KRA-U23

KRA
Rapid Test Device
(Urine)

For forensic use only

INTENDED USE

The KRA Rapid Test Device (Urine) is a rapid visual immunoassay for the qualitative presumptive detection of KRA kratom and its metabolite in human urine specimens at the cut-off concentrations listed below:

Parameter	Calibrator	Cut-off (ng/mL)
KRA	7-hydroxymitragynine	500

INTRODUCTION

Mitragyna speciosa is a tropical evergreen tree in thecoffee family native to Southeast Asia. M. speciosa isindigenous to Thailand, Indonesia, Malaysia, Myanmar, and Papua New Guinea, where it has beenused in traditional medicines since at least thenineteenth century. Kratom has opioid properties andsome stimulant-like effects.

Mitragynine is classified as a kappa-opioid receptor agonist and is roughly 13 times more potent than morphine. Mitragynine is thought to be responsible for the opioid-like effects

Kratom, due to its opioid-like action, has been used for treatment of pain and opioid withdrawal. Animal studies suggest that the primary mitragynine pharmacologic action occurs at the mu and delta-opioid receptors, as well as serotonergic and noradrenergic pathways in the spinal cord. Stimulation at post-synaptic alpha-2 adrenergic receptors, and receptor blocking at 5-hydroxytryptamine 2A may also occur. The 7-hydroxymitragynine may have a higher affinity for the opioid receptors. Partial agonist activity may be involved.

PRINCIPLE

The KRA Rapid Test Device (Urine) has been designed to detect kratom and its metabolite through visual interpretation of color development in the Device. The membrane was immobilized with KRA conjugates on the test region, and the sample pad was pre-coated with colored anti- KRA antibodies colloidal gold conjugates. After specimens were added, the gold-conjugates move along the membrane chromatographically by capillary action and antibodies get to the test region. If there is no drug molecule in the urine the antibody gold conjugate would attach to the drug conjugate to form a visible line. Therefore, the formation of a visible precipitant in the test region occurs when the urine is negative for the drug. If KRA are present in the urine, the drug antigen competes with the immobilized drug conjugate on the test region for limited antibody sites. In case of sufficient concentration of the drug, it fills the limited antibody binding sites. This will prevent attachment of the colored antibody-colloidal gold conjugate to the drug conjugate zone on the test region. Therefore, absence of the colored band on the test region indicates a positive result. Appearance of a colored band at the control region serves as a procedural control. This indicates that proper volume of specimen has been added and membrane wicking has occurred.

REAGENTS

Each test consists of a reagent Device mounted in a plastic housing. The amount of each antigen and/or antibody coated on the Device is less than 0.001 mg for antigen conjugates and goat anti-rabbit IgG antibodies, and less than 0.0015 mg for antibody components.

The control zone of each test contains goat anti-rabbit IgG antibody. The test zone of each test contains drug-bovine protein antigen conjugate, and the conjugate pad of each test contains monoclonal anti-drug antibody and rabbit antibody-colored particle complex.

MATERIALS

Materials Provided

- Test Devices
- Disposable pipettes
- Package insert

Materials Required but Not provided

- Positive and negative controls
- Centrifuge
- Timer

PRECAUTIONS

- For forensic use only.
- Do not use after the expiration date indicated on the package. Do not use the test if the foil pouch or canister is damaged. Do not reuse tests.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not completely guarantee the absence of transmissible pathogenic agents. It is therefore, recommended that these products be treated as potentially infectious, and handled by observing usual safety precautions (e.g., do not ingest or inhale).
- Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
- Read the entire procedure carefully prior to testing.
- Do not eat, drink or smoke in the area where specimens and kits are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow standard procedures for the proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- Humidity and temperature can adversely affect results.
- Used testing materials should be discarded in accordance with local regulations.

STORAGE AND STABILITY

- The kit should be stored at 2-30°C until the expiry date printed on the sealed pouch or canister.
- The test must remain in the sealed pouch or closed canister until use.
- **Do not freeze.**
- Kits should be kept out of direct sunlight.
- Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

SPECIMEN COLLECTION AND STORAGE

- The KRA Rapid Test Device (Urine) is intended for use with human urine specimens only.
- Urine collected at any time of the day may be used.
- Urine specimens must be collected in clean, dry containers.
- Turbid specimens should be centrifuged, filtered, or allowed to settle and only the clear supernatant should be used for testing.
- Perform testing immediately after specimen collection. Do not leave specimens at room temperature for prolonged periods. Urine specimens may be stored at 2-8°C for up to 2 days. For long term storage, specimens should be kept below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Avoid repeated freezing and thawing of specimens.

If specimens are to be shipped, pack them in compliance with all applicable regulations for transportation of etiological agents.

PROCEDURE

Bring tests, specimens, and/or controls to room temperature (15-30°C) before use.

1. Remove the test from its sealed pouch, and place it on a clean, level surface. Label the test with patient or control identification. For best results, the assay should be performed within one hour.
2. Using the provided disposable pipette, transfer 3 drops of specimen (approximately 120 µL) to the specimen well (S) of the device and start the timer.
   Avoid trapping air bubbles in the specimen well (S), and do not add any solution to the result area.
   As the test begins to work, color will migrate across the membrane.
3. Wait for the colored band(s) to appear. The result should be read at 5 minutes. Do not interpret the result after 8 minutes.

INTERPRETATION OF RESULTS

POSITIVE: Only one colored band appears, in the control region (C). No colored band appears in the test region (T). A positive result indicates that the drug concentration exceeds the detectable level .

NEGATIVE: Two colored bands appear on the membrane. One band appears in the control region (C) and another band appears in the test region (T). A negative result indicates that the drug concentration is below the detectable level .

INVALID: Control band fails to appear. Results from any test which has not produced a control band at the specified read time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

1. The intensity of color in the test region (T) may vary depending on the concentration of analytes present in the specimen. Therefore, any shade of color in the test region (T) should be considered negative. Please note that this is a qualitative test only, and cannot determine the concentration of analytes in the specimen.
2. Insufficient specimen volume, incorrect operating procedure or expired tests are the most likely reasons for control band failure.

QUALITY CONTROL

- Internal procedural controls are included in the test. A colored band appearing in the control region (C) is considered an internal positive procedural control, confirming sufficient specimen volume and correct procedural technique.
- External controls are not supplied with this kit. It is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS OF THE TEST

1. The KRA Rapid Test Device (Urine) is for forensic use, and should be only used for the qualitative detection of KRA.
2. This assay provides a preliminary analytical test result only. 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 National Institute on Drug Abuse (NIDA). Clinical consideration and professional judgment should be applied to any test result, particularly when preliminary positive results are indicated.
3. There is a possibility that technical or procedural errors as well as other substances and factors may interfere with the test and cause false results.
4. Adulterants, such as bleach and/or alum, in urine specimens may produce erroneous results regardless of the analytical method used. Therefore, please preclude the possibility of urine adulteration prior to testing.
5. A positive result indicates the presence of a KRA only, and does not indicate or measure intoxication.
6. A negative result does not at any time rule out the presence of KRA in urine, as they may be present below the minimum detection level of the test.

PERFORMANCE CHARACTERISTICS

A. Accuracy

The accuracy of the KRA Rapid Test Device (Urine) was compared and checked against commercially available tests with a threshold value at the same cut-off levels. Urine samples taken from volunteers claiming to be non-users were examined under both tests. The results were >95.0% in agreement.

B. Reproducibility

The reproducibility of the KRA Rapid Test Device (Urine) was verified by blind tests performed at four different locations. Samples with KRA concentrations at 50% of the cut-off were all determined to be negative, while samples with KRA concentrations at 150% of the cut-off were all determined to be positive.

C. Precision

Test precision was determined by blind tests with control solutions. Controls with KRA concentrations at 50% of the cut-off yielded negative results, and controls with KRA concentrations at 150% of the cut-off yielded positive results.

D. Specificity

The following tables list the concentrations of compounds (ng/mL) above which the KRA Rapid Test Device (Urine) identified positive results at 5 minutes.

KRA **related compounds**	**Concentration (ng/ml)**
7-hydroxymitragynine	500
Mitragynine	6000

The following compounds yielded negative results up to a concentration of 100 µg/mL:

Acetaminophen	Diazepam	Morphine Sulfate
Acetone	4-Dimethylaminoantipyrine	Myoglobin
Acetylsalicylic acid	Diphenhydramine	Nalophine
Albumin	Dopamine	Nicotine
Amitriptyline	Ecgonine HCL	Niacinamide
Amobarbital	Ecgonine Methyl Ester	Nortriptyline
Amphetamine	EDDP	Omeprazole
Ampicillin	Efavirenz	Oxalic Acid
Ascorbic Acid	Ephedrine	Oxycodone
Atropine Sulfate	(+/-)-Epinephrine	Oxymorphone
Benzocaine	Erythromycin	Oxazepam
Benzoylecgonine HCL	Ethanol	Pantoprazole
Bilirubin	Furosemide	Penicillin-G
Bup-3-B-glucuronide	Glucose	Pentobarbital
Buprenorphine	Hemoglobin	Pheniramine
Butalbital	Hippuric acid	d-Propoxyphene
Caffeine	Hydrocodone	Phencyclidine
Cannabidiol	Hydromorphone	Phenylephrine
Cannabinol	HU-211	B-Phenylethylamine
Chloroquine	Ibuprofen	Procaine
(+)-Chlorpheniramine	Immipraime	Pseudoephedrine
(+/-)-Chlorpheniramine	(+/)-Isoproterenol	Quinidine
+/- CP 47,497	11-hydroxy-delta-9-THc	Ranitidine
Cocaine	11-nor-Ac-THC-9-COOH	Riboflavin
Codeine	Ketamine	RSC-4-N-5-hydroxlpenfyl
Cotinine	Lansoprazole	Secobarbital
Creatine	Lidocaine	Sodium Chloride
Delta-8-tetrahydrocannabinol	MDA	Sulindac
Dexbrompheniramine	MDMA	Theophylline
Dextromethorphan	Methadone	Trimipramine
Dextrose	Methamphetamine	Tyramine
JWH-200	JWH-250	Urea

LITERATURE REFERENCES

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GLOSSARY OF SYMBOLS

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		Do not reuse