

C. Precision

The precision of the SureStep Smoke Check 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

D. Specificity

The specificity for the SureStep Smoke Check 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 ug/ml
The following compounds were found not to cross-react when tested at concentrations up to 100 µg/ml.	
Acetaminophen	DL-Homatropine
Acetylsalicylic Acid	Hydrocodone
Albumin	Hydromorphone
Amiripityline	(+/-)-Isoproterenol
D-Amphetamine	Lidocaine
Ampicillin	Maprotiline
Aspartame	Methadone
Aspirin	Meperidine
Atropine	Methadol
Benzocaine	Methamphetamine
Benzylecgonine	Methapyriene
(+)-Brompheniramine	Methaqualone
Caffeine	Methylphenidate
Chloroquine	(+/-)3,4-MDMA
(+)-Chlorpheniramine	Morphine
(+/-)-Chlorpheniramine	Morphine-3-β-d-glucuronide
Chlorprothixene	(1S,2S)-(-)-N-Methyl-Ephedrine
Codaine	Naloxone
Creatine	Naltrexone
r-Cyclohexatin	β-Naphthaleneacetic acid
Cyclobenzaprine	(+)-Naprofen
(-)-Deoxyephedrine	Nortriptyline
Dextromethorphan	Nicotinic Acid
4-Dimethylaminoantipyrine	Oxalic Acid
5,5-Diphenylhydantoin	Penicillin-G
Diazepam	Pentobarbital
Dopamine	Perphenazine
Doxylamine	Pheniramine
Egogrine Methyl Ester	Phenobarbital
EDDP	Phenothiazine
(-)-Ephedrine	L-Phenylephrine
(+)-Ephedrine	α-Phenylethylamine
(+/-)-Ephedrine	Phentermine
(+/-)-Epinephrine	(+/-)-Phenylpropranolamine
Erythromycin	Primidone
Furosemide	Procaine
Glucose	Promethazine
Guaiacol Glyceryl Ether	d-Propoxyphene
Hemoglobin	Secobarbital

Sodium Chloride	D(+)-Trehalose
Tenocyclidine	Trifluoperazine
Δ ⁹ -Tetrahydrocannabinol	Tyramine
Theophylline	Triprolidine Hydrochloride
Thioridazine	Vitamin C
Trimethobenzamide	

BIBLIOGRAPHY OF SUGGESTED READING

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SureStep™

Smoke Check Test

One-Step Cotinine Test

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

For determination of smoking status only.
Not Intended For Medical Diagnostic Use.

INTENDED USE

The SureStep Smoke Check 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 of 200 ng/ml. This product is used to obtain a visual, qualitative result and is intended for the determination of smoking status only.

This assay provides only a preliminary analytical test 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 consideration and professional judgment should be applied to all test results, particularly when preliminary positive results are indicated.

SUMMARY

Tobacco smoking results in the absorption of nicotine through the lung and buccal/nasal epithelium, after which nicotine is metabolized into about 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 relatively long half life of approximately 17 hours. Therefore, the detection of cotinine is 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, EIA and RIA. These methods usually require special equipment and complicated operation procedures.

The SureStep Smoke Check 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 immunochromatological 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.



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Revision: C

PRINCIPLE

The SureStep Smoke Check 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 urine moves 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 the limited antibody sites. 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 different antigen/antibody reaction is added to the membrane strip at the control region (C) to indicate that 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) A sufficient volume of urine has been added, and 2) that proper flow was obtained.

STORAGE AND STABILITY

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

PRECAUTIONS

- 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.

REAGENTS AND MATERIALS SUPPLIED

- Individually wrapped test devices which include one disposable pipette each.

- One instruction sheet.

MATERIAL REQUIRED BUT NOT PROVIDED

- Specimen collection container.
- Timer.

SPECIMEN COLLECTION AND HANDLING

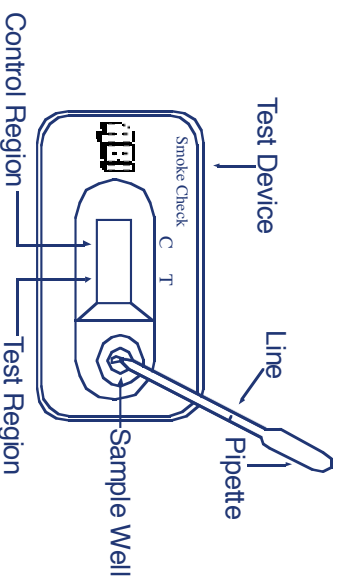
The urine specimen must be collected in a clean dry container, either plastic or glass. Fresh urine does not require any special handling or pretreatment. Test should be performed soon after the urine specimen is collected, preferably during the same day. The specimen may be refrigerated at 2-8°C for 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.

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

TEST PROCEDURE

Review "Specimen Collection" instructions. If refrigerated, test device, patient 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.

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 result after 8 minutes.



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 line region (T).

Invalid:

No colored line appears in the 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 (C) does not appear, the test result is invalid and the assay should be repeated.



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

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 level of consumption.
- 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 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. Before using a new kit with patient specimens, positive and negative controls should be tested. 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

A. Accuracy

The accuracy of the SureStep Smoke Check 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 presumed non-smoker 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 2,000 ng/ml (as determined by a commercially available immunoassay) were tested using the SureStep Smoke Check Test. All fifty (50) specimens were determined positive by the SureStep Smoke Check Test, representing 100 % agreement with the commercially available immunoassay.

B. Reproducibility

The reproducibility of the SureStep Smoke Check 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.