RAPID COTININE TEST

FOR THE QUALITATIVE ASSESSMENT OF COTININE IN HUMAN URINE

Catalog Number: 1L44S3, 1L44C3

INTENDED USE

Rapid Cotinine Test is an immunochromatography based one step in vitro test. It is designed for qualitative determination of cotinine in human urine specimens at cut-off level of 200 ng/ml. This assay has not been evaluated in the point of care location and is for use by Healthcare Professionals 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

Cotinine is an alkaloid found in tabacco and is also a major metabolite of nicotine. Cotinine is used as a biomarker for exposure to tobacco smoke and has also been sold as an anti-depressant under the brand name of Scotine. Cotinine has an in vivo half-life of approximately 20 hours, and is typically detectable for several days after the use of tobacco. The level of cotinine is proportionate to the amount of exposure to tobacco smoke. In urine, values between 11 ng/ml and 30 ng/ml may be associated with light smoking or passive exposure. The cotinine levels in active smokers typically reach 500 ng/ml or more.

TEST PRINCIPLE

Rapid Cotinine 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, 200 ng/ml of cotinine, 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. Rapid Cotinine 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 cotinine protein antigen conjugates.
   Control zone: contains Goat anti-rabbit IgG antibody.
   Conjugate pad: contains rabbit anti-cotinine antibody.

MATERIALS REQUIRED BUT NOT PROVIDED

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

STORAGE AND STABILITY

The test device should be stored at 2 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 in vitro diagnostic and 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 2-8ºC 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 Rapid Drugs of Abuse 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 (Catalog Number: 1L44S3)

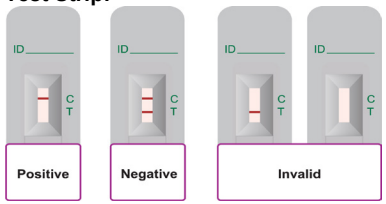
1. Bring all materials and specimens to room temperature.
2. Remove the test strip from the sealed foil pouch.
3. Dip the strip into the urine specimen with the arrow pointing toward the sample. The sample level should not be higher than the arrow pointed maximum line.
4. Hold the strip in the urine until a reddish color appears at the test area (approximately 20 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 to 10 minutes after adding the sample.
   Test results may not be accurate after 10 minutes.

For Test Card (Catalog Number: 1L44C3)

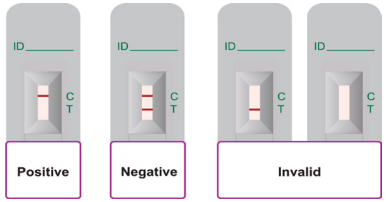
1. Bring all materials and specimens to room temperature.
2. Remove the test card from the sealed foil pouch.
3. Place the transfer pipette in the specimen and depress the bulb to withdraw a sample.
4. Hold the pipette in a vertical position over the sample well of the test card and deliver 3 drops (120-150 μl) of sample in to the sample well.
5. Read the results at 5 to 10 minutes after adding the sample.
   Test results may not be accurate after 10 minutes.

INTERPRETATION OF RESULTS

Test Strip:



Test Card:



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 cotinine 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 buprenorphine 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 other substances in certain foods and medicines may interfere with the test and cause false results. Please refer "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

Cotinine test is a qualitative assay. It identifies cotinine in human urine at a concentration of 200 ng/ml or higher. The concentration of the ketamine cannot be determined by this assay. The test is intended to distinguish negative result from presumptive positive result. All positive results must be confirmed using an alternate method, preferably GC/MS.

PERRFOMANCE CHARACTERISTICS

A. Sensitivity

The cut-off concentration (sensitivity level) of cotinine test card is determined to be 200 ng/ml of cotinine

B. Accuracy

The accuracy of the Rapid Cotinine Test was evaluated in cotinine spiked urine specimens. Forty (40) urine specimens were spiked with cotinine from 100 to 2000 ng/ml. 30 samples with cotinine concentration between 300 and 2500 ng/ml were all found positive (100% agreement), 10 samples with cotinine concentration between 100 and 150 ng/ml were found negative.

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.

D. Specificity

The specificity for cotinine 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 cotinine 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 cotinine 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

3. Specificity

Clonazepam test is specific with clonazepam and its metabolites. It does not cross with many other benzodiazepiens. The following table lists the cross reaction of clonazepam test with the benzodiazepines.

Compounds	Concentration	Cross reactivity
Cotinine	200 ng/ml	100%
S(-)Nicotine	10,000 ng/ml	2%

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-Δ^8–THC-9-COOH (10 µg/ml)* 11-nor-Δ^9–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. Clonazepam Official FDA information, side effects and uses (2010)

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