P-5836-E

AccuSign® THC

One-Step THC Test

For *In Vitro* Use Only

Simple One-Step Immunoassay for the Qualitative Detection of THC Metabolites in Urine

Catalog No.	DOA-201-35	35 Test Kit
	DOA-201-10	10 Test Kit

Intended Use

AccuSign® THC is a simple, one-step immunochromatographic assay for the rapid, qualitative detection of THC metabolites in urine. The test is intended for use in the qualitative detection of cannabinoids in human urine with a cutoff at 50 ng/mL for 11-nor-Δ^9-THC-9-COOH.

The* AccuSign® THC *test provides only a preliminary analytical result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography, mass spectrometry (GC/MS) is the preferred confirmatory method. Other chemical confirmatory methods are available. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.[1]

Summary and Explanation

THC (Δ^9-tetrahydrocannabinol) is the primary active ingredient in cannabinoids (marijuana). When ingested or smoked, it produces euphoric effects. Users have impairment of short term memory and THC use slows learning. Also, it may cause transient episodes of confusion, anxiety, or even frank toxic delirium. Long term, relatively heavy use may be associated with behavioral disorders. The peak effect of smoking THC occurs in 20-30 minutes and the duration is 90-120 minutes after one cigarette. Elevated levels of urinary metabolites are found within hours of exposure and remain detectable for 3-10 days after smoking. The main metabolite excreted in the urine is 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid.[2]

Principle

The **AccuSign® THC** test is based on the principle of the highly specific immunochemical reactions between antigens and antibodies which are used for the analysis of specific substances in biological fluids. The test relies on the competition for binding to the antibodies between drug conjugates and drugs which may be present in the urine sample. In the test procedure, a sample of urine is placed in the sample well of the device, and the sample is allowed to migrate upward. If drug is present in the urine sample, it competes with the drug conjugate, which is immobilized on the membrane, for the limited antibodies present in the form of dye-antibody conjugate. When a sufficient amount of drug is present, the drug will saturate the antibodies, and the dye-antibody conjugate cannot bind to the drug conjugate on the membrane. Therefore, a drug-positive urine sample will not generate a line at the Test position in the Result Window, indicating a positive result from positive drug competition, while a negative urine sample will generate a line at the Test position in the Result Window, indicating a negative result from an absence of competition with free drug.

In addition, the test card has a procedural control built into the system, at the Control position in the Result Window. The control line is immobilized with polyclonal anti-mouse antibody; therefore, it will capture monoclonal antibody-dye conjugates that pass the region, showing a colored line at the Control (validation) position. The line works as a procedural control, confirming that proper sample volume was used and the reagent system worked. If insufficient sample volume is used, there may not be a control line, indicating the test is invalid.

Materials Provided

The **AccuSign® THC** test kit contains all the reagents necessary to perform the assay.

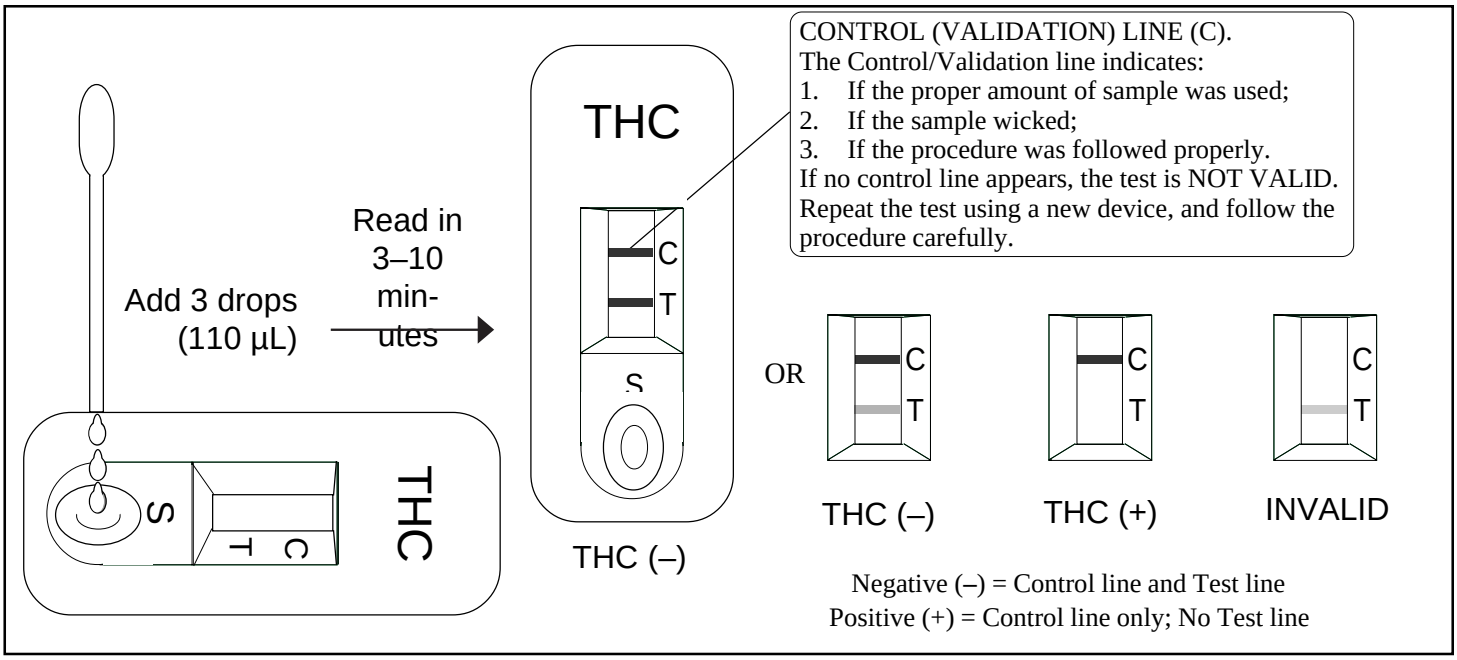
- **AccuSign® THC** device. The test device contains a membrane strip coated with drug conjugate and a dye pad containing anti-THC antibody-dye conjugate in a protein matrix.
- Disposable sample dispenser.
- Instructions for use.

Precautions

- For *in vitro* diagnostic use only.
- Avoid cross contamination of urine samples by using a new urine specimen container and dropper for each urine sample.
- This test kit does not contain any HIV or hepatitis infective components.
- Urine specimens are potentially infectious. Proper handling and disposal methods should be established according to good laboratory practices.
- The **AccuSign®** device should remain in its original sealed pouch until ready for use. Do not use the test if the pouch is damaged or the seal is broken.
- Do not use the test kit after the expiration date.

Storage and Stability

The **AccuSign® THC** test kit should be stored at 2–30°C (36–86°F) in the original sealed pouch. The expiration dating given was established under these storage conditions.



Specimen Collection and Preparation

Approximately 110 µL of urine sample is required for each test. Fresh urine specimens do not require any special handling or pretreatment. Specimens should be collected in a clean glass or plastic container. If testing will not be performed immediately, specimens should be refrigerated (2–8°C) or frozen. Specimens should be brought to room temperature before testing.

Specimens containing a large amount of particulate matter may give inconsistent test results. Such specimens should be clarified by centrifuging or allowing to settle before testing.

Test Procedure

The test procedure consists of adding the urine sample to the Sample well of the device and watching for the appearance of colored lines in the result window.

Test Protocol

1. For each test, open one **AccuSign® THC** pouch and label the **AccuSign®** device with the patient ID.
2. Holding the dropper vertically, dispense 3 drops (110 µL) of the urine sample into the Sample well (**S**).
3. Read the result after 3 minutes, but within10 minutes.

Interpretation of Results

Negative: The appearance of a reddish-purple Control line (**C**) and a line next to T indicates a negative test result; i.e., no drug above the cutoff level has been detected. The color intensities of the Control line and the Test line may not be equal. *Any faint Test line in the Result window, visible in 10 minutes, should be interpreted as negative. A negative test result does not indicate the absence of drug in the sample; it only indicates the sample does not contain drug above the cutoff level in qualitative terms.*

Positive: The appearance of only a reddish-purple Control line and no distinct line next to T indicates the test result is positive for THC (i.e., the specimen contains THC at a concentration above the cutoff level). *A positive test result does not provide any indication of the level of intoxication or urinary concentration of the drug in the sample; it only indicates the sample contains drug above the cutoff level in qualitative terms.*

Invalid: A distinct Control line (**C**) should always appear. The test is invalid if no Control line forms at the **C** position. Such tests should be repeated with a new **AccuSign® THC** test device.

Limitations

- The test is designed for use with unadulterated human urine only.
- There is a possibility that factors such as technical or procedural errors, as well as other substances in the urine sample which are not listed in Table 4 below, may interfere with the test and cause erroneous results.
- Adulterants, such as bleach and/or alum, in urine specimens may produce erroneous results regardless of the method of analysis. If adulteration is suspected, the test should be repeated with a new sample.
- The test result read after 10 minutes may not be consistent with the original reading obtained within the 10 minute reading period. The test must be read within 10 minutes of sample application.
- Prolonged passive smoking of THC may produce a positive result.

User Quality Control

Internal Control: Each **AccuSign® THC** test device has a built-in control. The Control line is an internal positive proce

dural control. A distinct reddish-purple Control line should appear at the Control position in the Result Window, if the test procedure is performed properly, an adequate sample volume is used, the sample and reagent are wicking on the membrane, and the test reagents at the control line and the conjugate-color indicator are reactive. In addition, if the test is performed correctly and the device is working properly, the background in the Result window will become clear and provide a distinct result. This may be considered an internal negative procedural control.

The positive and negative procedural controls contained in each **AccuSign® THC** test device satisfy the requirements of testing a positive control and a negative control on a daily basis. If the Control line does not appear at the Control position in the Result Window, the test is invalid and a new test should be performed. If the problem persists, contact PBM for technical assistance.

External Control: External controls may also be used to assure that the reagents are working properly and that the assay procedure is followed correctly. It is recommended that a control be tested at regular intervals as good laboratory testing practice. For information on how to obtain controls, contact PBM's Technical Services.

Expected Values

AccuSign® THC is a qualitative assay. The amount of THC metabolites present in the urine cannot be estimated by the assay. The assay results distinguish positive from negative samples. Positive results indicate the samples contain cannabinoids above the cutoff concentration.

Performance Characteristics

The National Institute on Drug Abuse has suggested that the screening cutoff for positive samples be 50 ng/mL for THC. The **AccuSign® THC** test has been shown to detect THC metabolite, 11-nor-Δ^9-THC-9-COOH, in urine at the cutoff of 50 ng/mL.

The accuracy of **AccuSign® THC** was evaluated in comparison to a commercially available immunoassay (Syva® EMIT® II). A total of 1001 samples was tested by both procedures. Complete agreement was observed in 99% of the samples, as shown below (Table 1).

Table 1. Accuracy: Comparison of AccuSign® THC with Syva® EMIT® II

		Syva® EMIT® II (THC)		
		Positive	Negative	TOTAL
AccuSign® THC	Positive	327	5	332
	Negative	13	655	668
TOTAL		340	660	1000
		Relative Sensitivity	Relative Specificity	
THC		96.2% (327/340)	99.2% (655/660)	

In a separate study, **AccuSign® THC** was evaluated against specimens confirmed as positive by GC/MS. The results below demonstrate the excellent correlation of **AccuSign® THC** with GC/MS (99% agreement, Table 2).

Table 2. Accuracy: Comparison of AccuSign® THC with GC/MS Assay

		AccuSign® THC	GC/MS
THC	Positive	87	88
	Negative	1	0

Precision and Accuracy

The precision of the **AccuSign® THC** assay was determined by carrying out the test with serially diluted standard drug solutions. Ninety-five percent of the samples containing 50% over the cutoff level of the drug consistently showed positive results.

The study also included over 40 samples containing ± 25% of cutoff level. These results were found to be consistently in agreement with predicate test results.

Distribution of Random Error:

Twenty blind samples prepared by spiking various concentrations of THC were separately tested by two operators. The test results from the two operators showed complete agreement.

Reproducibility

The reproducibility of the test results of the **AccuSign® THC** test was examined at three different sites using a total of 15 blind controls, consisting of 5 negative samples, 5 moderately positive samples (i.e., a concentration 1.5 times the cutoff level), and 5 strongly positive samples (i.e., a concentration 3 times the cutoff level). The results obtained at these three sites with these controls demonstrated 100% agreement with each other.

Specificity

The following table lists compounds that are detected by the **AccuSign® THC** test. The specificity of the **AccuSign® THC** test was determined by adding various drugs and drug metabolites to drug-negative urine specimens and testing with the **AccuSign® THC** test kit. The results are expressed in terms of the concentration required to produce a positive result (Table 3).

Table 3. Specificity

Compound	Concentration (ng/mL)	% Cross-reactivity
Cannabinol	50,000	0.1
11-nor-Δ^8-THC-9-COOH	250	20
11-nor-Δ^9-THC-9-COOH	50	100
Δ^8-THC	25,000	0.2
Δ^9-THC	15,000	0.3
11-hydroxy-Δ^9-THC	10,000	0.5

The following compounds show no cross-reactivity when tested with **AccuSign® THC** at a concentration of 100 µg/mL (Table 4).

Table 4. Non Cross-Reacting Compounds

4-Acetamidophenol
Acetophenetidin (Phenacetin)
N-Acetylprocainamide
Acetylsalicylic acid
Aminopyrine
Amitryptyline
Amobarbital
Amoxapine
Amoxicillin
D,L-Amphetamine
l-Amphetamine
Apomorphine
Aspartame
Atropine
Benzilic acid
Benzoic acid
Benzoylecgonine
Benzphetamine
Butabarbital
Cannabidiol
Chloralhydrate
Chloramphenicol
Chlordiazepoxide
Chlorothiazide
Chlorpromazine
Chlorquine
Cholesterol
Clomipramine
Clonidine
Cocaine hydrochloride
Codeine
Cortisone
(–) Cotinine
Creatinine
Deoxycorticosterone
Dextromethorphan
Diazepam
Diclofenac
Diethylpropion
Diflunisal
Digoxin
Diphenhydramine
Domperidone
Doxylamine
Ecgonine hydrochloride
Ecgonine methylester
(+) Ephedrine
(±) Ephedrine
(–) Ephedrine
(–) Ψ Ephedrine
Erythromycin
ß-Estradiol
Estrone-3-sulfate
Ethyl-p-aminobenzoate
Fenoprofen
Furoxmide
Gentisic acid
Glucuronide
Glutethimide
Guaifenesin
Hippuric acid
Hydralazine
Hydrochlorothiazide
Hydrocodone
Hydrocortisone
Hydromorphone
O-Hydroxyhippuric acid
3-Hydroxytyramine
Ibuprofen
Imipramine
Iproniazid
(–) Isoproterenol
Isoxsuprine
Ketamine
Ketoprofen
Labetalol
Levorphanol
Lidocaine
Loperamide
Loxapine succinate
Maprotiline
Meperidine
Meprobamate
Methadone
p-Hydroxymethamphetamine
Methaqualone
Methoxyphenamine
(±) 3,4-Methylenedioxyamphetamine
(±) 3,4-Methylenedioxymethamphetamine
Methylphenidate
Methyprylon
Morphine-3-ß-D-glucuronide
Nalidixic acid
Nalorphine
Naloxone
Naltrexone
Naproxen
Niacinamide
Nifedipine
Norcodein
Norethindrone
Noroxymorphone
D-Norpropoxyphene
(–) Norpseudoephedrine
Noscapine
Nylidrin
D,L-Octopamine
Oxalic acid
Oxazepam
Oxolinic acid
Oxycodone
Oxymetazoline
Oxymorphone
Papaverine
Penicillin-G
Pentazocaine
Pentobarbital
Perphenazine
Phencyclidine
Phendimetrazine
Phenelzine
Phenobarbital
Phentermine
Phentoin
L-Phenylephrine
ß-Phenylethylamine
Phenylpropanolamine
Prednisolone
Prednisone
Procaine
Promazine
Promethazine
D,L-Propanolol
Propiomazine
D-Propoxyphene
D-Pseudoephedrine
Quinidine
Quinine
Rantidine
Salicylic acid
Secobarbital
Serotonin
Sulfamethazine
Sulindac
Temazepam
Tetracycline
Tetrahydrocortisone
Tetrahydrozoline
Thebaine
Thiamine
Thioridazine
D,L-Thyroxine
Tolbutamide
Triamterene
Trifluoperazine
Trimethoprim
Trimipramine
Tryptamine
D,L-Tryptophan
Tyramine
D,L-Tyrosine
Uric acid
Verapamil
Zomepirac

References

1. Hawks RL, Chiang CN, eds. *Urine Testing for Drugs of Abuse*, Rockville, MD: National Institute for Drug Abuse (NIDA), Research Monograph 73; 1986.
2. Tietz, Norbert W. *Textbook of Clinical Chemistry.* W.B. Saunders Company. 1986.

Symbols Key

	Manufactured by
CE	CE Mark
EC REP	Authorized Representative
IVD	*In Vitro* Diagnostic Medical Device
REF	Catalog Number
	Consult Instructions for Use
LOT	Batch Code
EXP YYYY-MM-DD	"Use By" date in year-month-day format
2°C (35°F) Min / 30°C (86°F) Max	Temperature Limitation
Σ n	Contains sufficient for <n> tests
	Do not reuse
CONT	Contents
DEV	Test Device
PIP	Transfer Pipette
IFU	Instructions for Use
TEST DRUG	One-step immunochromatographic Assay for the Detection of Drugs of Abuse in Urine
TEST THC	Marijuana Test

EC REP

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