator (2-30 °C). The test is valid only until the expiration date printed on the package. Keep from freezing.

Specimen Collection and Preparation

+ **Urine collection:** The urine specimen must be collected in a clean and dry container. It could be collected at any time of the day.

+ **Specimen storage:** The urine specimen can be stored at 2-8 °C for more than 48 hours, and frozen for long-term storage at -20 °C (at most 2 weeks). Frozen specimens must be thawed and mixed well before testing.

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Contents and Required Supplies

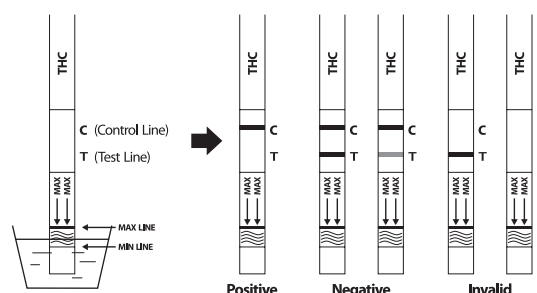
Items found in the kit: + Brochure +Test strip

Required items not included in the kit: +Specimen collection container +Timer

User Manual

Before testing, let the test strip and the urine specimen reach room temperature (18-30 °C).

- Bring the pouch to room temperature, before opening it. Remove the test strip from the sealed pouch, and use it as soon as possible.
 - Dip the test strip vertically into the urine specimen in the direction indicated by the arrows up to the MAX line, and hold it for at least 10-15 seconds (See illustration).
 - Place the test strip on a flat, non-absorbent surface, set the timer and wait for the colored line to appear. Interpret the results within 5 minutes.
- + Do not interpret the results after 10 minutes.



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Result Interpretation

Negative: Two lines appear, one line should be in the control area (C) and another colored line should appear in the test area (T). This negative result indicates that the Marijuana concentration is below the detectable level of 50 ng/mL.

+ **Attention:** The color shade in the testing area may vary, but it should be considered negative whenever there is even a faint colored line.

Positive: A colored line should appear in the control area (C) and no line should be seen in the test area (T). This positive result shows that the concentration of Marijuana is above the detectable level of 50 ng/mL.

Invalid: The control line (C) is not formed. insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem is not solved, stop using the strip and contact the after-sales service immediately.

Quality Control

The quality control of the method is included in the test, and the colored line appearing in the control area (C) is considered as an inner controller which confirms the sufficient volume of the specimen and the correct execution of the test. The test strip does not come with control standards. It is recommended to do the test with positive and negative controls in an accredited laboratory to confirm the method and the correctness of the test.

Limitations

1. Marijuana Rapid Test strip provides only preliminary analytical and qualitative results, and therefore, a secondary analytical method must be employed to confirm the results. For the confirmation of the results of this test, gas chromatography-mass spectrophotometry (GC-MS) is preferred.
2. There is a possibility of error in this technique. As some interfering substances in the urine specimen may lead to wrong results.
3. Adding substances like bleaches or white alum in the urine specimen may cause a wrong result, despite the analytical method used, and should the substances remain, the test should be performed with another urine specimen.

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4. A positive result indicates the presence of substances or their metabolites. A negative result, on the other hand, may not necessarily indicate the absence of substances in the urine. The drug level may be lower than the level detectable by the test.

5. The test is not able to detect the abuse of certain therapeutic cases.

+ **Expected values:** if Marijuana concentration is less than 50 ng/mL, the result will be negative and if the concentration is greater than 50 ng/mL, the result will be positive. The cut-off value of the test is 50 ng/mL.

Executive Feature

Accuracy: The results obtained from Hana Gene's THC Rapid Test strip were compared with those of an available commercial kit. In a study on 200 urine specimens (90 positive and 110 negative), Hana Gene's kit detected 89 of the specimens positive and 108 negative. The results are presented in table below:

Method		Hana Gene Result		Total Result
The THC Rapid Test Dipstick	Results	Positive	Negative	
	Positive	89	2	91
	Negative	1	108	109
Total Result		90	110	200

Sensitivity: > 98.8% (93.9 % ~ 99.9%) **Specificity:** 98.1% (93.5% ~ 99.7%)
Accuracy: 99.5% (95.6% ~ 99.6%) 95% Confidence Intervals

In order to compare the results of Hana Gene's Marijuana Rapid test kit, using TLC method, 200 clinical specimens (95 positive; 105 negative) were collected, and Hana Gene's kit detected 91 positive and 100 of the specimens negative. The results are shown in the table below:

Method		Rapid Test		Total Result
The THC Rapid Test Dipstick	Results	Positive	Negative	
	Positive	91	5	96
	Negative	4	100	104
Total Result		95	105	200

Sensitivity: 95.7% (89.5 % ~ 98.8%) **Specificity:** 95.2% (89.2% ~ 98.4%)
Accuracy: 95.5% (91.6% ~ 97.9%) 95% Confidence Intervals

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Analytical Sensitivity:

Some amount of drug-free urine specimens was prepared in the following concentrations of 11-nor- Δ^9 -THC-9COOH:

0 ng/mL, 25 ng/mL, 37.5 ng/mL, 50 ng/mL, 62.5 ng/mL, 75 ng/mL and 150 ng/mL. The results show more than 99% accuracy in 50% of specimens greater than the cut-off level and 50% below cut-off value. This information is summarized in the table below:

11-nor- Δ^9 -THC-9 COOH Concentration (ng/mL)	Percent of Cut-off	n	Visual Result	
			Negative	Positive
0	0	30	30	0
25	-50%	30	30	0
37.5	-25%	30	21	9
50	Cut-off	30	9	21
62.5	+25%	30	1	29
75	+50%	30	0	30
150	3X	30	0	30

Analytical Features:

The following table shows the compounds positively identified by the Marijuana test strip in the urine specimen within 5 minutes.

Compound	Concentration (ng/mL)
Cannabinol	20,000
11-nor- Δ^8 -THC-9 COOH	30
11-nor- Δ^9 -THC-9 COOH	50
Δ^8 -THC	15,000
Δ^9 -THC	15,000

Precision

A study was carried out in three different centers by untrained people making use of three different lots of the product in order to evaluate the intra-rater, inter-rater, and inter-individual reliability. A panel of coded specimens containing certain Marijuana concentrations was used in each center, at 0 ng/mL, 25% above and below the minimum detection limit, and 50% above and below the cut-off level.

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11-nor- Δ^9 -THC-9 COOH Concentration (ng/mL)	n per site	A		B		C	
		-	+	-	+	-	+
0	10	10	0	10	0	10	0
25	10	9	1	10	0	10	0
37.5	10	8	2	10	0	9	1
62.5	10	0	10	0	10	0	10
75	10	0	10	0	10	0	10

Effect of Urinary Specific Gravity

11-nor- Δ^9 -THC-9COOH with concentrations of 25 ng/mL and 75 ng/mL was added to 15 urine specimens with low, normal, and high specific gravity. Then pure specimens and specimens with certain 11-nor- Δ^9 -THC-9COOH concentrations were checked by the test strip. The results show that the specific weight and gravity do not affect the test results.

Effect of Urinary pH

The pH of the negative diluted urine specimen was changed from 5 to 9, and the specimens were given 11-nor- Δ^9 -THC-9COOH concentrations of 25 ng/mL and 75 ng/mL. The adjusted pH of the urine was done in a duplicate rapid Marijuana test whose results show that the pH range does not interfere with the test.

Cross Reactivity

In order to assess the interferences with positive and negative marijuana test results, a study was carried out. The following compounds did not show any interference in the test results with 100 µg/ml Marijuana concentration:

Acetamidophenol Deoxycorticosterone (+)3,4-Methylenedioxy- Prednisolone Acetophenetidin Dextromethorphan amphetamine Prednisone N-Acetylprocainamide Diazepam (+)3,4-Methylenedioxy- Procaine Acetylsalicylic acid Diclofenac methamphetamine Promazine Aminopyrine Diflunisal Methylphenidate Promethazine Amitriptyline Digoxin Methpyrrolon D,L-Propanolol Amobarbital Diphenhydramine Morphine-3- D-Propoxyphene Amoxicillin Doxylamine β -D-glucuronide D-Pseudoephedrine Ampicillin Ecgonine hydrochloride Nalidixic acid Quinidine L-Ascorbic acid Ecgonine methylester Nalorphine Quinine D,L-Amphetamine (-)- ψ -Ephedrine Naloxone Ranitidine L-Amphetamine Erythromycin Naltrexone Salicylic acid Apomorphine β -Estradiol Naproxen Secobarbital Aspartame Estrone-3-sulfate Niacinamide

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Serotonin (5-Hydroxytryptamine) Atropine Ethyl-p-aminobenzoate Nifedipine Sulfamethazine Benzilic acid Fenoprofen Norcodein Sulindac Benzoic acid Furosemide Norethindrone Temazepam Benzoyllecgonine Gentisic acid D-Norpropoxyphene Tetracycline Benzphetamine Hemoglobin Noscapine Tetrahydrocortisone, Bilirubin Hydralazine D,L-Octopamine 3-Acetate ($\pm$)-Brompheniramine Hydrochlorothiazide Oxalic acid Tetrahydrocortisone Caffeine Hydrocodone Oxazepam 3 (β -D-glucuronide) Cannabidiol Hydrocortisone Oxolinic acid Tetrahydrozoline Chloralhydrate O-Hydroxyhippuric acid Oxycodone Thebaine Chloramphenicol 3-Hydroxytryptamine Oxymetazoline Thiamine Chlordiazepoxide Ibuprofen p-Hydroxy-Thioridazine Chlorothiazide Imipramine methamphetamine D, L-Thyroxine ($\pm$) Chlorpheniramine Iproniazid Papaverine Tolbutamine Chlorpromazine ($\pm$) Isoproterenol Penicillin-G Triamterene Chlorquine Isoxsuprine Pentazocine Trifluoperazine Cholesterol Ketamine Pentobarbital Trimethoprim Clomipramine Ketoprofen Perphenazine Trimipramine Clonidine Labetalol Phencyclidine Tryptamine Cocaine hydrochloride Levorphanol Phenelzine D, L-Tryptophan Codeine Loperamide Phenobarbital Tyramine Cortisone Maprotiline Phentermine D, L-Tyrosine (-) Cotinine Meprobamate L-Phenylephrine Uric acid Creatinine Methadone β -Phenylethylamine Verapamil Methoxyphenamine Phenylpropanolamine Zomepirac

References

Hawks RL, CN Chiang. Urine Testing for Drugs of Abuse. National Institute for Drug Abuse (NIDA), Research Monograph 73, 1986 2. Baselt RC. Disposition of Toxic Drugs and Chemicals in Man. 2nd Ed. Biomedical Publ., Davis, CA. 1982; 488



Factory: No. 119, 5th Golestan St., Baharestan Industrial Town, 5th Kilometer of Karaj-Qazvin highway, Alborz, Iran



Tel: (+9826) 3618 1000

info@hanagene.com

Support: (+98) 993 321 2207

www.hanagene.com