**NicAlert Saliva**

**但是他**

**Product Insert**

**NEVER PLACE NICALERT™ STRIP IN MOUTH**

**INTENDED USE**

NicAlert™ is intended for in vitro diagnostic professional use for the semi-quantitative determination of cotinine in saliva for the purpose of determining if an individual has been exposed to tobacco products such as cigarettes, pipes, or chewing tobacco within the past 48 hours. The cutoff concentration for the NicAlert™ test is 10 ng/mL. Second hand smoke exposure (environmental tobacco smoke) may cause a positive result in a non-smoker user of tobacco products. The NicAlert™ Positive and Negative Controls are intended for in vitro diagnostic use for the quality control of the NicAlert™ test.

**BACKGROUND**

The knowledge and awareness of the health hazards associated with exposure to tobacco products, especially smoking cigarettes, is well established. Cotinine smoking has been identified as one of the most significant causes of death and disease in the U.S. (Surgeon General's Report on the Health Benefits of Smoking, Year 2000). Smoking has been cited as being responsible for 87% of deaths from lung cancer, 21% of deaths from coronary heart disease, 10% of deaths from stroke, and 82% of deaths from chronic obstructive pulmonary disease. Significantly elevated risks of disease and death are also associated with other forms of tobacco use such as pipe and cigar smoking and the use of chewing tobacco.

As an adjacent to self-reporting of smoking behavior, and as a more objective approach, the assay of cotinomial markers is of established importance. Nicotine is not a reliable indicator of smoking status as it has a comparatively short half-life (1-4 hours). Cotinine has been shown to be more sensitive and specific than CO monitoring for measuring smoking status. The reference method for measuring cotinine is Gas Chromatography/Mass Spectrometry (GC/MS), or Liquid Chromatography / Mass Spectrometry (LC/MS/MS).

**PRINCIPLE OF THE TEST**

NicAlert™ is an immunochromatographic assay that uses monoclonal antibody-coated gold particles and a series of easy steps that allow quantification of cotinine. It employs patented technologies. The U.S. Patent Nos. 5,527,686; 5,710,009; 6,087,185 and 6,121,008. The sample collection elution of the strip contains gold particles coated with monoclonal antibodies to cotinine, a relatively long-lived metabolite of nicotine. The distance the gold migrates on the strip is shown by a clear color change and provides an accurate measure of the amount of cotinine in the sample.

**MATERIALS PROVIDED**

NicAlert™ Strip: Each NicAlert™ test strip is individually packaged in a sealed plastic labeled plastic pouch. (REF 83607003388)

Funnel

Saliva Tube container

Snap on top absorbent material

Each test strip is composed of the following:

a) 5 mm X 90 mm nitrocellulose impregnated with:
   - Mouse monoclonal antibodies reactive to cotinine, conjugated to colloidal gold particles
   - Rabbit anti-mouse polyclonal antibodies reactive to mouse antibodies
   - 50mM sodium phosphate buffer pH 7.2, bulking agents, stabilizers.
   - Cotton pads, filters.

**OPTIONAL MATERIALS**

Containers:

a. NicAlert™ Negative Control (cotinine 0 ng/mL)

b. NicAlert™ Low Positive Control (cotinine 400 ng/mL)

c. NicAlert™ High Positive Control (cotinine 2000 ng/mL)

The NicAlert™ Positive and Negative Controls are synthetic saliva-based liquid and are ready to use. These Controls each contain a known concentration of cotinine (Negative: 0 ng/mL, Low Positive: 400 ng/mL, High Positive: 2000 ng/mL). The NicAlert™ Positive Control is prepared by spiking known concentrations of cotinine into the NicAlert™ Negative Control, which is synthetic saliva with no detectable amount of cotinine by LC/MS. See sale date and for opened and closed exhalation.

Materials Required But Not Provided:

A timer or clock.

**QUALITY CONTROL**

Good laboratory practice recommends periodic use of quality control procedures. The use of controls from other commercial vendors is also recommended. Users should follow the applicable regulatory guidelines concerning the running of external quality controls. The NicAlert™ Positive and Negative Test Controls are used as the diagnostic use for the quality control of the NicAlert™ test. The NicAlert™ Negative Controls consist of cotinine-free synthetic saliva. The NicAlert™ Positive Controls consist of cotinine-free synthetic saliva spiked with cotinine to concentrations of 400 ng/mL, cotinine (Low Positive Control, NicAlert™ Low 400 ng/mL, cotinine (High Positive Control, NicAlert™ Low 2000 ng/mL, cotinine (High Positive Control). NicAlert™ Low 2000 ng/mL, cotinine (High Positive Control). NicAlert™ Low 2000 ng/mL.

**STORAGE**

Store NicAlert™ at room temperature, out of direct sunlight, in the sealed pouches. The test strip can be used up to 45 minutes after the expiration date indicated on the label. Once the package is opened, the strip should be used within 10 minutes. NicAlert™ Positive and Negative Controls should be used at a 1:1 dilution. After opening, do not use the Controls if the controls become cloudy or altered in appearance.

**WARNINGS AND PRECAUTIONS**

1. Do not use any part of the NicAlert™ strip in your mouth.

2. Treat samples as a potential biohazard and discard appropriately after testing.

3. Wear gloves when handling test strips for 10 minutes after opening the pouch.

4. Discard any samples if contamination is suspected and obtain another sample.

5. Do not use saliva that has been collected in a non-Saliva Tube container.

6. Test samples at room temperature.

The NicAlert™ strip should not be used on cloudy or pink urine samples.

**TESTING SALIVA SAMPLES WITH NICALERT™**

Before Starting

Before starting, you will need:

- A NicAlert™ strip in its pouch with a valid expiry date
- A timer or clock
- A water, timer, or clock, and:
  - A clear non-bonded surface (such as a counter or a plastic surface) on which to place the NicAlert™ strip (do not use absorbent materials such as paper towels, etc.).
  - A laminated instruction card or used to.

**PROCEDURE**

1. ***Cayla*** Handle any saliva sample as if it was a potential biohazard. Discard appropriately after testing.

   1. Open the NicAlert™ package (1) and lay the NicAlert™ strip flat on a non-absorbent surface (do NOT put the NicAlert™ on paper or Kleenex). The plastic laminated instruction card can be used to mark the test area for this purpose.

   2. Tear open the NicAlert™ Saliva Collection Kit and remove the funnel, the saliva tube container, and the snap-on top for the saliva tube container.

   3. Place the funnel in the saliva tube container and separate saliva into the funnel, enough to fill at least 1/2 of the saliva tube container. (3) Then discard the funnel. Slip on the top of the saliva tube container. Squeeze 8 drops from the inverted saliva tube directly onto the white padded end of the NicAlert™ strip. (4)

   4. Snap on top of the saliva tube container.

   5. Place NicAlert™ strip on the saliva tube container. Squeeze 8 drops from the inverted saliva tube directly onto the white padded end of the NicAlert™ strip. (4)
Table 1: GC-MS Determination of Smoking Status (50 ng/mL cut-off) vs. saliva NicAlert™ determination for the 2 Operators at each Site

<table>
<thead>
<tr>
<th>Site 1</th>
<th>Operator 1</th>
<th>Operator 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-MS</td>
<td>Non-Smoker (NicAlert™ = 0)</td>
<td>Smoker (NicAlert™ = 31)</td>
</tr>
<tr>
<td>Non-Smoker (&lt; 50 ng/mL)</td>
<td>47</td>
<td>2</td>
</tr>
<tr>
<td>Smoker (≥ 50 ng/mL)</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100%</td>
<td>98.7%</td>
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<tr>
<td>Specificity</td>
<td>95.9%</td>
<td>91.8%</td>
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<table>
<thead>
<tr>
<th>Site 2</th>
<th>Operator 1</th>
<th>Operator 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-MS</td>
<td>Non-Smoker (NicAlert™ = 0)</td>
<td>Smoker (NicAlert™ = 31)</td>
</tr>
<tr>
<td>Non-Smoker (&lt; 50 ng/mL)</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>Smoker (≥ 50 ng/mL)</td>
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</tr>
<tr>
<td>Sensitivity</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
<td>96.7%</td>
</tr>
</tbody>
</table>

Cross-Reactants
Negative Saliva was spiked with compounds related to cotinine at 100,000 ng/mL.
Nicotinic Acid – Hazy no distinct bands
Nicotinic Acid n-oxide – no crossreactivity
Niacinamide – no crossreactivity
Nicotine – Trap Level 3

Reproducibility
Three saliva control levels were tested in duplicate by three readers over three days.

Reader 1

<table>
<thead>
<tr>
<th>0 ng/mL</th>
<th>150 ng/mL</th>
<th>2000 ng/mL</th>
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<tbody>
<tr>
<td>Test 1 Day 1</td>
<td>0</td>
<td>3</td>
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<tr>
<td>Test 2 Day 1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Test 1 Day 2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Test 2 Day 2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Test 1 Day 3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Test 2 Day 3</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

REFERENCES