The COT Rapid Test Device (Urine) is intended for use with human urine specimens only. Urine collected at any time of the day may be used. Urine specimens must be collected in clean, dry containers. Turbid specimens should be centrifuged, filtered, or allowed to settle and only the clear supernatant should be used for testing. Perform testing immediately after specimen collection. Do not leave specimens at room temperature for prolonged periods. Urine specimens should be stored at 2-8°C for up to 2 days. For long term storage, reagents should be kept below -20°C. Bring specimens to room temperature prior to testing. Freeze specimens must be completely melted and mixed well prior to use. Avoid repeated freezing and thawing of specimens. If specimens are to be shipped, pack them in compliance with all applicable regulations for transportation of biological agents.

**PRINCIPLE**

The COT Rapid Test Device (Urine) detects Cotinine through visual interpretation of color development on the device. Drug conjugates are immobilized on the test region of the membrane. During testing, the specimen reacts with antibodies conjugated to colored particles and proceeds on the sample pad. The mixture then migrates through the membrane by capillary action, and interacts with reagents on the membrane. If there are insufficient drug molecules in the specimen, the antibody-colored particle conjugate will bind to the drug conjugates, forming a colored band at the test region of the membrane. Therefore, a colored band appears in the test region when the urine is negative for the drug. If drug molecules are present in the urine above the cut-off concentration of the test, they compete with the immobilized drug conjugate on the test region for limited antibody binding sites. This will prevent attachment of the antibody-colored particle conjugate to the test region. Therefore, the absence of a colored band at the test region indicates a positive result. The appearance of a colored band at the control region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

**MATERIALS**

**Materials Provided**
- Package insert
- Timer
- Solution A (carrier solution)
- Solution B
- Specimen collection container
- Cleaning solution

**Materials Required but Not provided**
- COT (Cotinine) 200
- COT-2U3
- COT Rapid Test Device (Urine)
- Negative control
- Positive control
- Buffer A
- Buffer B
- Urea
- Sodium acetate
- Electrophoresis tank
- Stain
- Destain

**PRECAUTIONS**

- For professional in vitro diagnostic use only.
- Do not use after expiration date indicated on the package. Do not use the test if its foil pouch is damaged. Do not reuse tests.
- This kit may contain products of animal origin. Certified knowledge of the origin and/or sanitary status of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore, recommended that these products be treated as potentially infectious, and that all observing the usual safety precautions (do not ingest or inhale).
- Avoid cross-contaminating the specimen by using a new specimen collection container for each specimen obtained. Read the entire procedure carefully prior to performing any tests.
- Do not eat, drink or smoke in the area where the specimens and kits are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- Humidity and temperature can adversely affect results.
- The used testing materials should be discarded in accordance with local, state and/or federal regulations.

**STORAGE AND STABILITY**

- The kit should be stored at 2-30°C until the expiry date printed on the sealed pouch. The test must remain in the sealed pouch until use.
- Do not freeze.
- External controls are not supplied with this kit. It is recommended that positive and negative controls be used as a good laboratory practice to confirm the test procedure and to verify proper test performance.

**LIMITATIONS OF THE TEST**

- Internal procedural controls are included in the test. A colored band appearing in the control region (C) is considered an internal positive procedural control, confirming sufficient specimen volume and proper procedural technique.
- External controls are not supplied with this kit. It is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

**PERFORMANCE CHARACTERISTICS**

- **A. Accuracy**
  - The accuracy of the COT Rapid Test Device (Urine) was checked and compared against commercially available tests with a threshold value at the same cut-off levels. Urine samples taken from volunteers consisting of cigarette smokers and non-smokers were examined under both tests. The results were >99.9% in agreement.
- **B. Reproducibility**
  - The reproducibility of the COT Rapid Test Device (Urine) was verified by blind tests performed at four different locations. Samples with Cotinine concentrations at 50% of the cut-off were all determined to be negative, while samples with Cotinine concentrations at 200% of the cut-off were all determined to be positive.
- **C. Precision**
  - Test precision was determined by blind tests with control solutions. Controls with Cotinine concentrations at 50% of the cut-off yielded negative results, and controls with Cotinine concentrations at 150% of the cut-off yielded positive results.
- **D. Specificity**
  - The following tables list the concentrations of compounds (mg/mL) above which the COT Rapid Test Device (Urine) identified positive results at 3 minutes.

<table>
<thead>
<tr>
<th>Cotinine 200 related compounds</th>
<th>Concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-Epinephrine</td>
<td>Chlorpheniramine</td>
</tr>
<tr>
<td>(+/-)-Naproxen</td>
<td>Dextromethorphan</td>
</tr>
<tr>
<td>(+/-)-Ephedrine</td>
<td>Dextrophan tartrate</td>
</tr>
<tr>
<td>4-Dimethylamino-&lt;sub&gt;2&lt;/sub&gt;aterine</td>
<td>Phenylalanine</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>Dopamine</td>
</tr>
<tr>
<td>Acetone</td>
<td>Ethanol</td>
</tr>
<tr>
<td>Albutol</td>
<td>Ethanol</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>Fenoldoxime</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>Glucose</td>
</tr>
<tr>
<td>Aspartame</td>
<td>Guaiacol Glycephosphate</td>
</tr>
<tr>
<td>Aspirin</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>Benzocaine</td>
<td>Isoindoline</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>(+/-)-Isoprotoren</td>
</tr>
<tr>
<td>Benzocaine tartrate</td>
<td>Methadone</td>
</tr>
<tr>
<td>Caffeine</td>
<td>Methadone</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>Vitamin C (Ascorbic Acid)</td>
</tr>
</tbody>
</table>

- **QUALITY CONTROL**
  - Internal procedural controls are included in the test. A colored band appearing in the control region (C) is considered an internal positive procedural control, confirming sufficient specimen volume and proper procedural technique.
  - External controls are not supplied with this kit. It is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

**INTRODUCTION**

Cotinine is the first-stage metabolite of nicotine, a toxic alkaloid that produces stimulation of the autonomic ganglia and central nervous system when humans. In nicotine is a drug to which virtually every member of a tobacco-smoking society is exposed whether through direct contact or second-hand inhalation. In addition to tobacco, nicotine is also commercially available as the active ingredient in smoking replacement therapies such as nicotine transdermal patches and nasal sprays. In a 24-hour urine, approximately 5% of a nicotine dose is excreted as unchanged drug with 10% as cotinine and 35% as hydroxocotinine; the concentrations of other metabolites are believed to be less than 5%. While cotinine is thought to be an inactive metabolite, it’s elimination profile is more stable than that of nicotine which is largely urine pH dependent. As a result, cotinine is considered a good biological marker for determining nicotine use. The plasma half-life of nicotine is approximately 60 minutes following paternal administration. Nicotine and cotinine are rapidly eliminated by the kidney; the window of detection for cotinine in urine at a cutoff level of 200 ng/mL is expected to be up to 2-3 days after nicotine use.

**PROCEDURE**

Bring tests, specimen, and/or controls to room temperature (15-30°C) before use.

1. Remove the test from its sealed pack and place it in a clean, level surface. Label the test with patient or control identification. For best results, the assay should be performed within one hour.
2. Using the provided disposable pipette, transfer 3 drops of specimen (approximately 120 µL) to the specimen well (S) of the device and start the timer.
3. Avoid trapping air bubbles in (S). Avoid the test well (T) and do not add any solution to the result area.
4. As the test begins to work, color will migrate across the membrane.
5. Wait for the colored band(s) to appear. The result should be read at 5 minutes. Do not interpret the result after 10 minutes.

<table>
<thead>
<tr>
<th>GLOSSARY OF SYMBOLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalog number</td>
</tr>
<tr>
<td>Consult instructions for use</td>
</tr>
<tr>
<td>In vitro diagnostic medical device</td>
</tr>
<tr>
<td>Manufacturer</td>
</tr>
<tr>
<td>Do not reuse</td>
</tr>
<tr>
<td>CE marking according to IVD Medical Devices Directive 98/79/EC</td>
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</tbody>
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