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

BIBLIOGRAPHY OF SUGGESTED READING

1. Zevin S, Jacob P and Benowitz N. Cotinine effects on nicotine metabolism.
3. Benowitz NL. Cotinine as biomarker of environmental tobacco smoke exposure.
   Epidemiologic Reviews, 1996 18(2):188-204.

Catalog # 5000 Package Insert Part #32-8000 Revision: C

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.

<table>
<thead>
<tr>
<th>Conc. (ng/ml)</th>
<th>Number Tested</th>
<th>Correct results</th>
<th>% Correct result</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>400</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

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.

- (-)-Nicotine 350 µg/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
- Amitriptyline
- (+/-)-Isoproterenol
- D-Amphetamine
- Lidocaine
- Ampicillin
- Pheniramine
- Ecgonine Methyl Ester
- Phenobarbital
- EDDP
- Phenothiazine
- (-)-Ephedrine
- L-Phenylephrine
- (+)-Ephedrine
- (+/-)-Epinephrine
- (+/-)-Phenylpropanolamine
- Erythromycin
- Primidone
- Furosemide
- Procaine
- Glucose
- Promethazine
- Guaiacol
- Glyceryl Ether
- d-Propoxyphene
- Hemoglobin
- Secobarbital

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APPLIED BIOTECH, INC.
DIAGNOSTIC PRODUCTS

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 for the qualitative detection of cotinine in urine, after a visual, qualitative result is obtained. The assay provides only a preliminary analytical result; a more specific alternative chemical method, such as high performance liquid chromatography (HPLC), may be required to confirm the result.

This assay provides preliminary analytical results only and is intended for the determination of smoking status only. It is not intended for medical diagnostic use. For determination of cotinine in urine, the protocol is to conduct a visual, qualitative test with spiked controls. The control at 100 ng/ml gives a negative result. The control at 400 ng/ml gives a positive result.

SUMMARY
Tobacco smoking results in the absorption of nicotine through the lung and buccal/nasal epithelium, after which nicotine is metabolized in the liver and excreted in the urine. Cotinine is a major metabolite of nicotine and is more stable in urine than nicotine itself. The SureStep Smoke Check Test is a lateral flow, one-step immunoassay for the qualitative detection of cotinine in human urine. It is based on the principle of highly specific and sensitive antibodies that specifically recognize cotinine in urine. The assay provides only a preliminary analytical result; a more specific alternative chemical method, such as high performance liquid chromatography (HPLC), may be required to confirm the result.
**SPECIMEN COLLECTION AND HANDLING**

**MATERIAL REQUIRED BUT NOT PROVIDED**

- One instruction sheet.
- One seat.

**REAGENTS AND MATERIALS SUPPLIED**

- A complete test kit, including:
  - Two (2) SureStep Smoke Check test devices
  - Two (2) individually wrapped test devices
  - One (1) disposable pipette
  - One (1) sample collection container

**PERFORMANCE CHARACTERISTICS**

**A. Accuracy**

- The kit is designed for use with human urine only.
- It is not intended for use with any other body fluid.

**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 positive. Of the sixty (60) samples with cotinine concentrations of 400 ng/ml of cotinine, all were determined positive.

**C. Sensitivity and Specificity**

- Sensitivity: 100%
- Specificity: 100%

**STORAGE AND STABILITY**

- The SureStep Smoke Check Test is stable for the duration of the shelf-life.
- It should be stored at room temperature 2-30°C.
- It should be protected from freezing.
- Samples should 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 a longer time, however, it is recommended to equilibrate to room temperature before testing.

**PRECAUTIONS**

- Specimens that have been refrigerated must be equilibrated to room temperature prior to testing.
- 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 a longer time, however, it is recommended to equilibrate to room temperature before testing.

**TEST PROCEDURE**

1. Remove the test device from its protective pouch (bring the test 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 the result after 2 minutes.

- Negative: No colored line appears in the control region.
- Positive: A positive result is identified when a pink colored band appears in the test region above the cut off concentration. It does not indicate or measure level of consumption.

**INTERPRETATION OF RESULTS**

- A positive result indicates only that the presence of cotinine 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 kit is designed for use with human urine only.
- It is not intended for use with any other body fluid.
- The kit is not designed to detect the presence of other substances or factors not listed that 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.

**QUALITY CONTROL**

- Good laboratory practice recommends the use of control materials to ensure proper kit performance. Before using a new kit, control materials should be tested to confirm satisfactory performance. They should be re-tested at regular intervals as recommended by the manufacturer. When testing the positive and negative controls, use the same assay procedure as with a urine specimen.

**INTERPRETATION OF RESULTS**

- Control Region

- Test Line:

- Positive: A positive result is identified when a pink colored band appears in the test region above the cut off concentration. It does not indicate or measure level of consumption.

- Negative: No colored line appears in the control region.

- Invalid: If no color appears in the control region, the test result is invalid and the kit should be repeated.