

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 50 ng/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 synthetic 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 often 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 30°C 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.

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 2-8°C 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 methylphenidate concentration 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 methylphenidate level 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 50 ng/ml or higher. The 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.

PERFORMANCE CHARACTERISTICS

A. Sensitivity

The cut-off concentration (sensitivity level) of K2/Spice Test is determined to be 50 ng/mL of JWH-018 5-pentanoic acid metabolite and 50 ng/mL of JWH-073 4-butanoic acid metabolite respectively.

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 50 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 37.5 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.

Donor	JWH-018 Pentanoic Acid			JWH-073 Butanoic Acid		
	K2/Spice Test	Lab confirmation	Result (ng/ml)	K2/Spice Test	Lab confirmation	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
	25	42							42	42	42
	75	42	40	41	40	2	1	2			

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	50 ng/ml	100%
JWH-073 butanoic acid	50 ng/ml	100%

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	EDDP	Perphenazine
4-Acetamidophenol	Ephedrine	Penicillin G
Acetylsalicylic acid	Epinephrine	Pentobarbital
Amikacin	Fentanyl	Phencyclidine (PCP)
Amitriptyline	Fluoxetine	Phenobarbital
Amobarbital	Genetic acid	Phenylethylamine-α
Amphetamine	Guaiacol glycer ester	Phenylpropranolamine
Arteron	Hetaion	Promethazine
Aspartame	Histamine	Propranolol
Ascorbic acid	Homatrophine	Propoxyphene
Atenolol	Hydrochlorothiazide	Propriptyline
Atenolol	Hydroxyzine	Quetiapine fumarate
Atrophine	Ibuprofen	Quinine antidine
Benzoylcegonine	Imipramine	Ranitidine
Buprenorphine	isoproterenol	Salicylic acid
Bup-3-β-glucuronide	Ketamine	Secobarbital
Butabarbital	Lidocaine	Sertral
Butalbital	MDA	Sertraline
Caffeine	MDMA	Tetracycline
Camphor	Meperidine	Tetrahydrozoline
Cannabidiol	Methylphenidate	Theophylline
Cannabinol	Methamphetamine	Thioridazine
Cetirizine	Methadol	Trifluoperazine
Chloroquine	Methadone	Trihexyphenidyl
Cortisone	Methaqualone	Trimipramine
Chlorpheniramine	Morphine	Tryptophen
Cocaine	Nalbuphine	Tyramine
Codeine	Nalophine	Venlafaxine
Cotinine	Nalxone	Verapamil
Despiramine	Natrexone	11-nor-A ¹ -THC-9-COOH (10 µg/ml)*
Dextromethorphan	Neomycin	11-nor-A ¹ -THC-9-COOH (10 µg/ml) *
Deoxyephedrine	Niacinamide	
Digitoxin	Nicotine	
Digoxin	Noigertaline	
Diphenhydramine	Nortriptyline	
Diphenylhydramine	Orphenadine	
Doxylamine	Oxcarbazepine	
Eggonine	Oxycodone	
Eggonine methyl ester	Oxymorphone	

*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>.
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Express Diagnostics Int'l, Inc.
1550 Industrial Drive
Blue Earth, MN 56013 USA



CEPartner4U
Esdoornlaan 13
3951 DB Maarn
The Netherlands



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