DRUGCHECK® K2/Spice Test

FORENSIC USE ONLY

FOR THE QUALITATIVE ASSESSMENT OF SYNTHETIC CANNABIS (K2) IN HUMAN URINE

INTENDED USE

The DrugCheck K2/Spice Test is an immunochromatography-based one step in vitro test. It is designed for qualitative determination of synthetic cannabis JWH-018 and JWH-073's major metabolites in human urine specimens at cut-off level of 25 ng/ml. In addition, other synthetic cannabis compound metabolities can be detected, MAM2201 (100ng/ml); JWH-0398 (200ng/mL); and JWH-210 (300ng/mL). This assay has not been evaluated in the point of care location and is for forensic use only.

This assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) has been established as the preferred confirmatory method by the Substance Abuse Mental Health Services Administration (SAMHSA). Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are indicated.

SUMMARY AND EXPLANATION

Synthetic cannabis is a psychoactive herbal and chemical product that, when consumed, mimics the effects of cannabis. It is best known by the brand name K2 and Spice, both of which have largely become genericized trademarks used for refer to any systhetic cannabis product. The studies suggest that synthetic cannabinoid intoxication is associated with acute psychosis, worsening of previously stable psychotic disorders, and also may have the ability to trigger a chronic (long-term) psychotic disorder among vulnerable individuals such as those with a family history of mental illness. A large and complex variety of synthetic cannabinoids, most ofter cannabicyclohexanol, JWH-018, JWH-073, or HU-210, are used. As of March 1, 2011, five cannabinoids, JWH-018, JWH-073, CP-47, JWH-200 and cannabicyclohexanol are illegal in US because these substances have the potential to be extremely harmful and, therefore, pose an imminent hazard to the public safety.

TEST PRINCIPLE

The K2/Spice Test is based on the principle of specific immunochemical reaction between antibodies and antigens to analyze particular compounds in human urine specimen. The assay relies on the competition for binding antibody between drug conjugate and free drug which may be present in the urine specimen being tested. When drug is present in the urine specimen, it competes with drug conjugate for the limited amount of antibody-dye conjugate. When the amount of drug is equal or more than the cut-off it will prevent the binding of drug conjugate to the antibody. Therefore, a positive urine specimen will not show a colored band on the test line zone, indicating a positive result, while the presence of a colored band indicates a negative result.

A control line is present in the test window to work as procedural control. This colored band should always appear on the control line zone if the test device is stored in good condition and the test is performed appropriately.

MATERIALS PROVIDED

1. Instructions for use.
2. K2/Spice Test: The amount of each coated antigen and/or antibody on the strip is less than 1.0 mg for antigen conjugate and is less than 1.0 mg for goat anti-rabbit IgG antibody.

Test zone: contains K2 protein antigen conjugates.

Control zone: contains goat anti-rabbit IgG antibody.

3. Conjugate pad: contains mouse anti-K2 monoclonal antibody.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Urine collection container.
2. Timer or clock.

STORAGE AND STABILITY

The test device should be stored at 4 to 30ºC and will be effective until the expiration date stated on the package. The product is humidity-sensitive and should be used immediately after being open. Any improperly sealed product should be discarded.

PRECAUTIONS

1. For forensic use only.
2. Do not use the product beyond the expiration date.
3. Handle all specimens as potentially infectious.
4. Humidity sensitive product, do not open foil pouch until it is ready to be tested.
5. Use a new urine specimen cup for each sample to avoid cross contamination.
6. The immunoassy reaction between anitbody and antigen requires ions to react as designed. Tap water (or drinkng water) generally speaking does not contain these ions and will not create the necessar reaction. Therefore, water is not a good specimen to use as a negative control test and will cause the strip to give an erroneous result.

SPECIMEN COLLECTION AND PREPARATION

It is required that approximately 120-150 µl of sample for each test. Fresh urine specimens do not need any special handling or treatment. Specimens should be collected in a clean, dry, plastic or glass container. If the assay is not performed immediately, urine specimen may be refrigerated at 2-8ºC or frozen up to 7 days. Specimens should be thawed and brought to room temperature before testing. Urine specimens exhibiting a large amount of precipitate or turbidity should be centrifuged or allowed to settle before testing. Avoid contact with skin by wearing gloves and proper laboratory attire.

QUALITY CONTROL

Good Laboratory Practice recommends the daily use of control materials to validate the reliability of device. Control materials should be assayed as clinical specimen and challenging to the assay cutoff concentration, e.g., 25% above and below cutoff concentration. If control values do not fall within established range, assay results are invalid. Control materials which are not provided with this test kit are commercially available.

The K2/Spice Test provides a built-in process control with a different antigen/antibody reaction at the control region (C). This control line should always appear regardless the presence of drug or metabolite. If the control line does not appear, the test device should be discarded and the obtained result is invalid. The presence of this control band in the control region serve as 1) verification that sufficient volume is added, 2) that proper flow is obtained.

PROCEDURE

For Test Strip

1. Bring all materials and specimens to room temperature.
2. Remove the single test strip or device from the sealed foil pouch.
3. Dip the strip into the urine specimen with the arrow pointing toward the sample. The sample level should not be higher than the arrow pointed maximum line.
4. Hold the strip in the urine until a reddish color appears at the test area (approximately 15 seconds).
5. Withdraw the strip and place it face up on a clean, non-absorptive surface or leave the strip in urine if the urine level is not higher than arrow pointed maximum line.
6. Read the results at 5-10 minutes minutes after adding the sample.

Test results may not be accurate after 10 minutes.

INTERPRETATION OF RESULTS

Negative: Two colored bands form. The appearance of two colored bands, one in test line zone and the other in control line zone, indicates negative results. The negative result indicates that the concentrations of metabolites of JWH-18 and/or JWH-73 in the specimen is either zero or less than cut-off level.

Positive: One colored band forms. One colored band appears in control line zone. No colored band is found in test line zone. This is an indication that the concentrations of metabolites of JWH-18 and/or JWH-73 in the specimen is above the cut-off level.

Invalid: If there is no colored band in control line zone, the test result is invalid. Retest the sample with a new device.

Note: A borderline (+/-) in test line zone should be considered negative result.

LIMITATION OF PROCEDURE

The assay is designed for use with human urine only. A positive result with any of the tests indicates only the presence of a drug/metabolite and does not indicate or measure intoxication.

There is a possibility that technical or procedural error as well as other substances in certain foods and medicines may interfere with the test and cause incorrect results. Please refer to the "SPECIFICITY" section for lists of substances that will produce either positive results, or that do not interfere with test performance. If a drug/metabolite is found present in the urine specimen, the assay does not indicate frequency of drug use or distinguish between drug of abuse and certain foods and medicines.

EXPECTED RESULTS

The K2/Spice Test test is a qualitative assay. It identifies JWH-018 pentanoic acid or JWH-073 butanoic acid in human urine at a concentration of 25 ng/mL or higher and other K2 compounds at the indicated level: MAM2201 (100ng/ml), JWH-398 (200ng/mL), JWH-210 (300ng/mL). The exact concentration of the synthetic cannabis cannot be determined by this assay. The test is intended to distinguish a negative result from a presumptive positive result. All positive results must be confirmed using an alternate method, preferably GC/MS. **NOTE: This test has been determined to cross react with the drug Lamictal (Lamotrigine). Specimen from individuals taking this prescription drug will likely register a false positive for K2. All presumptive positive screens should be sent to a confirmation lab for analysis.**

PERFORMANCE CHARACTERISTICS

A. Sensitivity

The cut-off concentration (sensitivity level) of K2/Spice Test is determined to be 25 ng/mL of JWH-018 5-pentanoic acid metabolite and 25 ng/mL of JWH-073 4-butanoic acid metabolite respectively. Other K2 compounds can be detected at the concentration level as follows: MAM2201(100ng/mL), JWH-398 (200ng/mL) and JWH-210 (300ng/mL).

B. Accuracy

1. The accuracy of the K2/Spice Test was evaluated in K2 spiked urine specimens. Forty (40) K2 urine specimens were spiked with JWH-018 pentanoic acid or JWH-73 butanoic acid from 10 to 150 ng/mL; 30 samples with JWH-018 pentanoic acid or JWH-073 butanoic acid concentration between 25 and 150 ng/mL were all found positive (100% agreement); 10 samples with JWH-018 pentanoic acid or JWH-073 butanoic acid concentration between 10 and 18.75 ng/mL were found negative.

2. A second study was conducted by a third-party laboratory using known positive urine from six (6) donors. Each specimen was screened with the K2/Spice Test. The laboratory then processed and tested each specimen following forensic guidelines.

	JWH-018 Pentanoic Acid			JWH-073 Butanoic Acid		
Donor	K2/Spice Test	Lab confir-mation	Result (ng/ml)	K2/Spice Test	Lab confir-mation	Result (ng/ml)
1	Positive	Positive	1.5	Negative	-	-
2	Positive	Positive	65.2	Positive	Positive	2.3
3	Positive	Positive	107.1	Positive	Positive	7.0
4	Positive	Positive	116.4	Positive	Positive	3.3
5	Positive	Positive	269.8	Positive	Positive	6.8
6	Positive	Positive	396.4	Positive	Positive	20.4

C. Precision

The precision study was performed by three individuals observing the test result to determine the random error of visual interpretation. The test results were found to have no significant differences between the three observers.

Device	Control Con. ng/ml	No. of Tested	No. of positive			No. of borderline			No. of negative		
			1	2	3	1	2	3	1	2	3
JWH-018 and JWH-073	0	42							42	42	42
	12.5	42							42	42	42
	37.5	42	41	42	41	1		1			

D. Specificity

The specificity for K2/Spice Test test was tested by adding various drugs, drug metabolites, and other compounds that are likely to be present in urine. All compounds were prepared in drug-free normal human urine.

1. Interference testing

The K2/Spice Test performance at cut-off level is not affected when pH and Specific Gravity ranges of urine specimen are at 5.0 to 8.0 and 1.005 to 1.035.

The following substances were tested and confirmed not to interfere with K2/Spice Test at the listed concentrations.

Glucose	2000 mg/dL
Human albumin	2000 mg/dL
Human hemoglobin	10 mg/dL
Urea	4000 mg/dL
Uric acid	10 mg/dL

2. Specificity

K2/Spice Test is specific with JWH-018 pentanoic acid and JWH-073 butanoic acid.

Compounds	Concentration	Cross reactivity
JWH-018 pentanoic acid	25 ng/ml	100%
JWH-073 butanoic acid	25 ng/ml	100%
MAM2201 N-pentanoic acid	100ng/ml	50%
JWH-398 N-pentanoic acid	200ng/ml	25%
JWH-210 N-5-Carboxypentyl	300ng/ml	17%

Each listed substance that may be found in the urine was evaluated and indicated negative result at concentration of 100 µg/ml or higher unless it is specified.

Acetaminophen
4-Acetamidophenol
Acetylsalicylic acid
Amikacin
Amitriptyline
Amobarbital
Amphetamine
Arterenol
Aspartame
Ascorbic acid
Atenolol
Atenonal
Atrophine
Benzoyolecgonine
Buprenorphine
Bup-3-β-glucuronide
Butabarbital
Butalbital
Caffeine
Camphor
Cannabidiol
Cannabinal
Cetirizine
Chloroquine
Cortisone
Chlorpheniramine
Cocaine
Codeine
Cotinine
Despiramine
Dextromethorphan
Deoxyephedrine
Digitoxin
Digoxin
Diphenhydramine
Diphenhydramine
Doxylamine
Ecgonine
Ecgonine methyl ester
EDDP
Ephedrine
Epinephrine
Fentanyl
Fluoxetine
Gentisic acid
Guaiacol glycer ester
Hertoine
Histamine
Homatrophine
Hydrochlorothiazide
Hydroxyzine
Ibuprofen
Imipramine
Isoproterenol
Ketamine
Lidocaine
MDA
MDMA
Meperidine
Methylphenidate
Methamphetamine
Methadol
Methadone
Methaqualone
Morphine
Nalbuphine
Nalophine
Nalxone
Natrexone
Neomycin
Niacinamide
Nicotine
Noigertaline
Nortriptyline
Orphenadine
Oxcarbazepine
Oxycodone
Oxymorphone
Perphenazine
Penicillin G
Pentobarbital
Phencyclidine (PCP)
Phenobarbital
Phenylethylamine-α
Phenylpropanolamine
Promethazine
Propranolol
Propoxyphene
Protriptyline
Quetiapine fumarate
Quinine antidine
Ranitidine
Salicylic acid
Secobarbital
Sertral
Sertraline
Tetracycline
Tetrahydrozoline
Theophyline
Thioridazine
Trifluoperazine
Trihexyphenidyl
Trimipramine
Tryptophen
Tyramine
Venlafaxine
Verapamil
11-nor-Δ⁹ –THC-9-COOH (10 µg/ml)*
11-nor-Δ⁹ –THC-9-COOH (10 µg/ml) *

*The highest level to be tested is as indicated.

REFERENCES

1. Urine testing for drugs of abuse, NIDA Research Monograph 73 (1986).
2. InfoFacts-Club drugs, NIDA , May 2006, http://www.nida.nih.gov/infofacts/clubdrugs.html.
3. Drug Fact Sheet, DEA, January 2012, http://www.dea.gov.
4. K2/Spice Test screening and confirmation study, Clinical Reference Laboratory, February 2012, pp. 2-7.

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