OneStep

Tricyclic Antidepressants (TCA)

RapiCard™ InstaTest

Cat # 121025-1

FOR THE QUALITATIVE ASSESSMENT OF NORTRIPTYLINE IN HUMAN URINE

For in vitro Diagnostic and Forensic Use

INTENDED USE

The Cortez Diagnostics, Inc TCA RapiCard™ InstaTest is an immunochromatography based one step in vitro test. It is designed for qualitative determination of Nortriptyline in human urine specimens above a cut-off level of 1000 ng/ml. This assay may be used in the point of care setting.

This assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) has been established as the preferred confirmatory method by the Substance Abuse Mental Health Services Administration (SAMHSA). Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are indicated.

SUMMARY AND EXPLANATION

Tricyclic Antidepressants (TCA) are a group of antidepressant drugs that contain tricyclic structure, TCA are commonly used for the treatment of depressive disorders, it can be taken by oral or injection, TCAs are
metabolized in the liver, both TCAs and their metabolites are excreted in urine for up to 10 days. Therefore, Cortez TCA is able to detect TCAs and their metabolites, such as nortriptyline, etc.

**TEST PRINCIPLE**

The Cortez Diagnostics, Inc. TCA RapiCard™ InstaTest is based on the principle of specific immunochemical reaction between antibodies and antigens to analyze particular compounds in human urine specimen. The assay relies on the competition for binding antibody between drug conjugate and free drug which may be present in the urine specimen being tested. When drug is present in the urine specimen, it competes with drug conjugate for the limited amount of antibody-dye conjugate. When the amount of drug is equal or more than the cut-off, 1000 ng/ml, it will prevent the binding of drug conjugate to the antibody. Therefore, a positive urine specimen will not show a colored band on the test line zone, indicating a positive result, while the presence of a colored band indicates a negative result.

A control line is present in the test window to work as procedural control. This colored band should always appear on the control line zone if the test device is stored in good condition and the test is performed appropriately.

**MATERIALS PROVIDED**

1. Instructions for use.
2. Cortez TCA test device. The amount of each coated antigen and/or antibody on the strip is less than 1.0 mg for antigen conjugate and is less than 1.0 mg for goat anti-mouse IgG antibody.
   - Test zone: contains TCA bovine protein antigen conjugates.
   - Control zone: contains Goat anti-mouse IgG antibody.

**MATERIALS REQUIRED BUT NOT PROVIDED**

1. Urine collection container.
2. Timer or clock.

**STORAGE AND STABILITY**

The test device should be stored at 2 to 30°C and will be effective until the expiration date stated on the package. The product is humidity-sensitive and should be used immediately after being open. Any improperly sealed product should be discarded.

**PRECAUTIONS**

1. For in vitro diagnostic and forensic use only.
2. Do not use the product beyond the expiration date.
3. Handle all specimens as potentially infectious.
4. Humidity sensitive product, do not open foil pouch until it is ready to be tested.
5. Use a new urine specimen cup for each sample to avoid cross contamination.

**SPECIMEN COLLECTION AND PREPARATION**

It is required that approximately 150 µl of sample for each test. Fresh urine specimens do not need any special handling or treatment. Specimens should be collected in a clean, dry, plastic or glass container. If the assay is not performed immediately, urine specimen may be refrigerated at 2-8°C or frozen up to 7 days. Specimens should be thawed and brought to room temperature before testing. Urine specimens exhibiting a large amount of precipitate or turbidity should be centrifuged or allowed to settle before testing. Avoid contact with skin by wearing gloves and proper laboratory attire.
QUALITY CONTROL
Good Laboratory Practice recommends the daily use of control materials to validate the reliability of device. Control materials should be assayed as clinical specimen and challenging to the assay cutoff concentration, e.g., 25% above and below cutoff concentration. If control values do not fall within establish range, assay results are invalid. Control materials which are not provided with this test kit are commercially available.

The Cortez Drugs of Abuse Test provides a built-in process control with a different antigen/antibody reaction at the control region (C). This control line should always appear regardless the presence of drug or metabolite. If the control line does not appear, the test device should be discarded and the obtained result is invalid. The presence of this control band in the control region serve as 1) verification that sufficient volume is added, 2) that proper flow is obtained.

PROCEDURE
1. Bring all materials and specimens to room temperature.
2. Remove the test strip from the sealed foil pouch.
3. Place the transfer pipette in the specimen and depress the bulb to withdraw a sample.
4. Hold the pipette in a vertical position over the sample well of the card and deliver 3 drops (120-150ul) of sample into the sample well.
5. Read the results at 5 minutes after adding the sample. **Do not interpret the result after 5 minutes.**

INTERPRETATION OF RESULTS

Negative:
Two colored bands form. The appearance of two colored bands, one in test line zone and the other in control line zone, indicates negative results. The negative result indicates that the nortriptyline concentration in the specimen is either zero or less than cut-off level.

Positive:
One colored band forms. One colored band appears in control line zone. No colored band is found in test line zone. This is an indication that the nortriptyline level in the specimen is above the cut-off level.

Invalid:
If there is no colored band in control line zone, the test result is invalid. Retest the sample with a new device.

*Note: A borderline (+-) in test line zone should be considered negative result.*

LIMITATION OF PROCEDURE

The assay is designed for use with human urine only. A positive result with any of the tests indicates only the presence of a drug/metabolite and does not indicate or measure intoxication. There is a possibility that technical or procedural error as well other substances in certain foods and medicines may interfere with the test and cause false results. Please refer “SPECIFICITY” section for lists of substances that will produce either positive results, or that do not interfere with test performance. If a drug/metabolite is found present in the urine specimen, the assay does not indicate frequency of drug use or distinguish between drug of abuse and certain foods and medicines.

EXPECTED RESULTS

The Cortez Diagnostics, Inc. TCA RapiCard™ InstaTest is a qualitative assay. It identifies nortriptyline in human urine at a concentration of 1000 ng/ml or higher. The concentration of the nortriptyline cannot be determined by this assay. The test is intended to distinguish negative result from presumptive positive result. All positive results must be confirmed using an alternate method, preferably GC/MS.
PERFORMANCE CHARACTERISTICS

A. Accuracy
The accuracy of the Cortez TCA test was evaluated in comparison to GC/MS at a cut-off of 1000 ng/ml of nortriptyline. 100 urine specimens with GC/MS confirmed nortriptyline concentration were evaluated in this study. The results are summarized and presented below:

<table>
<thead>
<tr>
<th>Cortez TCA Test</th>
<th>No. of Tested</th>
<th>(-)</th>
<th>Near cutoff negative (between –25% and c/o)</th>
<th>Near cutoff positive (between c/o and +25%)</th>
<th>GC/MS Positive (greater than +25% cut off)</th>
<th>Percent agreement with GC/MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>53</td>
<td>2</td>
<td>7</td>
<td>25</td>
<td>91.4</td>
<td>N=98</td>
</tr>
<tr>
<td>Negative</td>
<td>53</td>
<td>8</td>
<td>3</td>
<td>0</td>
<td>95.3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>103</td>
<td>10</td>
<td>10</td>
<td>25</td>
<td>N=98</td>
<td></td>
</tr>
</tbody>
</table>

Positive % agreement: 97.8, Negative % agreement: 94.5.
Four specimens were found discrepant between the Cortez TCA and GC/MS method. When compared those data, 75% (3 out of 4) of the discrepancy specimens were found between –25% and +25% cut-off concentration (750 – 1250 ng/ml).

B. Sensitivity
The cut-off concentration (sensitivity level) of Cortez TCA test is determined to be 1000ng/ml.

C. Precision
The precision study was performed by three individuals observing the test result to determine the random error of visual interpretation. The test results were found to have no significant differences between the three observers.

<table>
<thead>
<tr>
<th>Device</th>
<th>Control Con. ng/ml</th>
<th>No. of Tested</th>
<th>No. of positive</th>
<th>No. of positive &amp; TCA</th>
<th>No. of borderline #</th>
<th>No. of negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1*</td>
<td>2*</td>
<td>3*</td>
<td>1*</td>
</tr>
<tr>
<td>TCA</td>
<td>500</td>
<td>42</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>42</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>42</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1250</td>
<td>42</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>42</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>0</td>
</tr>
</tbody>
</table>

D. Specificity
The specificity for Cortez TCA test was tested by adding various drugs, drug metabolites, and other compounds that are likely to be present in urine. All compounds were prepared in drug-free normal human urine.

1. Interference testing
The Cortez TCA test performance at cut-off level is not affected when pH and Specific Gravity ranges of urine specimen are at 4.0 to 9.0 and 1.005 to 1.035.
The following substances were tested and confirmed not to interfere with Cortez TCA test at the listed concentrations.

- Glucose: 2000 mg/dl
- Human albumin: 2000 mg/dl
- Human hemoglobin: 10 mg/dl
- Urea: 4000 mg/dl
- Uric acid: 10 mg/dl

2. Specificity

The following table lists compounds that are detected by Cortez PCP test which produced positive results when tested at levels equal or greater than the concentrations listed below:

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Con. (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nortriptyline</td>
<td>1000</td>
</tr>
<tr>
<td>Protriptyline</td>
<td>1000</td>
</tr>
<tr>
<td>Imipramine</td>
<td>1000</td>
</tr>
<tr>
<td>Desipramine</td>
<td>1000</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>1000</td>
</tr>
<tr>
<td>Doxepin</td>
<td>1000</td>
</tr>
<tr>
<td>Nordoxepin</td>
<td>1000</td>
</tr>
<tr>
<td>Promazine</td>
<td>500</td>
</tr>
<tr>
<td>Trimopramine</td>
<td>2000</td>
</tr>
</tbody>
</table>

Each listed substance that commonly found in the urine was evaluated and indicated negative result at concentration of 100 µg/ml.

Acetaminophen 4-Acetamidophenol Acetylsalicylic acid Amikacin
Amitriptyline Amobarbital Amphetamine Arterenol
Aspartame Ascorbic acid Atrophine Caffeine
Camphor Chloroquine Chlopheniramine Cortisone
Deoxyephedrine Dextromethorphan Digitoxin Digoxin
Diphenhydramine Ecgonine Ecgonine methyl ester Ephedrine
Epinephrine Gentisic Guaiacol glycer ester Histamine
Hydrochlorothiazide Homatrine Imipramine Ibuprofen
Isoproterenol Ketamine Lidocaine Meperidine
Methadone Methamphetamine 3,4±MDMA Methaqualone
Methylphenidate Neomycin Niacinamide Oxazepam
Perphenazine Penicillin G Phenylethylamine-α Phenylpropanolamine
Promethazine Pseudoephedrine Quinine antidine Salicylic acid Tetracycline
Tetrahydrozoline Theophylline 11-nor-Δ⁸–THC-9-COOH (10 µg/ml) Tryptophan
11-nor-Δ⁸–THC-9-COOH (10 µg/ml)Thioridazine Trifluoperazine
Tyramine Perphenazine Chlopromazine Clomipramine

REFERENCES


<table>
<thead>
<tr>
<th>Date Adopted</th>
<th>Reference No.</th>
</tr>
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<tbody>
<tr>
<td>2004-11-19</td>
<td>DA-Tricyclic Antidepressants (TCA)-2009</td>
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