DFE, Inc.

SmokeCheck Test

One-Step Cotinine Test

Instructions

INTENDED USE

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

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

SUMMARY AND EXPLANATION OF THE TEST

Tobacco smoking results in the absorption of nicotine through the lung and buccal/nasal epithelium, after which nicotine is metabolized into 20 metabolites excreted in urine. Cotinine, a major metabolite, accumulates in the body with regular smoking. It is reported that cotinine is stable in body fluids and has a relative long half life of approximately 17 hours. Therefore, the detection of cotinine is less dependent on the timeof 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, EIA and RIA. These methods usually require special equipment and complicated operation procedures.

The SmokeCheck 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 a simple and convenient test for the rapid qualitative detection of cotinine in human urine at 200 ng/ml cut-off concentration.

PRINCIPLE OF THE TEST

The SmokeCheck Test applies the principle of a competitive immunoassay. The test device contains a membrane strip that is precoated 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 colored antibody colloidal gold conjugate and urinemoves chromatographically by capillary action across the membrane. This solution migrates to the 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 indicates a **negative** result (non-smoker). When cotinine is present in urine, it competes with cotinine on the test band region for limited antibody sites. When 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 in the test region indicates a **positive** result (smoker).

A differentantigen/antibody reaction is added to the membrane strip at the control region (C) to indicate the test has been performed properly. This control line should always appear, regardless of the cotinine status in the urine. This means that **negative** urine will have **two** pink colored bands, and **positive** urine will have only **one** pink colored band. The pink colored control band serves as an indicator that 1) sufficient volume of urine has been added, and 2) that proper flow was obtained.

MATERIALS PROVIDED

1. Individually wrapped test devices which include one disposable pipette each.
2. Test Instructions.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Specimen collection container
2. Timer

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic and professional use only.
2. Do not use the test device beyond the expiration date.
3. Avoid cross contamination of urine samples by using a new specimen collection container and specimen pipette for each urine sample.
4. Urine specimens may be infectious; properly handle and dispose of all urine and urine reaction devices in a biohazard container.
5. Visually inspect the foil package to ensure it has not been compromised before beginning the test. If the package does not reach you intact, the integrity of the device may be compromised.

STORAGE AND STABILITY

The test should be stored refrigerated or at room temperature 2°-30°C (36°-86°F); do not freeze. If stored at 2°-8°C (36°F-46°F), allow the test kit to reach room temperature (15°-30°C) before performing the test. Each device will be stable until the expiration date as printed on the foil package.

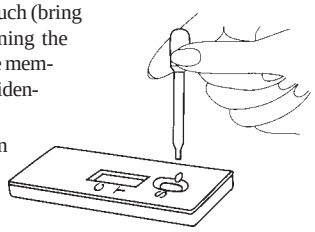
SPECIMEN COLLECTION AND PREPARATION

The urine specimen must be collected in a clean dry container, either plastic or glass. 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.

TEST PROCEDURE

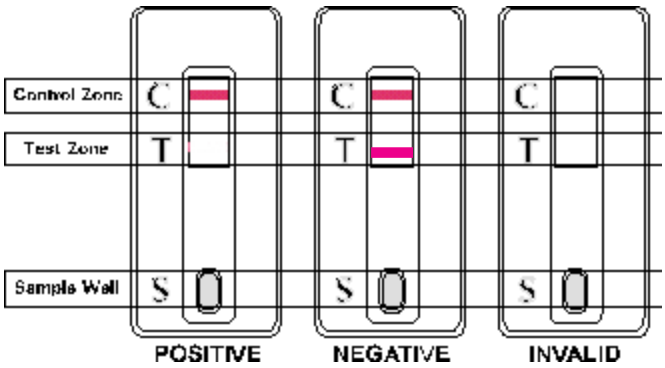
Review "Specimen Collection" Instructions. If refrigerated, test device, patient samples and controls should be brought to room temperature (15-30C) prior to testing. Do not open pouches until ready to perform the assay.

1. Remove the test device from its 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.
2. Draw the urine sample to the line marked on the pipette (approximately 0.2 ml). Dispense the entire contents into the sample well. Use a separate pipette and device for each sample or control.



3. Read result between 3 to 8 minutes after the addition of samples. Do not read results after 8 minutes.

INTERPRETATION OF RESULTS



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

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 lineintensity may be weaker or stronger than that of the control line.

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

QUALITY CONTROL

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

LIMITATIONS OF THE TEST

1. This product is designed to be used for the detection os in human urine only.
2. There is a possibility that technical or procedural errors as well as other substances or factors beyond the control of the manufacturer 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.
3. If it is suspected that the samples have been mislabeled or deteriorated, a new specimen should be collected and the test should be repeated.

PERFORMANCE CHARACTERISTICS

1. **Accuracy:** The accuracy of the SmokeCheck Test was evaluated in comparison to a commercially available immunoassay at a cut-off concentration of 200 ng/ml for cotinine. One hundred and twenty samples (120) collected from presumed non-smokers volunteers were tested by both methods with 100% agreement.

   In a separate study, fifty (50) urine specimens with cotinine concentrations ranging from 300 ng/ml to 2000 ng/ml (as determined by a commercially available immunoassay) were tested using the SmokeCheck Test. All fifty (50) specimens were determined positive by the SmokeCheck Test, representing 100% agreement with the commercially available immunoassay.

2. **Reproducibility:** The reproducibility of the SmokeCheck Test was evaluated at four different sites using blind controls. Of the sixty (60) samples with cotinine concentrations of 100 ng/ml, all were determined negative. Of the sixty (60) samples with cotinine concentrations of 400 ng/ml of cotinine, all were determined positive.

3. **Precision**: The precision of the SmokeCheck Test was determined by conducting the test with spiked controls. The control at 100 ng/ml gave a negative result. The control at 400 ng/ml gave a positive result.

Conc. (ng/ml)	Number Tested	Correct Results	% Correct Result
100	50	50	100
400	50	50	100

4. **Specificity:** The specificity for the SmokeCheck 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 cotinine free normal human urine.

   The following structurally related compounds produced positive results when tested at levels equal to or greater than the concentrations listed below:

Compound	**Concentration**
(-) Nicotine	350 µg/ml

The following compounds were found not to cross-react when tested at concentrations up to100 µg/ml.

Acetaminophen
Acetylsalicyclic Acid
Albumin
Amitriptyline
D-Amphetamine
Ampicillin
Aspartame
Aspirin
Benzocaine
Benzoylecgonine
(+)-Chlorpheniramine
(+/-) Chlorpheniramine
Chlorprothixene
Codeine
Creatine
r-Cyclodexirin
Cyclobenzaprine
(-)-Deoxyephedrine
Dextromethorphan
4-Dimethylaminoantipyrine
5,5-Diphenylhydantoin
Diazepam
Dopamine
Doxylamine
Ecgonine Methyl Ester
EDDP
(-)-Ephedrine
(+)-Ephedrine
(+/-)-Ephedrine
(+/-)-Epinephrine
Erylthromycin
Furosemide
Glucose
GuaiacolGlyceryl Esther
Hemoglobin
DL-Homatropine
Hydrocodone
Hydromorphone
(+/-) Isoproterenol
Lidocaine
Maprotiline
Methadone
Meperidine
Methadol
Methamphetamine
Methapyriline
Methaqualone
Methylphenidate
(+/-)3,4-MDMA
Morphine
Morphine-3-β-d-glucuronide
(1S,2S)-(-)-N-Methyl-Ephedrine
Naloxone
Naltrexone
β Naphtaleneacetic Acid
(+)-Naproxen
NortriptylineNicotinic Acid
Oxalic Acid
Penicillin G
Pentobarbital
Perphenazine
Pheniramine
Phenobarbital
Phenothiazine
L-Phenylephrine
α-Phenylethylamine
Phentermine
(+-)-Phenylpropanolamine
Primidone
Procaine
Promethazine
d-Propoxyphene
Secobarbital
Sodium Chloride
Tenocyclidine
Δ9-Tetrahydrocannabinol
Theophylline
Thioridazine
Trimethobenzamide
D(+)Trehalose
Trifluoperazine
Tyramine
Triprolidine Hydrochloride
Vitamin C

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