OneStep

Methadone Urine
RapiCard™ InstaTest

Cat. # 121070-1

FOR THE QUALITATIVE ASSESSMENT OF METHADONE IN HUMAN URINE
For in vitro Diagnostic and Forensic Use

INTENDED USE
The Cortez Diagnostics Inc. OneStep MTD RapiCard™ InstaTest is an immunochromatography based one step in vitro test. It is designed for qualitative determination of methadone in human urine specimens above a cut-off level of 300 ng/ml. The 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, such as GC/MS, must be used in order to obtain a confirmed analytical result. 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
Methadone is a synthetic opioid, clinically available. It is used clinically for the treatment of severe pain and in maintenance programs for morphine and heroine addicts.

Methadone acts on the central nervous and cardiovascular systems to produce respiratory and circulatory depression. Methadone also produces miosis and increases the tone of smooth muscle in the lower gastrointestinal tract while decreasing the amplitude of contractions. Acute higher doses induce analgesia, sedation, respiratory depression and coma.

After methadone administration, the major urinary excretion products are methadone and its metabolites, EDDP and EMDP. Large individual variations in the urine excretion of methadone are output of methadone from 5-22%. Typically, following a 5 mg oral dose, methadone and EDDP account for 5% of the dose in the 24-hour urine. In those individuals on maintenance therapy, methadone may account for 5 to 50% of the dose in the 24-hour urine and EDDP may account for 3 to 25% of the dose.
**TEST PRINCIPLE**
The Cortez OneStep MTD 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 mouse anti-methadone monoclonal antibody between methadone-protein conjugate and free drug which may be present in the urine specimen being tested.

When methadone is present in the urine specimen, it competes with methadone-protein conjugate for the limited amount of monoclonal antibody-colloidal gold conjugate. When the amount of methadone is equal or more than the cut-off, it will prevent the binding of methadone-protein 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 composed of Goat anti-Mouse IgG antibody 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 MTD 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 Methadone bovine protein antigen conjugates.
   - Control zone: contains Goat anti-mouse IgG antibody.
   - Conjugate pad: contains about 2 µg of colloidal gold-mouse anti-methadone monoclonal antibody conjugate.

**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.

**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. 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 up to 7 days or frozen. Specimens should be 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.
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.

The control line does not appear, the test should be discarded and the obtained result is invalid. You should always follow local, state and federal guidelines for running QC.

PROCEDURE
1. Bring all materials and specimens to room temperature.
2. Remove the test card 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 test card and deliver 2-3 drops (100-150 µl) of sample into the sample well.
5. Read the result 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 methadone level 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 methadone 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. This test has not been evaluated in a point of care setting.
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 OneStep MTD RapiCard™ InstaTest is a qualitative assay. It identifies methadone in human urine at a concentration of 300 ng/ml or higher. The concentration of the methadone 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 OneStep MTD RapiCard™ InstaTest was evaluated in comparison to GC/MS at a cut-off of 300 ng/ml of methadone. One hundred and nineteen urine specimens with confirmed methadone concentrations were evaluated in this study. Borderline readings were recorded as negative. The results are summarized and presented below:

<table>
<thead>
<tr>
<th>Cortez MTD Test</th>
<th>(-)</th>
<th>(+)</th>
<th>Percent agreement with GC/MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC/MS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC/MS Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(less than -25% cut off)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Near cutoff negative (between –25% and c/o)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Near cutoff positive (between c/o and +25%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC/MS Positive (greater than +25% cut off)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>9</td>
<td>49</td>
</tr>
<tr>
<td>Negative</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>9</td>
<td>49</td>
</tr>
<tr>
<td>Percent agreement with GC/MS</td>
<td>100</td>
<td>96.7</td>
<td></td>
</tr>
</tbody>
</table>

Positive % agreement: 96.7, Negative % agreement: 100. Two specimens were found discrepant between the Cortez MTD and GC/MS method. When compared those data, 100% (2 out of 2) of the discrepancy specimens were found between cut-off and +25% cut-off concentration (300 – 375 ng/ml).

B. Sensitivity
The cut-off concentration (sensitivity level) of Cortez MTD test is determined to be 300ng/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 1*</th>
<th>No. of positive 2*</th>
<th>No. of positive 3*</th>
<th>No. of borderline #1*</th>
<th>No. of borderline #2*</th>
<th>No. of borderline #3*</th>
<th>No. of negative 1*</th>
<th>No. of negative 2*</th>
<th>No. of negative 3*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTD</td>
<td></td>
<td>150</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>225</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21</td>
<td>28</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>375</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>450</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>

D. Specificity
The specificity for Cortez MTD 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 OneStep MTD RapiCard™ InstaTest performance at cut-off level is not affected when pH and Specific Gravity ranges of urine specimen are at 4.5 to 8.0 and 1.005 to 1.035.

The following substances were tested and confirmed not to interfere with Cortez MTD 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 MTD test which produced positive results when tested at levels equal or greater than the concentrations listed below:

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Cut-off (ng/ml)</th>
<th>Cross reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methadone</td>
<td>300</td>
<td>100</td>
</tr>
<tr>
<td>Doxylamine</td>
<td>500,00</td>
<td>0.6</td>
</tr>
<tr>
<td>Norpropoxyphene</td>
<td>1,000</td>
<td>30</td>
</tr>
</tbody>
</table>

The following compounds show no cross-reactivity at concentration up to 100 µg/ml unless specified.

Acetaminophen Acetylsalicylic acid Amikacin Amitriptyline
Amobarbital Amphetamine Arterenol Aspartame
Ascorbic acid Atrophine Benzoic acid Benzylecgonine
Butabartital Caffeine Camphor Chlopeniramine
Chloroquine Cocaine Cortisone Deoxyephedrine
Dextromethorphan Diazepam Digitoxin Digoxin
Diphenhydramine Ecgonine Ecgonine methyl ester Ephedrine
Epinephrine Gentiisic acid Guaiacol glycer ester Histamine
Homatrophone Hydrochlorothiazide Ibufrofen Imipramine
Isoproterenol Ketamine Lidocaine 3,4±MDA
Methamphetamine Meperidine Methaqualone Methylphenidate
Morphine Neomycin Niacinamide Oxazepam
Perphenazine Penicillin G Phencyclidine Phenobarbital
Phenylethylamine-α Phenylpropanolamine Promethazine Pseudoephedrin
Quinone antidine Salicylic acid Secobarbital Tetracycline
Tetrahydrozoline Theophyline 11-nor-∆^8-THC-9-COOH(10 µg/ml)
11-nor-∆^9-THC-9-COOH(10 µg/ml) Thioridazine Trifluoperazine
Tyramine Tryptophan Propoxyphene

REFERENCES

1. Urine testing for drugs of abuse, NIDA Research Monograph 73 (1986)

<table>
<thead>
<tr>
<th>Date Adopted</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008-08-09</td>
<td>DA-Methadone Urine-2009</td>
</tr>
</tbody>
</table>

CORTEZ DIAGNOSTICS, INC.
23961 Craftsman Road, Suite D/E/F, Calabasas, CA 91302
Tel: (818) 591-3030 Fax: (818) 591-8383
ISO 13485-2003

Revision Date: 1/20/09