Cotinine

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FOR PROFESSIONAL USE ONLY

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

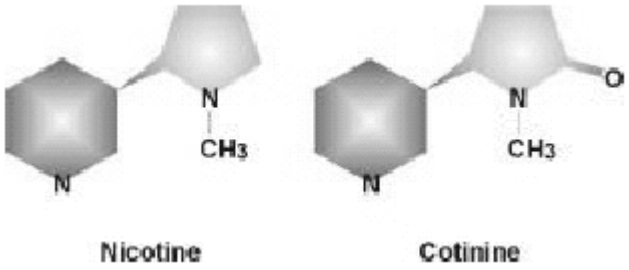
INTENDED USE

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 used in order to obtain a confirmed analytical result.

SUMMARY

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 metabolized 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 150 ng/ml cut-off concentration.

PRINCIPLE

The Cotinine Card Test applies the principle of competitive Ag-Ab 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 colored 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 preci-pitant 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 band 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 colored antibody-colloidal gold conjugate at the test line region. Therefore, absence of the color band on the test region indicates a positive result (smoker). A control band 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 colored bands, and positive urine will have only one band. The presence of this colored band 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 kit out of a damaged pouch.

REAGENTS AND MATERIALS SUPPLIED

Individually wrapped test devices each including one disposable pipette each. Each test device contains a membrane coated with drug-protein conjugate and a colloidal gold conjugate pad coated with cotinine antibody. Instruction sheet.

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 pretreatment. 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

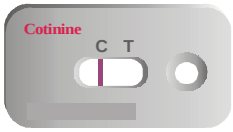
- Review "Specimen Collection" instructions. Test device, samples, and controls should be brought to room temperature (20- 30 °C) prior to testing. Do not open pouches until ready to perform the assay. Do not use kit out of a damaged pouch.
- Remove the test device from the protective pouch (bring the 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 to the line marked on the pipette (approximately 0.2 ml). Dispense the urine into the sample well. 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 cutoff 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:

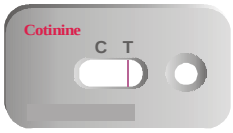
Both the test line (T) and the control line (C) should appear in the viewing window. The control line (C) indicates proper performance of the device. The test line intensity may be weaker or stronger than that of the control line.

Positive:



Only one Colored line appears in the control line region(C). No colored line appears in the test region(T).

Invalid:



No colored line appears in the control region (C). Under no circumstances should a positive result be identified unless the control line (C) appears in the viewing area. If the control line (C) does not appear, the test result is inconclusive and should be repeated.

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 error 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 mislabeled 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 Card Test 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 rpesumed non-smoker volunteers, has 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 SureStickTM Smoke Check with 100 % agreement.

Reproducibility

The reproducibility of the Cotinine Card Test 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 Card Test 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 Card Test 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
Acetylsalicylic Acid
Amitriptyline
D-Amphetamine
Ampicillin
Aspartame
Aspirin
Atropine
Benzocaine
Benzoylecgonine
[+]-Brompheniramine
Caffeine
Chloroquine
(+)-Chlorpheniramine
(+/-)-Chlorpheniramine
Chlorprothixene
Codeine
Creatine
Cyclobenzaprine
r-Cyclodextrin
(-)-Deoxyephedrine
Dextromethorphan
Diazepam
4- Dimethylaminoantipyrine
5,5-Diphenylhydantoin
Dopamine
Doxylamine
Ecgonine methyl ester
(+)-Ephedrine
(+/-)-Ephedrine
(+/-)-Epinephrine
Erythromycin
EDDP
Furosemide
Glucose
Guaiacol glyceryl ether
DL-Homatropine
Hydrocodone
Hydromaphone
Lidocaine
Meperidine
Maprotiline
(+/-)3,4-MDMA
Methadol
Methamphetamine
Methaqualone
Methadone
Methapyrilene
(1R,2S)-(-)-N-Methyl-Ephedrine
Morphine
Morphine-3+d-glucuronide
Naloxone
Naltrexone
(+)-Naproxen
p-Naphthaleneacetic acid
Nortriptyline
Nicotinic Acid

Oxalic Acid
Penicillin-G
Pentobarbital
Pheniramine
Phenobarbital
Phenothiazine
L-Phenylephrine
p-Phenylethylamine
(+/-)-Phenylpropanolamine
Phertermine
Procaine
Promethazine
d-Propoxyohene
Secobarbital
Sodium Chloride
(+/-)-Soproterenol
Tenocyclidine
11-nor-D9 – Tetrahydrocannabinol-9-carboxylic acid
Theophylline
Thioridazine
D(+)Trehalose
Trifluoperazine
Trimethobenzamide
Triprolidine Hydrochloride
Tyramine
Vitamin C

REFERENCES

1. Zevin S, Jacob P and Benowitz N. Cotinine effects on nicotine metabolism. Clinical Pharmacology & Therapeutics, 1997 June 61(6): 649-54.

2. Zuccaro P, Pichini S, Altieri I, Rosa M, Pellegrini M and Pacifici R. Interference of nicotine metabolites in cotinine deter-mination by RIA. Clinical Chemistry, 1997 Jan 43(1): 180-1.

3. Benowitz NL. Cotinine as biomarker of environmental tobacco smoke exposure. Epidemiologic Reviews, 1996 18(2): 188-204.

4. Curvall M and Vala FK. Nicotine and metabolites: analysis and levels in body fluids. Edited by Gorrod JW and Wahren J. Published in 1993 by Chapman & Hall, London. ISBN 0 142 55740.

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