INTENDED USE

The DRG International, Inc Marijuana (THC) Screen Test is an in vitro qualitative, lateral flow, immuno-chromatographic urinary assay for the rapid detection of cannabinoids and their metabolites, notably: 11-nor-Δ-9-THC-9-COOH (as well as 11-nor-Δ-8-THC-9-COOH, Δ-8-THC-9-COOH, Δ-9-THC-9-COOH and 11-hydroxy-Δ-9-THC, all termed "THC") in human urine at the Substance Abuse Mental Health Services Administration (SAMHSA) cut-off limit of 50 ng THC/ml. This test is designed to obtain a visual, qualitative result and intended for professional use only. It is not intended for quantitative analysis, or for over-the-counter sale. The test provides only preliminary analytical data. A more specific, quantitative alternative method is required to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) has been established as the preferred confirmatory method by SAMHSA. Clinical considerations and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are indicated.

SUMMARY AND EXPLANATION OF TEST

Cannabinoids are central nervous system stimulants that alter mood and sensory perceptions, produce loss of coordination, impair short-term memory, and yield symptoms of anxiety, paranoia, depression, confusion, hallucination, and increased heart rate. Individuals who overdose on cannabinoids could develop tolerance and physiologic dependency, leading to THC abuse. For cannabinoid use of 1 to 3 or more hours, the urinary clearance rate may last from 1 to 7 days after the last drug use. The prominent Δ9 THC metabolite, 11-nor-Δ 9 THC-9-carboxylic acid (i.e., Δ9-THC-9-COOH) is the primary urinary marker for detecting marijuana use. Urinary screening for any drug of abuse usually detects the presence of the parent compounds and their drug metabolites, such as Δ9-THC-9-COOH. The Marijuana (THC) Screen Test is a rapid, visual, lateral flow, competitive, immuno-chromatographic assay for the qualitative detection of cannabinoids and their metabolites (i.e., THC) in human urine. These in vitro screening tests are based on immunoassay principles and designed specifically for the assay and identification of THC and its metabolites in urine at the cut-off level of 50ng THC/ml or higher, as set by SAMHSA.

PRINCIPLE OF THE PROCEDURE

The Marijuana (THC) Screen Test is a competitive immunoassay in which chemically modified and bound THC conjugate competes with urinary cannabinoids and their metabolites for limited, specific THC antibody binding sites. Each test device contains a sample reaction unit, a pink-colored, antibody-colloidal gold conjugate unit pre-labeled with specific, mouse monoclonal antibody, and a chromatographic membrane pre-coated with the drug conjugate in the device test region. When free drug is absent from urine or at concentrations lower than the urinary detection cut-off level, the pink-colored, antibody-colloidal gold conjugate is free to bind to the immobilized drug conjugate pre-coated on the chromatographic membrane test region.

As a result, a pink band will form in the test region indicating a negative result. When drug is present in urine at concentrations at or exceeding the detection cut-off level, it will bind to limited epitopes on the pink-colored, antibody-colloidal gold conjugate, completely competing with drug conjugate in the test region. As a result, no band forms in the test region, indicating a positive result.

The Marijuana (THC) Screen Test also provides a built-in process control. A pink-colored band should always appear in the control region, regardless of the presence of any urinary THC. This pink-colored control band verifies that: 1) sufficient urine volume was added, and 2) proper flow was obtained. If the control band is missing, the test was not performed correctly or failed to function correctly. In summary, negative urine will produce two pink colored bands, one in the control region and one in the test region. Positive urine will produce one pink-colored band in the control region only.

REAGENTS AND MATERIALS SUPPLIED

1. Test device with the following components: a sample reaction unit, a pink-colored, colloidal gold conjugate unit, and a chromatographic membrane unit. A disposable sample pipette comes with the test card.
2. Test Instructions for The Marijuana (THC) Screen Test device.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Urine sample collection containers.
2. Timer or clock with setting of 5.0 minutes or more.
3. For each urine sample, a disposable pipette is required to disperse at least 4 drops of urine into the sample
reaction area of the test card or test strip.

WARNINGS AND PRECAUTIONS

1. For *in vitro* use. In the United States, this kit is intended for Research Use Only.
2. **Avoid cross contamination of urine samples by using a new sample collection container and pipette for each urine sample**
3. Urine specimens may be potentially infectious. Avoid contact with skin by wearing gloves and proper laboratory attire. Properly handle and dispose of all used test devices in an approved biohazard container.
4. Do not use a test device beyond the expiration date imprinted on the outside of the foil pouch.

STORAGE AND STABILITY

- The test devices supplied can be stored at room temperature or refrigerated (2-28°C) and will remain stable until the expiration date.
- Do Not Freeze.

SAMPLE COLLECTION AND PREPARATION

The fresh urine samples should be collected in a clean, dry container such that same-day testing may be performed. Urine specimens may be refrigerated at 2-8°C for 48hours, or frozen at -20°C for assaying at a later date. Specimens that have been refrigerated or frozen must be equilibrated to room temperature before testing. Urine samples exhibiting visible precipitates should be filtered, centrifuged or allowed to settle so that clear aliquots can be obtained for testing.

ASSAY PROCEDURE

1. Do not open foil pouch until ready to begin testing. Refrigerated test device should be allowed to come to room temperature (15-28°C) before pouch is opened.
2. Remove the test device from the sealed foil pouch by tearing along the notch.
3. For test strip: Immerse the strips in urine with the arrow end pointing towards the urine. Do not introduce urine above the maximum level, as indicated by the arrows.
   For test card: Draw the urine sample into the pipette and dispense 4 drops (approximately 0.2ml) into the sample well of test device.
4. Read the test result at 5 minutes.

IMPORTANT

In order to prevent an incorrect reading, do not read the test results after more than 10 minutes. If the test is read after 10 minutes, the intensity of the colored lines may change or a new line may appear. To avoid confusion, discard the test device after interpreting the result.

INTERPRETATION OF RESULTS

Negative

Two colored bands appear, one in the control region and one in the test region. A negative result indicates that the free drug is absent or at concentrations lower than the detection level of the test.

Positive

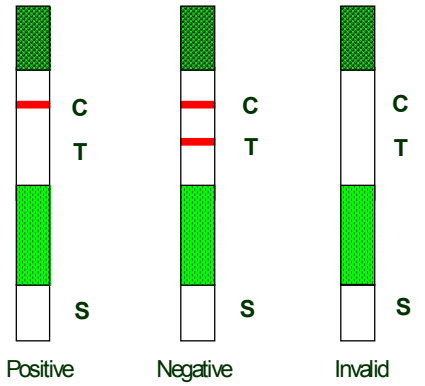
One colored band appears in the control region with no apparent band appearing in the test region. A positive result indicates that the free drug is present in the urine at concentrations at or exceeding the detection cut-off level of the test.

Invalid

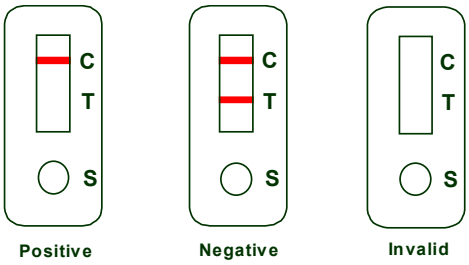
No band appears in control region, or a band appears in the test region only. An invalid test result may be due to improper assay procedures or damage to the device. If results are invalid, the assay is inconclusive and the specimen should be re-tested using a new test device.

Note: The test band intensity may be weaker or stronger than that of the control band, but a very faint band in the test region indicates that the free drug concentration is near the cut-off level in the urine. The specimen should be re-tested or confirmed with a more specific method before a positive determination is made.

TEST STRIPS



TEST CARDS



PROCESS AND QUALITY CONTROL

Good laboratory practice requires use of control materials that ensure proper test device performance and reliability. Quality control standards are available for the validation of device functionality from commercial sources such as BioRad, Alltech, and Biopool. When testing the quality of control standards, use the same assay procedure as with a urine sample. The SAMHSA recommended guidelines for drugs of abuse screening test devices indicate that controls should contain the drug of abuse analyte at levels at least 25% above the SAMHSA cut-off values.

AFTER TESTING

Urine specimens may be potentially infectious. Avoid contact with skin by wearing gloves and proper laboratory attire. Properly handle and dispose of all used test devices in an approved biohazard container. The residual urine should be disposed of in a medically approved manner after the completion of all testing, including the confirmatory assay.

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The Marijuana (THC) Screen Test has been designed for the detection of cannabinoids and their metabolites (mainly 11-nor-Δ 9-THC-9-COOH) in urine at the detection sensitivity of 50 ng/ml, as suggested for immunoassay methods by SAMHSA. In sensitivity studies performed, samples with concentrations of THC equal to or lower than 30 ng/ml were identified as negative for all samples.

Concentrations of THC equal to or higher than 50 ng/ml were identified as positive results for all samples.

PRECISION

In order to determine the precision of both The Marijuana (THC) Screen Test Card and Test Strip, both within-lot and inter-lot reproducibility studies were performed. Results of the within-lot reproducibility studies clearly showed excellent repeatability for all 3 batches of positive and negative urine samples, using one lot of Marijuana (THC) Screen Test Card devices and one of Test Strip devices. The results of these tests clearly demonstrate that there is no appreciable inter-lot variation when testing both positive and negative samples across three (3) different lots of Marijuana (THC) Screen Test Strip and Test Card devices.

SPECIFICITY STUDY

The specificity of The Marijuana (THC) Screen Test device was tested for compounds related to, or not associated with, THC as prepared in drug-free, normal human urine. The following compounds returned positive results with The Marijuana (THC) Screen Test at levels at or greater than concentrations listed below.

11-nor-Δ9-THC-9-COOH	50 ng/ml
11-nor-Δ8-THC-9-COOH	250 ng/ml
Cannabinol	50000 ng/ml
Δ8-THC	25000 ng/ml
Δ9-THC	15000 ng/ml
11-hydroxy-Δ9-THC	10000 ng/ml

The following compounds were found not to have any impact on negative results when tested at levels of up to 100 µg drug/ml (100,000 ng drug/ml):

COMPOUNDS	
Acetaminophen (N-Acetyl-p-aminophenol)	Imipramine
Amitriptyline	(+/-)-Isoproterenol
Ampicillin	(1R,2S)-(-)-N-Methyl-Ephedrine
Aspartame (Asp-Phe Methyl Ester)	Lidocaine
Aspirin (Acetylsalicylic Acid)	(+)-Naproxen
Atropine	(+/-)-Norephedrine
Benzocaine (Ethyl p-Aminobenzoate)	Penicillin-G (Benzylpenicillin)
Caffeine	Pheniramine
Chloroquine	Phenothiazine (Thiodiphenylamine)
(+)-Chlorpheniramine	L-Phenylephrine
(+/-)-Chlorpheniramine	-Phenylethylamine
Dexbrompheniramine ([+]-Brompheniramine)	([+/-]-Phenylpropanolamine)
Dextromethorphan	Procaine

4-Dimethylaminoantipyrine	Quinidine
Dopamine (3-Hydroxytyramine)	Ranitidine
(+/-)-Ephedrine	Sulindac
(-)-Ephedrine	Thioridazine
(+)-Epinephrine	Trifluoperazine
Erythromycin	Trimethobenzamide
Furosemide	Tyramine
Guaiacol Glyceryl Ether	Vitamin C (L-Ascorbic Acid)

ASSAY COMPARISONS & EQUIVALENCY

Accuracy and equivalency comparisons of both The Marijuana (THC) Screen Test Card and Test Strip were evaluated against 168 individual in-house laboratory urine samples, as well as against 341 individual external SAMHSA-certified clinical laboratory urine samples. The results have been tabulated below.

Table 1. THC Card vs. Emit® II THC

THC	EMIT II (+)	EMIT II (-)	Row Totals
(+)	159	3	162
(-)	9	338	347
Col. Totals	168	341	509

When compared to Emit® II Assay, the percent agreement with Marijuana (THC) Screen Test Card positive samples was 159/168 or 94.6%. Negative samples recovered at 338/341 or 99.1%, while the overall relative accuracy obtained was 497/509 or 97.6%.

Table 2. THC Strip vs. Emit® II THC

THC	EMIT II (+)	EMIT II (-)	Row Totals
(+)	160	0	160
(-)	8	341	349
Col. Totals	168	341	509

When compared to Emit® II Assay, the percent agreement with Marijuana (THC) Screen Test Strip positive samples was 160/168 or 95.2%. Negative samples recovered at 341/341 or 100%, while the overall relative accuracy obtained was 501/509 or 98.4%.

Table 3. THC Card vs. GC/MS

THC	GC/MS (+)	GC/MS (-)	Row Totals
(+)	107	4	111

(-)	0	284	284
Col. Totals	107	288	395

When compared to the GC/MS data, the relative sensitivity or percent agreement of Marijuana (THC) Screen Test Card positive samples with the external clinical study was 107/107 or 100%. Negative samples recovered a relative specificity of agreement of 284/288 or 98.6%. Finally, the overall relative accuracy obtained was 501/509 or 98.4%.

Table 4. THC Strip vs. GC/MS

THC	GC/MS (+)	GC/MS (-)	Row Totals
(+)	107	5	112
(-)	0	283	283
Col. Totals	107	288	395

When compared to the GC/MS data, the relative sensitivity or percent agreement of Marijuana (THC) Screen Test Strip positive samples with the external clinical study was 107/107 or 100%. Negative samples recovered a relative specificity of agreement of 283/288 or 98.3%. Finally, the overall relative accuracy obtained was 390/395 or 98.7%.

LIMITATIONS OF PROCEDURE

1. This test is designed to be used for the medical or forensic in vitro detection of THC in human urine. In the United States, this kit is intended for Research Use Only.
2. This test only provides a qualitative screening for THC in urine. It is not to be used for the quantitative determination of THC in urine. A positive result with this test indicates only the presence of THC and should be confirmed by GC/MS. It does not indicate intoxication.
3. There is a possibility of false positive results due to assay procedural errors or the presence of other substances as interfering factors in the urine, some of which are listed.
4. See the specificity lists of substances that will produce positive results at high levels, versus those substances that do not interfere with test performance.
5. Adulterants, such as bleach or other strong oxidizing agents, may produce erroneous test results when added to urine specimens, regardless of the analysis method. If an adulteration is suspected, another urine specimen should be used.

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