**2. Controls:**

- forceps, tweezers or gloves to hold the strip when dipping it into the urine sample.
- You may also prefer to use:
  - a clean nonabsorbent surface (such as a countertop or a plastic surface) on which to place the clean, leak-proof, disposable container for the urine sample;
  - Before Starting TESTING URINE SAMPLES WITH NICALERT™

**3. Use the NicAlert™ strip within 10 minutes of opening the pouch.**

**WARNINGS AND PRECAUTIONS**

- NicAlert™ is intended for in vitro diagnostic use for the quality control of the NicAlert™ test.
- NicAlert™ Human Urine 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. Cigarette smoking has been identified as one of the most significant risk factors in the world. (J. Cigarettes—Health and Tobacco Control in the Community, 2000) Smoking has been cited as being responsible for 87% of deaths from lung cancer, 25% of deaths from coronary heart disease, and 50% of deaths from chronic obstructive pulmonary disease. Significantly elevated risks of disease and death are associated with other forms of tobacco use such as pipe and cigar smoking and the use of chewing tobacco.

As an adjunct to self-reporting of smoking behavior, and as a more objective approach, the assay of biochemical markers of tobacco use has become increasingly important. Urine tests are a reliable indicator of smoking status as it has a comparatively short half-life. Cotinine is a major metabolite of nicotine and has a relatively long half-life (24-48 hours). Cotinine has been shown to be more sensitive and specific than CO monitoring for measuring smoking status. The reference method used for measuring cotinine is Gas Chromatography/Mass Spectrometry (GC/MS), or Liquid Chromatography/Mass Spectrometry (LC/MS).

**PRINCIPLE OF THE TEST**

NicAlert™ is an immunochromatographic assay that uses monoclonal antibody-coated gold particles and a series of avidity traps that allow quantification. It employs patented technologies. (U.S. Patent Nos. 5,272,688; 5,109,000; 6,087,185 and 6,121,065.) The sample collection end of the strip contains gold particles coated with monoclonal antibody 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**

1. NicAlert™ Strip: Each NicAlert™ test strip is individually packaged in a sealed labeled plastic pouch.
2. Each NicAlert™ strip is color-coded for easy identification.
   - a) 5 mm X 90 mm nitrocellulose impregnated with:
     - 2 μg monoclonal antibodies reactive to cotinine, conjugated to colloidal gold particles.
     - 2 μg anti-cotinine polyclonal antibodies reactive to cotinine.
     - 1% Cobalt Blue 406 #2 dye.
     - Carboxycholesterol, isocaproylcarboxynorcotinine, carboxyphenylethylcotinine bromide.
     - 50 μM sodium phosphate buffer pH 7.2, bulking agents, stabilizers.
   - b) Paper filter, filter.

**2. Controls:**

-a. NicAlert™ Negative Control (cotinine 0 ng/mL).
-b. NicAlert™ Low Positive Control (cotinine 400 ng/mL).
-c. NicAlert™ High Positive Control (cotinine 4000 ng/mL).

The NicAlert™ Positive and Negative Controls are human urine-based liquid and are ready to use. These Controls contain known concentration of human urine containing 0 ng/mL, Low Positive: 400 ng/mL, High Positive: 4000 ng/mL. The NicAlert™ Positive Control is prepared by spiking in appropriate concentrations of nicotine into NicAlert™ Level 0 (control) which is preserved human urine with no detectable amount of cotinine by LC/MS/MS. Negative Controls are intended for in vitro diagnostic use for the quality control of the NicAlert™ test. The NicAlert™ Negative Controls consist of cotinine-free urine. The NicAlert™ Positive Controls consists of cotinine-free urine spiked with cotinine to concentrations of 400 ng/mL, cotinine level 4 control, NicAlert™ level 4 (2000 ng/mL) and 2000 ng/mL, cotinine (High-Positive Control, NicAlert™ level 9). The NicAlert™ controls are intended for clinical use.

Materials Required But Not Provided:

- A timer or clock.
- A bathroom scale.
- A timer or clock.
- A clean, leak-proof, disposable container for the urine sample.

**QUALITY CONTROL**

Good laboratory practice recommends periodic use of quality control procedures. It is recommended that the NicAlert™ Human Urine Positive and Negative Test Controls be run at least once per day. NicAlert™ Positive and Negative Controls are intended for in vitro diagnostic use for the quality control of the NicAlert™ test. The NicAlert™ Negative Controls consist of cotinine-free urine. The NicAlert™ Positive Controls consists of cotinine-free urine spiked with cotinine to concentrations of 400 ng/mL, cotinine level 4 control, NicAlert™ level 4 (2000 ng/mL) and 2000 ng/mL, cotinine (High-Positive Control, NicAlert™ level 9). The NicAlert™ controls are intended for clinical use.

**STORAGE**

Store NicAlert™ at room temperature, out of direct sunlight, in the sealed pouches. The test strips can be used up until the expiration date indicated on the label. Once the package is opened, the strip should be used within 4 hours. Store and discard in a sealed surface (such as a countertop or a plastic surface) on which to place the NicAlert™ strip. The test strips are stable at room temperature for at least two years when stored properly. NicAlert™ should not be exposed to direct sunlight or excessive heat.

**WARRANTIES AND PRECAUTIONS**

1. Do not put any part of the NicAlert™ strip in your mouth.
2. Test samples as a potential biohazard and discard appropriately after testing.
3. Use the NicAlert™ strip within 10 minutes of opening the pouch.
4. Do not consume or handle food or drink near the NicAlert™ strip or when performing the test.
5. The NicAlert™ strip should not be used on cloudy or pink urine samples.

**COLLECTING AND STORING THE URINE SAMPLE**

**BEFORE STARTING**

Before testing, you will need:
- a NicAlert™ strip in its pouch with a valid expiry date,
- clean, leak-proof, disposable container for the urine sample,
- a watch, timer, or clock,
- a clean nonabsorbent surface (such as a countertop or a plastic surface) on which to place the NicAlert™ strip.
- NicAlert™ Positive and Negative Controls should be stored at 2°-8°C. After opening, do not use the Controls if the contents become cloudy or altered in appearance.

Caution: Handle any urine sample as if it was a potential biohazard. Discard appropriately after testing.

- Use only leak-proof, disposable container such as a urine specimen container. Label the container if more than one person is being tested at a time. Do not re-use containers.
- Collect appropriately after testing.
- Collect at least 25 ml of (1 fluid oz.) urine. The person providing the sample should wash their hands before and after providing the sample. The person should collect a mid-stream sample by collecting the sample after a few seconds of the start of the urine stream. Discard the first few drops of collection. Urine samples can be collected for up to 3 days if refrigerated at 39°F (4°C) immediately after collection or stored indefinitely if frozen at -20°F (-28°C) in airtight containers. Do not use the sample if contamination is suspected and obtain another sample instead.

2. Visually check the urine sample for signs of contamination (foreign material such as bathroom tissue or hair, cloudiness, particles, blood, etc.). Do not use the sample if contamination is suspected and obtain another sample instead.

3. Test the sample within 4 hours of collection. Urine samples can be stored for up to 3 days if refrigerated at 39°F (4°C) immediately after collection or stored indefinitely if frozen at -20°F (-28°C) in airtight containers. Do not store any urine sample at a temperature above 86°F (30°C).

**HOW TO READ AND INTERPRET NICALERT™ RESULTS FOR URINE SAMPLES**

A reddish color must appear in at least one of the numbered zones (Levels 0 – 6) on the strip and there must not be any noticeable color in the area between the sample end (the exposed soft cotton end of the strip) and the Level 0. Otherwise the results are not valid and the test must be repeated with a new NicAlert™ strip. 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.

4. Find the lowest numbered zone (Level 0 – 6) on the strip with a reddish color in it. This lowest level is the NicAlert™ test result. Note: The strip may have more than one level in a given range. Use the lowest level. If needed, use latex or rubber gloves.

5. The NicAlert™ test result is the lowest numbered level on the strip with a reddish band or color in it. If there is doubt as to the lowest level, express the result as a range of 2 levels, eg. “1-2” or “5-6,” etc., or repeat the test.

6. For urine samples, a NicAlert™ result of:
- Level 3 or higher (Level 4, 5, or 6) indicates use of tobacco products.
- Level 1 (10-30 ng/mL) and Level 2 (30-100 ng/mL) indicates use of tobacco products;
- Levels 1 (10-30 ng/mL) and Level 2 (30-100 ng/mL) indicate no use of tobacco products;
- A borderline result at the cutoff (i.e. NicAlert™ level 2-3) could be a smoker or a non-smoker. Repeat testing with another urine sample is recommended.

**EXPRESSION OF NICALERT™ TEST RESULTS AS COTININE CONCENTRATION RANGES**

| Cotinine Concentration | Interpretation
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>0-10 μg/L</td>
<td>Non-user of tobacco products</td>
</tr>
<tr>
<td>10-200 μg/L</td>
<td>User of tobacco products</td>
</tr>
<tr>
<td>200-500 μg/L</td>
<td>User of tobacco products</td>
</tr>
<tr>
<td>500-1000 μg/L</td>
<td>User of tobacco products</td>
</tr>
<tr>
<td>1000-2000 μg/L</td>
<td>User of tobacco products</td>
</tr>
</tbody>
</table>

**LIMITATIONS OF THE PROCEDURE**

NicAlert™ is only intended for use with human urine. A positive result indicates tobacco product exposure and the presence of cotinine in the specimen. Enzyme results can be caused by technical or procedural errors or by adulteration or contamination of the sample. NicAlert™ should not be used if the urine is dark, red, or otherwise abnormally colored. The test should be delayed until the urine is clear and normal in appearance. The assay provides only a preliminary result. Clinical consideration and professional judgment must be applied to any test result, particularly in evaluating a preliminary positive result, a more accurate measure of the amount of cotinine in the sample.

**PERFORMANCE CHARACTERISTICS**

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