

ulti med Smoke Check Test One-Step Cotinine Test Cassette



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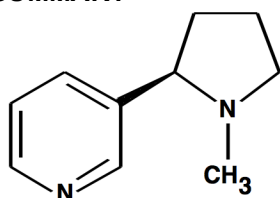
A visual one-step immunoassay for the qualitative detection of cotinine in human urine.

INTENDED USE

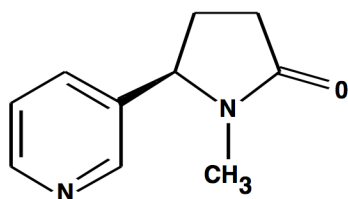
The Cotinine Card Test is a lateral flow, one-step immunoassay used for the qualitative detection of cotinine – the major metabolite of nicotine in human urine at a cut-off concentration of 200 ng/ml. This product is used to obtain a visual, qualitative result and is intended for professional use.

This assay provides only a preliminary analytical test result. A more specific alternative chemical method such as high pressure liquid chromatography (HPLC) or gas chromatography / mass spectrometry (GC/MS) must be applied in order to obtain a confirmed analytical result. Clinical consideration and professional judgement should be applied to all test results, particularly when preliminary positive results are indicated.

SUMMARY



Chemical structure of nicotine.



Chemical structure of cotinine.

The wide spread use of tobacco products has created huge costs to the society. Tobacco smoking results in the absorption of nicotine through the lung and buccal/nasal epithelium. There are about 20 metabolites of nicotine identified in urine. Cotinine is a major metabolite of nicotine, and it accumulates in the body with regular smoking. Nicotine and cotinine are metabolised by the same liver enzyme. It is reported that cotinine is stable in body fluids and has a relatively long half-life of approximately 17 hours, and is therefore less dependent on the time of sampling than that of nicotine and other metabolites. Cotinine has been widely used as a biomarker of tobacco exposure. Methods of analysis for cotinine in biological fluids include gas chromatography, gas chromatography-mass spectrometry, HPLC, HPLC-mass spectrometry and RIA. These methods usually require special equipment and complicated operation procedures. The Cotinine Card Test is a one step immunoassay that is used for the qualitative detection of cotinine in human urine. It is based on the principle of highly specific immunochemical reactions of antigens and antibodies. It is rapid, simple and convenient to be used for the qualitative detection of cotinine in human urine at 200 ng/ml cut-off concentration.

PRINCIPLE

The Cotinine Card Test applies the principle of competitive antigen-antibody binding. The test device contains a membrane strip that is pre-coated with cotinine antigen at the test line region. The cotinine antibody gold conjugate pad is placed at the end of the membrane. In cotinine free urine, the solution of coloured antibody-colloidal gold conjugate and urine moves chromatographically by capillary action across the membrane. This solution then migrates to the immobilized test line containing cotinine antigen and forms a visible line as the antibody complexes with the antigen. The formation of a visible precipitant in the test zone occurs when the test urine is negative (non-smoker). When cotinine is present in urine, it competes with cotinine that is pre-fixed on the test line region for the limited antibody sites on the antibody-colloidal gold conjugate. When a sufficient concentration of cotinine is present in urine, it will fill the limited antibody binding sites. This will prevent attachment of the coloured antibody-colloidal gold conjugate at the test line region. Therefore, absence of the coloured line on the test region indicates a positive result (smoker). A control line that has a different antigen/antibody reaction is added to the immunochromatographic membrane strip at the control region (C) to indicate that the test has performed properly. This control line should always appear, regardless of the cotinine status in the urine. This means that negative urine will have two coloured lines, and positive urine will have only one line. The presence of this coloured line in the control region serves as an indicator that 1) sufficient volume of sample has been added and 2) proper flow was obtained.

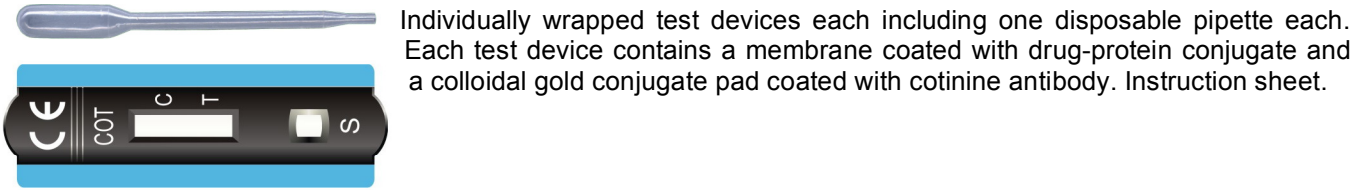
STORAGE AND STABILITY

The test kit may be refrigerated or stored at room temperature of 2-30°C (36-86°F) in the sealed pouch for the duration of the shelf life.

PRECAUTIONS

For laboratory use only. Urine specimens may be potentially infectious. Proper handling and disposal methods should be established. Avoid cross-contamination of urine samples by using a new specimen collection container and specimen pipette for each urine sample. Do not use the test, if the pouch is damaged.

REAGENTS AND MATERIALS SUPPLIED



MATERIAL REQUIRED BUT NOT PROVIDED

- 1. Specimen collection container.
- 2. Timer.

SPECIMEN COLLECTION AND HANDLING

The Cotinine Card Test is designed for urine specimens. Fresh urine does not require any special handling or pre-treatment. Test should be performed soon after the urine specimen is collected, preferably during the same day. The specimen may be refrigerated at 2-8 °C for 3 days, or frozen at -20 °C for a longer period of time. Specimens that have been refrigerated must be equilibrated to room temperature prior to testing. Specimens previously frozen must be thawed, equilibrated to room temperature, and mixed thoroughly prior to testing.

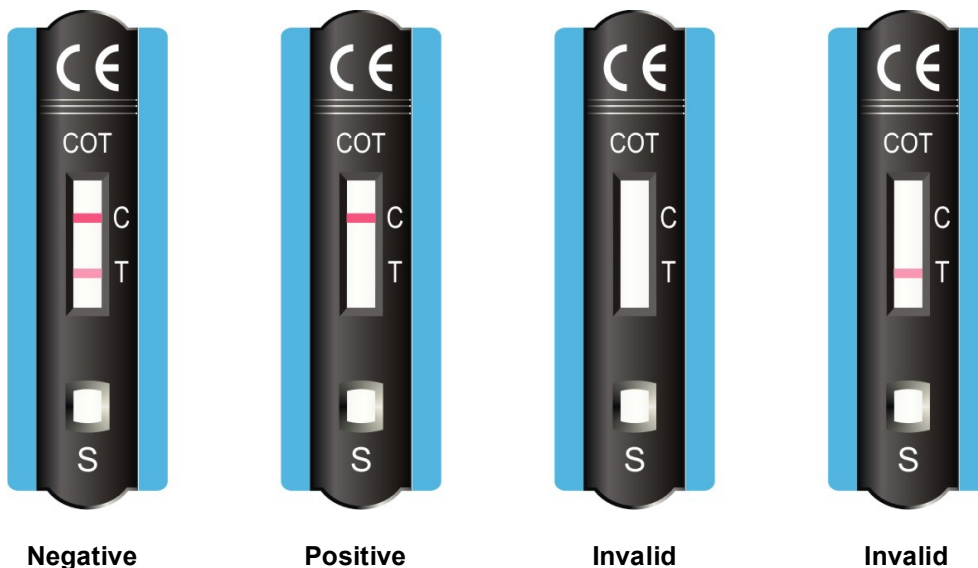
Note: Urine specimens, and all materials coming into contact with them, should be handled and disposed as if capable of transmitting infection. Avoid contact with skin by wearing gloves and proper laboratory attire.

TEST PROCEDURE

- Review "Specimen Collection" instructions. Test device, patient's samples, and controls should be brought to room temperature (20- 30 °C) prior to testing. Do not open pouches until ready to perform the assay. Do not use kit out of a damaged pouch.
- Remove the test device from the protective pouch (bring the device to room temperature before opening the pouch to avoid condensation of moisture on the membrane). Label the device with patient or control identification.
- Draw the urine sample into the pipette. Dispense three drops of urine into the sample well (approximately 100 µl). Use a separate pipette and device for each sample or control.
- Read result within 3 - 8 minutes after the addition of samples. Results may be inaccurate after 8 minutes.

Note: A very faint line on the test region indicates that the cotinine concentration in urine is near the cut-off level for the test. These samples should be retested or confirmed with a more specific method before a positive result is determined.

INTERPRETATION OF RESULTS



- Negative:*** Two lines appear, one red line in the control region (C), and another apparent red or pink line in the test region (T). This negative result indicates that the Cotinine concentration is below the detectable level (200 ng/ml).
- Positive:** One red line appears in the control region (C). No line appears in the test region (T). This positive result indicates that the Cotinine concentration exceeds the detectable level (200 ng/ml).
- Invalid:** Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test using a new test device. If the problem persists, discontinue using the lot immediately and contact distributor / manufacturer.

LIMITATIONS OF PROCEDURE

The assay is designed for use with human urine only. A positive result indicates only that the presence of cotinine is above the cut off concentration. It does not indicate or measure intoxication. There is a possibility that technical or procedural errors as well as other substances or factors not listed may interfere with the test and cause false results. See SPECIFICITY for lists of substances that will produce positive results, or that do not interfere with test performance. If it is suspected that the samples have been mislabelled or deteriorated, a new specimen should be collected and the test should be repeated.

QUALITY CONTROL

Good laboratory practice recommends the use of control materials to ensure proper kit performance. Quality control specimens are available from commercial sources. When testing the positive and negative controls, use the same assay procedure as with a urine specimen.

PERFORMANCE CHARACTERISTICS

Accuracy

The accuracy of the Cotinine Test Cassette was evaluated in comparison to a commercially available immunoassay at a cut off concentration of 200 ng/ml for cotinine. One hundred twenty samples (120), collected from presumed non-smoker volunteers, have been tested by both methods with 100 % agreement. In a separate study, fifty (50) urine samples, obtained from presumed smokers, were determined positive at cotinine concentrations ranging from 300 ng/ml to over 200 ng/ml by a commercially available immunoassay. All these 50 samples were found positive by Ulti Med Smoke Check with 100 % agreement.

Reproducibility

The reproducibility of the Cotinine Test Cassette was evaluated at four different sites using blind controls. Of the sixty (60) samples with cotinine concentration of 100 ng/ml, all were determined negative. Of the sixty samples with cotinine concentration of 400 ng/ml of cotinine, all were determined positive.

Precision

The precision of the Cotinine Test Cassette was determined by conducting the test with spiked controls. The control at 100 ng/ml should give a negative result.

Concentration [ng / ml]	Number	Correct results	Correct results [%]
100	50	50	100
400	50	50	100

Specificity

The specificity of the Cotinine Test Cassette was tested by adding various drugs, drug metabolites, and other compounds that are likely to be present in urine. All compounds were pre-pared in drug-free normal human urine. The following structurally related compounds produce positive results when tested at levels equal to or greater than 350 µg/ml.

(-)-Nicotine.

The following compounds were found not to cross-react when tested at concentrations up to 100 µg/ml.

Acetaminophen	Atropine	(+/-)-Chlorpheniramine
Acetylsalicylic Acid	Benzocaine	Chlorprothixene
Amitriptyline	Benzoyllecgonine	Codeine
D-Amphetamine	[+]-Brompheniramine	Creatine
Ampicillin	Caffeine	Cyclobenzaprine
Aspartame	Chloroquine	r-Cyclodextrin
Aspirin	(+)-Chlorpheniramine	(-)-Deoxyephedrine

Dextromethorphan	Methadol	p-Phenylethylamine
Diazepam	Methamphetamine	(+/-)-Phenylpropanolamine
4- Dimethylaminoantipyrine	Methaqualone	Phertermine
5,5-Diphenylhydantoin	Methadone	Procaine
Dopamine	Methapyrilene	Promethazine
Doxylamine	(1R,2S)-(-)-N-Methyl-	d-Propoxyphene
Ecgonine methyl ester	Ephedrine	Secobarbital
(+)-Ephedrine	Morphine	Sodium Chloride
(+/-)-Ephedrine	Morphine-3+d-glucuronide	(+/-)-Soproterenol
(+/-)-Epinephrine	Naloxone	Tenocyclidine
Erythromycin	Naltrexone	11-nor- Δ^9 -
EDDP	(+)-Naproxen	Tetrahydrocannabinol-9-
Furosemide	p-Naphthaleneacetic acid	carboxylic acid
Glucose	Nortriptyline	Theophylline
Guaiacol glyceryl ether	Nicotinic Acid	Thioridazine
DL-Homatropine	Oxalic Acid	D(+)-Trehalose
Hydrocodone	Penicillin-G	Trifluoperazine
Hydromaphone	Pentobarbital	Trimethobenzamide
Lidocaine	Pheniramine	Tripolidine Hydrochloride
Meperidine	Phenobarbital	Tyramine
Maprotiline	Phenothiazine	Vitamin C
(+/-)3,4-MDMA	L-Phenylephrine	

References

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