COLLECTING AND STORING THE URINE SAMPLE

1. Use a clean, 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.

2. Collect approximately 25 mL (1 fluid oz.) of urine. The person providing the sample should wash his or her 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 stream. The sample should be collected from the urine stream without any contamination of clothing, hands, or handles of the container. (see Figure 2)

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

4. Test the sample for the presence 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 -8°F (-27°C) for at least 6 months. Use a screw top jar or similar sealed container for storage. Allow refrigerated or frozen samples to reach room temperature before testing.

OPENING THE SEALED NICALERT™ PLASTIC POUCH

1. Open the NicAlert™ pouch by tearing at the slit on the side.

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

TESTING THE URINE SAMPLE WITH NICALERT™

1. Hold the strip by the handle, dip the sample end (the exposed soft cotton end of the strip) into the urine sample for 20 seconds (1/2 inch [1 cm]) or longer. Gently agitate the strip while holding it with forceps, tweezers, or gloves if preferred. Do not immerse the strip lower than the maximum sample line on the strip.

2. The NicAlert™ test result is the lowest level number 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.

3. For urine samples, a NicAlert™ result of:
   a. Level 3 or higher (Levels 3-5) indicates use of tobacco products.
   b. Levels 1-2 (Levels 1-2) indicates no detectable level of cotinine or tobacco product use.
   c. Levels 1-2 (Levels 1-2) indicates no detectable level of cotinine or tobacco product use.

4. 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.

5. A positive result indicates tobacco product exposure. The NicAlert™ test is 100 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 semi-quantitative determination of cotinine in urine for the purpose of determining if an individual has been exposed to tobacco products such as cigarettes. NicAlert™ test should be stored at ambient temperatures. The NicAlert™ test is only for use with human urine. A positive result indicates tobacco product exposure and discards appropriately after testing.

6. Each NicAlert™ test strip is individually packaged in a sealed plastic pouch. Each pouch contains a NicAlert™ strip for self-testing and discard appropriately after testing.

7. A NicAlert™ result should not be used on cloudy or pink urine samples.

8. For urine samples, a NicAlert™ result of:
   a. Level 3 or higher (Levels 3-5) indicates use of tobacco products.
   b. Levels 1-2 (Levels 1-2) indicates no detectable level of cotinine or tobacco product use.
   c. Levels 1-2 (Levels 1-2) indicates no detectable level of cotinine or tobacco product use.
   d. 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

<table>
<thead>
<tr>
<th>Level</th>
<th>Cotinine Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1-10 ng/mL</td>
</tr>
<tr>
<td>1</td>
<td>50-1000 ng/mL</td>
</tr>
<tr>
<td>2</td>
<td>500-10000 ng/mL</td>
</tr>
<tr>
<td>3</td>
<td>10000-200000 ng/mL</td>
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<td>4</td>
<td>200000-5000000 ng/mL</td>
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<td>5</td>
<td>5000000-10000000 ng/mL</td>
</tr>
<tr>
<td>6</td>
<td>10000000-50000000 ng/mL</td>
</tr>
<tr>
<td>7</td>
<td>Above 50000000 ng/mL</td>
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</table>

TABLE 1: COTININE CONCENTRATIONS FOR EACH LEVEL

<table>
<thead>
<tr>
<th>Level</th>
<th>Cotinine Concentration</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>1-10 ng/mL</td>
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<tr>
<td>1</td>
<td>50-1000 ng/mL</td>
</tr>
<tr>
<td>2</td>
<td>500-10000 ng/mL</td>
</tr>
<tr>
<td>3</td>
<td>10000-200000 ng/mL</td>
</tr>
<tr>
<td>4</td>
<td>200000-5000000 ng/mL</td>
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<tr>
<td>5</td>
<td>5000000-10000000 ng/mL</td>
</tr>
<tr>
<td>6</td>
<td>10000000-50000000 ng/mL</td>
</tr>
<tr>
<td>7</td>
<td>Above 50000000 ng/mL</td>
</tr>
</tbody>
</table>

LIMITATIONS OF THE TEST FOR THE URINARY COTININE

NicAlert™ is only intended for in vitro diagnostic use with human urine. A positive result indicates tobacco product exposure and the presence of cotinine in the sample. Enzyme immunoassay results can be affected by technical or procedural factors, 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, if possible, before testing. 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 test result. In order to obtain a positive test result, a more specific alternate chemical method is needed. Gas Chromatography/Mass Spectrometry (GC/MS) is the preferred method for quantitation.

PERFORMANCE CHARACTERISTICS

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

NicAlert™ Strips:
- Need to be handled as stated in procedure as if it were a potential biohazard. Discard appropriately after testing.
- Each NicAlert™ test strip is individually packaged in a sealed plastic pouch. Each pouch contains a NicAlert™ strip for self-testing and discard appropriately after testing.
- NicAlert™ strip should not be used on cloudy or pink urine samples.
- NicAlert™ strip should be stored at ambient temperatures.
- NicAlert™ strip is only for use with human urine. A positive result indicates tobacco product exposure.
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- NicAlert™ strip should be stored at ambient temperatures. 
### TABLE 3: ACCURACY STUDIES

#### A. Non-Smokers

<table>
<thead>
<tr>
<th>Cotinine Concentration (ng/mL)</th>
<th>Number of Samples</th>
<th>NicAlert™ Reading of &quot;0&quot;</th>
<th>NicAlert™ Reading of &quot;1&quot;</th>
<th>NicAlert™ Reading of &quot;2&quot;</th>
<th>NicAlert™ Reading of &quot;3&quot;</th>
<th>NicAlert™ Reading of &quot;4&quot;</th>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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#### B. Smokers

<table>
<thead>
<tr>
<th>Cotinine Concentration (ng/mL)</th>
<th>Number of Samples</th>
<th>NicAlert™ Reading of &quot;0&quot;</th>
<th>NicAlert™ Reading of &quot;1&quot;</th>
<th>NicAlert™ Reading of &quot;2&quot;</th>
<th>NicAlert™ Reading of &quot;3&quot;</th>
<th>NicAlert™ Reading of &quot;4&quot;</th>
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<td>0</td>
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</tr>
</tbody>
</table>

### REFERENCES


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