Acro Rapid Benzoylecgonine (Cocaine) Urine Test
For *in vitro* diagnostic use only
COCCS300

INTENDED USE

Acro Rapid Benzoylecgonine (Cocaine) Urine Test is a lateral flow, rapid immunoassay for the qualitative detection of benzoylecgonine in human urine at 300 ng/mL cut-off concentration. This assay is used to obtain a visual, qualitative result and is intended for laboratory use only.

This assay provides only a preliminary result. Clinical consideration and professional judgment must be applied to a drug test result, particularly in evaluating a preliminary positive result. In order to obtain a confirmed analytical result, a more specific alternate chemical method is needed. Gas Chromatography/Mass Spectroscopy (GC/MS) analysis is the preferred.

SUMMARY AND EXPLANATION

Cocaine is a potent central nervous system stimulant and a local anesthetic found in the leaves of the coca plant. The psychological effects induced by using cocaine are euphoria, confidence and sense of increased energy. These psychological effects are accompanied by increased heart rate, dilation of the pupils, fever, tremors and sweating. Cocaine is excreted in the urine primarily as Benzoylecgonine in a short period of time. Benzoylecgonine has a biological half-life of 5 to 8 hours, which is much longer than that of cocaine (0.5 to 1.5 hours), and can be generally detected for 24 to 60 hours after cocaine use and exposure.

The length of time following drug use for which a positive result may occur is dependent upon several factors, including the frequency and amount of drug, metabolic rate, excretion rate, drug half-life, the drug user's age, weight, activity and diet.

TEST PRINCIPLE

The Acro Rapid Benzoylecgonine (Cocaine) Urine Test is based on the principle of competitive immunochemical reaction between an immobilized drug-protein conjugate and the drug or drug metabolites, which may be present in the urine sample for limited antibody binding sites of a labeled drug antibody. The test contains a nitrocellulose membrane strip pre-coated with drug-protein conjugate in the test region and a wicking pad containing colored antibody-colloidal gold conjugate. During the test, the urine sample is allowed to migrate upward and hydrate the antibody-colloidal gold conjugate. The mixture then migrates along the membrane chromatographically by capillary action to the immobilized drug-protein band in the test region. When drug is absent in the urine, the colored antibody-colloidal gold conjugate and immobilized drug-protein bind specifically to form a visible line in the test region(test line). When benzoylecgonine is present in the urine sample, it will compete with the drug-protein conjugate for the limited antibody binding sites. The test line will be less intense with increasing drug concentration. When the drug is present in sufficient concentration in the urine sample, it will fill the limited antibody binding sites, which will inhibit attachment of the colored antibody-colloidal gold conjugate to the drug-protein conjugate in the test region. Therefore the presence of the test line indicates a **negative** result for benzoylecgonine and the absence of the test line indicates a **preliminary positive** result for benzoylecgonine.

Another visible line, control line, generated by a different antigen/antibody reaction is also present at a control region (C) next to the test region of the test strip. The control line should always appear, regardless of the presence of the drug or drug metabolites in the urine sample. This means that a **negative** urine sample will produce **two** lines (test line and control line), and a **positive** urine sample will generate only **one** line (control line). The presence of control line serves as a built-in control, which demonstrates that the test is performed properly.

REAGENTS & MATERIALS SUPPLIED

1. Test Cassette contains membrane-embedded reagents in a protein matrix containing sodium azide as a preservative.
2. Anti-Benzoylecgonine monoclonal antibody is from murine ascities.
3. Dropper. A transfer pipette is included with each test device inside the foil pouch.
4. Test Instructions

MATERIALS REQUIRED, BUT NOT PROVIDED

1. Timer
2. Sample container.

WARNINGS AND PRECAUTIONS

1. For laboratory *in vitro* diagnostic use only
2. Urine specimens may be potentially infectious. Proper handling and disposal procedures should be followed.
3. Avoid cross-contamination of urine samples. Use a new dropper or transfer pipette for adding each test sample.
4. Test device should remain sealed until ready for use.
5. Do not use the test kit after the expiration date.

STORAGE AND STABILITY

Store at 2-30°C (36-86°F) in the original sealed pouch. Do not freeze.

SPECIMEN COLLECTION AND HANDLING

Fresh urine does not require any special handling or pretreatment. A fresh urine sample should be collected in the container provided. Alternatively, a clean dry plastic or glass container may be used for specimen collection. If the specimen is not immediately tested after the specimen collection, the specimen may be refrigerated at 2-8°C for up to 2 days or frozen at -20°C for longer period of time. Specimens that have been refrigerated must be equilibrated to room temperature prior to testing. Frozen specimens must be thawed and mixed thoroughly prior to testing.

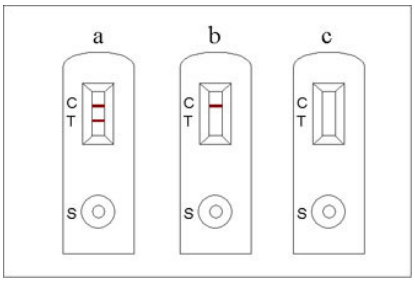
Note: Urine specimens and all materials coming in contact with the specimens should be handled and disposed as if capable of transmitting infection. Avoid contact with skin by wearing gloves and proper laboratory attire.

ASSAY PROCEDURE

1. Bring the test components and urine sample to room temperature (15° - 28º C) before testing. Do not open the foil pouch until ready to begin testing.
2. Open the foil pouch at the notch and remove the test device and dropper prior to testing. Place the device on a clean, level surface.
3. Hold the dropper vertically and dispense 3-4 drops (~ 100-120 µl) of urine sample without air bubbles into the sample well "S" of the test device.
4. Read the result between 5-10 minutes.

IMPORTANT: *The result must be read at five to ten minutes. Waiting more than ten minutes may cause the test result to be inaccurate.*

INTERPRETATION OF RESULTS



1. **Preliminary Positive**: a *rose-pink* color band appears in the Control Zone "C" but not in the Test Zone "T". A preliminary positive result indicates benzoylecgonine level in the urine sample is at or above the detection sensitivity of 300 ng/mL. The sample should be confirmed.
2. **Negative**: two horizontal *rose-pink* color bands appear, one in the Control zone "C" and one in the Test Zone "T". A negative result indicates benzoylecgonine level in the urine sample is below the detection sensitivity of 300 ng/mL.
3. **Invalid**: no *rose-pink* bands appear, or a band appears in the Test Zone "T", but not in the Control Zone "C". An invalid result may be due to improper testing procedures or deterioration of the kit components.

Note: There is no meaning attributed to line color intensity or width.

QUALITY CONTROL

An internal procedure control is included in the test device. A control line must form regardless of the presence or absence of drugs or metabolites. The presence of the line in the Control region indicates that a proper sample volume has been used. If the line in the Control region does not form, the test is considered invalid.

To ensure proper kit performance, it is recommended that the test devices be tested once a week or prior to use with external controls. External controls are available from commercial sources. It is important to make sure that the control values are within established limits. If the values of external control do not fall within established limits, the test results are invalid. Additional controls may be tested according to guidelines or requirement of local state, and/or federal regulations or accrediting organizations.

LIMITATIONS OF PROCEDURE

1. The assay is designed for use with human urine only.
2. A preliminary positive result indicates only the presence of benzoylecgonine and does not indicate or measure intoxication

3. 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 section for substances that will produce positive results, or that do not interfere with the test performance.
4. If adulteration is suspected, the test should be repeated with a new sample.
5. Certain over the counter or prescription medications (or certain foods) may cause false results.

PERFORMANCE CHARACTERISTICS

1. **Sensitivity**. The Acro Rapid Benzoylecgonine (Cocaine) Urine Test detects cocaine and its metabolites in urine at concentrations equal to or greater than 300 ng/mL.
2. **Specificity**. Interference of substances that may be present in urine specimens, as well as effect of sample pH and specific gravity was also studied.
a. Cross-reactivity of non-benzoylecgonine related compounds at concentrations much higher than normally found in the urine of people using or abusing them were tested using the assay devices. No cross-reactivity was detected with the substances listed in Table I.
b. Table II lists Benzoylecgonine related substances and concentrations that produced results approximately equivalent to the cutoff level for benzoylecgonine.
c. Varying sample pH within the range of 4 and 9 has no significant effect on the assay results.
d. Varying sample specific gravity within the range of 1.003 and 1.040 has no significant effect on the assay results.

Table-I: Compounds tested and found not to cross-react with the test at the indicated concentrations in urine.

Acetaminophen (100 ug/mL)
Acetone (100 ug/mL)
Albumin (500 ug/mL)
Ampicillin(100 ug/mL)
Ascorbic Acid (500 ug/mL)
Aspartame(100 ug/mL)
Aspirin (100 ug/mL)
Atropine (100 ug/mL)
Benzocaine (100 ug/mL)
Bilirubin (100 ug/mL)
Caffeine (100 ug/mL)
Chloroquine (100 ug/mL)
(+)-Chlorpheniramine (100 ug/mL)
(+/-)-Chlorpheniramine (100 ug/mL)
Creatine (500 ug/mL)
Dexbrompheniramine (100 ug/mL)
Dextromethrophan (100 ug/mL)
Diphenhydramine (100 ug/mL)
Dopamine (100 ug/mL)
(+/-)-Epinephrine (100 ug/mL)
Erythromycin (100 ug/mL)
Ethanol (0.2%)
Furosemide (100 ug/mL)
Glucose (500 ug/mL)
Guaiacol Glyceryl Ether (100 ug/mL)
Hemoglobin (500 ug/mL)
Ibuprofen (200 ug/mL)
(+/-)-Isoproterenol (100 ug/mL)
Ketamine (100 ug/mL)
Levorphanol (100 ug/mL)
Lidocaine (100 ug/mL)
(+)-Naproxen (100 ug/mL)
Niacinamide (100 ug/mL)
Nicotine (100 ug/mL)
(+/-)-Norephedrine (100 ug/mL)
Oxalic Acid (100 ug/mL)
Penicillin-G (100 ug/mL)
Pheniramine (100 ug/mL)
Phenothiazine (100 ug/mL)
1-Phenylephrine (100 ug/mL)
β -Phenylethylamine (100 ug/mL)
Procaine (100 ug/mL)
Quinidine (100 ug/mL)
Ranitidine (100 ug/mL)
Riboflavin (100 ug/mL)
Sodium Chloride (10,000ug/mL)
Sulindac (100 ug/mL)
Theophylline (100 ug/mL)
Tyramine (100 ug/mL)
4-Dimethylaminoantipyine (100 ug/mL)
(1R, 2S)-(-)-N-Methyl-Ephedrine (100 ug/mL)

Table-II: Concentration of cocaine-related compounds showing a positive response approximately equivalent to the cocaine cut-off set for the test.

Compound	**Concentration in ng/ml**
Cocaine	*300 ng/ml*
Benzoylecgonine	*300 ng/ml*
Cocaethylene	*7,500 ng/ml*
Ecgonine	*15,000 ng/ml*
Ecgonine Methyl Ester	*15,000ng/ml*

3. **Accuracy**. The accuracy study was performed by testing clinical benzoylecgonine urine samples with the device and comparing the results with a predicate immunoassay device and GC/

4. **Cut-off study**. The cut-off of the test was determined by the repetitive assaying of six levels of benzoylecgonine controls. The resultant data are summarized as follows:

Benzoylecgonine	# Tested	# Positive (+)	# Negative (-)	% Correct Results
0 ng/mL	60	0	60	100%
150 ng/mL	60	0	60	100%
225 ng/mL	60	16	44	73%
600 ng/mL	60	40	20	67%
375 ng/mL	60	45	15	75%
450 ng/mL	60	60	0	100%
600 ng/mL	60	60	0	100%

5. **Reproducibility**. The reproducibility was evaluated at four different sites. The Acro Rapid Benzoylecgonine (Cocaine) Urine Test was tested against blind-labeled urine controls containing 0, 150, 225, 375, 450 and 600 ng/mL benzoylecgonine at each site. The results are summarized as follows:

Test Sites	0 ng/mL		150 ng/mL		225 ng/mL		375 ng/mL		450 ng/mL		600 ng/mL	
	#	Result	#	Result	#	Result	#	Result	#	Result	#	Result
1	15	15-	15	15-	15	4+, 11-	15	12+, 3-	15	15+	15	15+
2	15	15-	15	15-	15	4+, 11-	15	11+, 4-	15	15+	15	15+
3	15	15-	15	15-	15	5+, 10-	15	11+, 4-	15	15+	15	15+
4	15	15-	15	15-	15	3+, 12-	15	11+, 4-	15	15+	15	15+
Total	60	60-	60	60-	60	16+, 44-	60	45+, 15-	60	60+	60	60+

MS analysis results.

Acro Rapid Test	GC/MS, Cutoff 300ng/ml				Percent Agreement with GC/MS
	Negative (<50% cutoff)	Near Cutoff Negative (– 50% to cutoff)	Near Cutoff Positive (cutoff to +50% cutoff)	Positive (> +50%)	
Positive	0	3	7	40	47/50 = 94%
Negative	60	7	3	0	67/70 = 96%

Total % agreement with GC/MS: 114/120=96%

6. **Stability Study.** As determined by temperature accelerated stability study method, the shelf life of the product under the specified storage condition is 24 months from the date of production.

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