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, pipe, 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-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 and daily smoking cigarettes, is well established. Tobacco 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-related effects of environmental tobacco smoke, Year 2000). Smoking has been cited as being responsible for 87% of deaths from lung cancer, 21% of deaths from coronary heart disease, 18% of deaths from stroke, and 82% of deaths from chronic obstructive pulmonary disease. Significantly elevated risks of disease and death are also associated with exposure to environmental tobacco smoke (environmental tobacco smoke) may cause a positive result in a non-user of tobacco products.

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 spit/expectorate saliva into the funnel, enough to fill at least 1/2 of the saliva tube container. 4. Then discard the funnel and top of the saliva tube container. SUPPLEMENT 8 drops from the inverted saliva tube directly onto the white padded end of the NicAlert™ strip. (4)

Testing the NicAlert™ strip: The NicAlert™ strip must appear in at least one of the numbered zones (Levels 0-6) on the strip. Note: The marking levels on the strip appear as an 68° (A), 55 (D), etc. This is in order to make the strip easier to read. If the color appears as an indistinct smear throughout the strip, the results are not valid and the test must be repeated with a new NicAlert™ strip.

Limitations of the Procedure
NicAlert™ saliva is for use with human saliva. A positive result indicates tobacco product exposure and the presence of cotinine in the sample. Errors may result from the use of technical or procedural errors or by contamination or interference of the sample. NicAlert™ saliva should not be used if the saliva appears to be abnormally colored or opaque. The appearance provides only a preliminary result. Clinical consideration and professional judgment must be applied to any test result, particularly in evaluating a preliminary positive result. In order to obtain a confirmed analytical result, a more specific alternate chemical method is needed. Gas Chromatography/Mass Spectrometry (GC/MS) is the preferred confirmation method.

Performance Characteristics
Sensitivity
Sensitivity has demonstrated that NicAlert™ strips have a shelf life of at least two years when stored at ambient temperatures.

Limit of Detection
Saliva samples were diluted to 20.10, 8.6, 4.2 and 0 ng/mL, cotinine and read in duplicate with NicAlert™ Strips.
### 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</td>
<td>Smoker</td>
</tr>
<tr>
<td></td>
<td>(NicAlert™ = 0)</td>
<td>(NicAlert™ &gt; 0)</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>
</tr>
</tbody>
</table>

### Site 2

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

**Cross-Reagents**

Negative Saliva was spiked with compounds related to cotinine at 100,000 ng/mL.

- Nicotinic Acid - Hazy no distinct bands
- Nicotine - No cross-reactivity
- 3-OH cotinine - 12-40% cross-reactivity in the NicAlert™ assay.

**Interfering Substances**

- Saliva Standards (0 ng/mL, 60 ng/mL, 150 ng/mL, and 2000 ng/mL) were spiked with:
  - Hemoglobin - No interference
  - Protein - No interference at (2.5 mg/mL)
  - pH 4.0 – Level 1 trap seen at all cotinine concentrations: 2.5, 4.5, 6.0 read as 1
  - pH 5.0 – Level 3 reads as 2, Level 6 reads as 4
  - pH 6.0 – Level 1 trap seen at all cotinine concentrations: 2, 3, 4, 5, 6, 7, 8 read as 1
  - Ascorbic Acid – Level 3 reads as 2, Level 6 reads as 4
  - Ethanol 10% solution – Traps 0, 1, 2, 3 normal, Traps 4, 5, 6 reading lowered by one
  - pH 7.0 and pH 8.0 run normally
  - 3-OH cotinine – Cross-reacted with cotinine in the NicAlert™ assay.

**Reproducibility**

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

**REFERENCES**