CONFIRM BIOSCIENCES

Synthetic Cannabis (K2/Spice and or K3 /Spice) Dip Card Tests

A rapid, one-step screening test for the qualitative detection of Synthetic Cannabis (K2/K3 Spice) in human urine.

Intended Use

The Synthetic Cannabis K2/K3 Dip Card Test is lateral flow chromatographic immunoassays for the qualitative detection of its drug metabolites in human urine at the following cut-off concentration:

Test	Calibrator	Cut-off
Synthetic Cannabinoid (K2)	JWH-018/JWH-073	50 ng/mL
Synthetic Cannabinoid (K3)	AB-Pinaca	25 ng/mL

The tests are used to obtain visual qualitative results and are intended **for forensic use only** to assist in the determination of drug compliance.

This assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed assay result. Liquid Chromatography/Mass Spectrometry (LC/MS) is the preferred confirmatory methods.

Summary and Explanation

Urine based screening tests for drugs of abuse range from simple immunoassay tests to complex analytical procedures. The speed and sensitivity of immunoassays have made them the most widely accepted method of screening urine for drugs of abuse.

Synthetic Canniboid, also known as "Spice", is a chemical product created for users to experience cannabis-like effects after smoking. This new form of "bio-drug" has gained popularity amongst the younger generation and can be obtained quite easily. Some studies suggest that synthetic cannabinoid intoxication is associated with acute psychosis and may trigger a chronic long-term psychotic disorder among vulnerable individuals such as those with a family history of mental illness. The K2/K3 Dip Card test is based on the principle of the highly specific immunochemical reactions of antigens and antibodies, which are used for the analysis of the specific compounds in human urine. The length of time following drug use for which a positive result may occur is dependent upon several factors including the frequency of use, amount of drug, metabolic rate, excretion rate, drug half-life, the drug user's age, weight, activity and diet.

Test Principle

The K2/K3 Dip Card test is a one-step competitive lateral flow immunoassay in which chemically modified drugs (drug-protein conjugates) compete for limited antibody binding sites with the drug which may be present in urine. The test device contains membrane strips which are pre-coated with drug-protein conjugates on the test band. The drug antibody-colloidal gold conjugate pad is placed at one end of the membrane on each test strip. In the absence of drug in the urine, the solution of the colored antibody-colloidal gold conjugate moves along with the sample solution chromatographically by capillary action across the membrane to the immobilized drug-protein conjugate zone on the test band region. The colored antibody-gold conjugate then attaches to the drug-protein conjugates to form visible lines as the antibody complex with the drug conjugate. Therefore, the formation of the visible precipitant in the test zone occurs when the test urine is negative for the drug. When the drug is present in the urine, the drug/metabolite antigen competes with drug-protein conjugate on the test band region for the limited antibody. When a sufficient concentration of the drug is present, it will fill the limited antibody binding sites which will prevent attachment of the colored antibody (drug-protein conjugate)-colloidal gold conjugate to the drug-protein conjugate zone on the test band region. Therefore, absence of the color band on the test region indicates a positive result.

A control band with a different antigen/antibody reaction is added to the membrane strip at the control region (C) to indicate that the test has performed properly. This control line should always appear regardless of the presence of drug or metabolite. If the control line does not appear the test device should be discarded.

Materials Provided

Each Synthetic Dip Card Test Kit contains:

1. Package Insert (PI)
2. 25 devices are packaged in each kit. Each test device contains the reagent strip housed in a separate strip channel in the plastic holder.

Materials Required But Not Provided

1. Specimen collection container
2. Timer
3. External urine controls (optional)

Warnings and Precautions

- For employment, insurance and Forensic use only
- The pouch containing the device should be sealed. Discard the test device if package is ripped or torn.
- Urine specimens may be potentially hazardous and should be handled in the same manner as an infectious agent.
- Avoid cross-contamination of urine samples by using a new container for a different urine sample. Do not reuse the container for different urine sample collection.

Product Storage

The pouched device should be stored at normal humidity and room temperature or refrigerated (2-30ºC) until the expiration date stated on the pouch. The product is sensitive to humidity and should be used immediately after being opened. Any test in an improperly sealed pouch should be discarded.

Specimen Collection and Handling

Urine Assay: The urine specimen must be collected in a clean and dry container. The urine sample can be collected and tested at any time of the day. Fresh urine does not require any special handling or pretreatment.
Urine Storage: It is recommended that the collected fresh urine be tested immediately. Fresh urine may be stored at room temperature (25ºC) for up to 4 hours or stored refrigerated (2-8ºC) for up to 48 hours prior to performing the test. Specimens that have been refrigerated must be brought to room temperature prior to testing.
Note: Urine specimens and all materials coming in contact with them should be handled and disposed of as if capable of transmitting infection. Avoid contact with skin by wearing gloves and proper laboratory attire.

Test Procedure

IMPORTANT: The test device and patient's sample, or controls should be brought to room temperature (15-30°C) prior to testing. Do not open pouches until ready to perform the assay.
Remove the test device from the sealed pouch and use it as soon as possible.

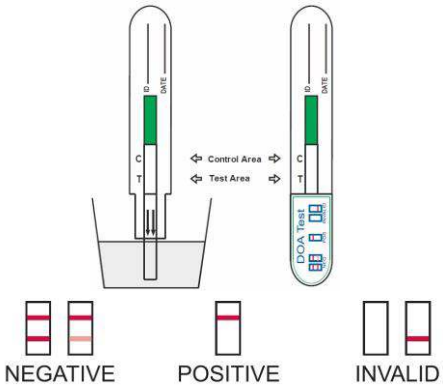
1. Remove the cap from the end of test card. Label the device with patient ID or control number.
2. With arrows pointing toward the urine specimen, immerse the strip(s) of the test card vertically in the urine specimen for at least 10 to 15 seconds.
3. Replace the cap and place the test card on a non-absorbent flat surface. Start the timer and wait for the colored line(s) to appear.
4. The result(s) should be read at 5 minutes. Do not interpret the result(s) after 8 minutes. Positive test results must be confirmed by another test method. Send the entire urine specimen to a toxicology laboratory for confirmation.

Interpretation of Results

Negative: A colored line appears in the control (C) region and a colored line appears in the test region (T). This negative result indicates that the drug concentration in the urine specimen is below the designated cut-off levels for the drug tested. The color intensity of the line for the drug may be weaker or stronger than that of the control line.

Positive: A colored line(s) appears in the control region (C). The absence of a colored line in the test region (T) indicates a positive result.

Invalid: No line appears in the control region (C). Under no circumstances should a positive sample be identified until the control line (C) forms in the viewing area. If the control line (C) does not form, the test result is inconclusive and the assay should be repeated with a new device.



Quality Control

A built-in procedural control is included in the test by using a different antigen/antibody reaction at the control region (C) on each test strip. This control line should always appear regardless of the presence of drug or metabolite. If the control line does not appear, the test device should be discarded. The presence of this control line in the control region serves as 1) verification that sufficient volume is added and 2) that proper flow is obtained.

Good Laboratory Practice recommends the use of control materials to ensure proper device performance. External controls are not provided in the kit. However, they are available from commercial sources and it is recommended that positive and negative controls be used to verify proper test performance. Use the same assay procedure as with a urine specimen. Users should follow the appropriate federal, state, and local guidelines concerning the running of external quality controls.

Limitations 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 other substances, as factors not listed, may interfere with the test and cause false results. See SPECIFICITY 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 drugs of abuse and certain foods and medicines.

Performance Characteristics
Cutoff Characterization

The sensitivity of the K2 Dip Card Tests were determined to be 50 ng/mL of JWH-018 5-Pentanoic acid metabolite. Other K2 compounds listed in the Specificity section can be detected at the indicated concentration level.
The sensitivityof the K3 test devices was determined to be 25 ng/mL of AB-Pinaca. Other K3 compounds listed in the Specificity section can be detected at the indicated concentration level.

Specificity

The specificity for the K2 Dip Card test has been tested by adding various drugs, drug metabolites, and other structurally related compounds that are likely to be present in normal human urine. The following compounds were found to produce positive results when tested at levels greater than the concentrations (in ng/mL) listed below:

JWH-018 5-Pentanoic Acid related compounds	Concentration (ng/mL)
JWH-018 5-Pentanoic acid	50 ng/mL
JWH-018 N-propanoic acid	25 ng/mL
JWH-073 N-butanoic acid	25 ng/mL
MAM2201 N-pentanoic acid	100 ng/mL
JWH-210 N-pentanoic acid	200 ng/mL
JWH-398 N-pentanoic acid	200 ng/mL

The specificity for the K3 Dip Card test has been tested by adding various drugs, drug metabolites, and other structurally related compounds that are likely to be present in normal human urine. The following compounds were found to produce positive results when tested at levels greater than the concentrations (in ng/mL) listed below:

AB-Pinaca and related compounds	Concentration (ng/mL)
AB-Pinaca	25 ng/mL
AB-Pinaca metabolite	25 ng/mL
AB-Fubica	25 ng/mL

Interference

The following compounds were found not to interfere when tested at the listed concentrations.

Human Albumin	2000 ng/mL
Human Hemoglobin	10 mg/dL
Glucose	2000 mg/dL
Urea	4000 mg/dL
Uric Acid	10 mg/dL

Effect of Urine pH

The pH ranges of 3.0 to 8.5 were prepared by adjusting the drug urine controls at ±25% and ±50% cut-off levels, respectively. The testing results demonstrate that the varying ranges of urine pH do not affect the test performance.

Effect of Urine Specific Gravity

The specific gravity (SG) ranges of 1.002, 1.010, 1.015, 1.020, 1.025 and 1.030 were prepared by adjusting the drug urine controls at ±25% and ±50% cut-off levels, respectively. The testing results with the K2 Dip Card tests demonstrate that the varying ranges of urine SG do not affect the test results.

Bibliography of Suggested Reading

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