



CORTEZ DIAGNOSTICS, INC.

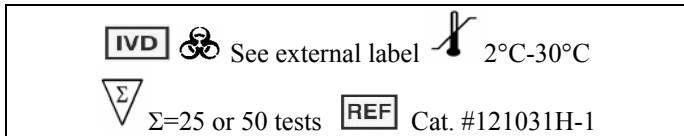
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OneStep TCA RapiDip IntaTest Cat. No. 121031H-1

INTENDED USE

This device is a one-step immunoassay intended to provide qualitative rapid detection of tricyclic antidepressants (TCA) at a cutoff concentration of 1000 ng/ml in human urine. It is for health care professional use only.

This assay provides only a preliminary result. Clinical consideration and professional judgment must be applied to any drug of abuse test result, particularly in evaluating a preliminary positive result. In order to obtain a confirmed analytical result, a more specific alternate chemical method is needed. Gas Chromatography/Mass Spectroscopy (GC/MS) is the preferred confirmation method.¹

SUMMARY AND EXPLANATION OF THE TEST

Tricyclic Antidepressants (TCA) are a group of antidepressant drugs that contain three fused rings in their chemical structure.² TCA can be taken orally or intramuscularly (IM). The progressive symptomatology of TCA includes agitation, confusion, hallucinations, hypertonicity, seizures, and EKG changes. The half-life of TCA varies from a few hours to a few days. The commonly used tricyclic antidepressants are excreted with a very low percentage of unchanged drugs in the urine, less than 1%. Therefore, detecting TCA or metabolites of TCA in human urine has been used for screening the abuse of TCA.^{3,4} This test is able to detect amitriptyline, desipramine, Imipramine and nortriptyline at a cut off level of 1,000 ng/ml.

PRINCIPLE OF THE PROCEDURE

This assay is a one-step lateral flow chromatographic immunoassay. The test strip includes: 1) a burgundy colored conjugate pad containing mouse anti-TCA antibodies coupled to colloidal gold (the immunogen is a blend of amitriptyline, desipramine, imipramine and nortriptyline); and 2) nitrocellulose membrane containing a Test (T) line a Control (C) line. The Test line is coated with TCA-BSA, and the Control line is coated with goat anti-mouse IgG antibody.

This test is a competitive binding immunoassay. The TCA metabolites in the urine specimen compete with the TCA-BSA, which is coated on the nitrocellulose membrane for the limited binding sites of the anti-TCA antibodies in the conjugate pad.

When an adequate amount of urine specimen is applied to the sample pad of the device, the urine specimen migrates by capillary action through the test strip. If the level of TCA and/or TCA metabolites in the urine specimen is below the cutoff (1000 ng/ml), the Test line should appear as a visible burgundy line. If the level of TCA metabolites in the urine specimen is at or above the cutoff, no Test line develops.

The Control line is coated with goat anti-mouse antibody, which should bind to the gold-antibody conjugate and form a burgundy color line regardless of the presence of TCA metabolites.

REAGENTS AND MATERIAL SUPPLIED

- 50 test devices, each sealed in a pouch with a desiccant.
- 1 package insert (Instructions for Use).

MATERIAL REQUIRED BUT NOT PROVIDED

- Specimen collection containers
- Timer

STORAGE AND STABILITY

Store the kit at room temperature 15-30°C (59-86°F). Each device may be used until the expiration date printed on the label if it remains sealed in its foil pouch containing a desiccant.

Do not freeze and/or expose the kit to temperatures over 30°C.

SPECIMEN COLLECTION

1. Each urine specimen must be collected in a clean container.
2. Specimens may be kept at 15-30°C (59-86°F) for at least 8 hours, at 2-8°C for up to 3 days and at -20°C or lower for long term storage. Do not mix specimens.

PRECAUTION

1. The instructions must be followed to obtain accurate results.
2. Do not open the sealed pouch, unless ready to conduct the assay.
3. Do not use expired devices.
4. Dispose of all specimens and used assay materials as potentially biohazardous.

ASSAY PROCEDURE

1. Refrigerated specimens and other test materials, including devices, **must be equilibrated to room temperature before testing.**
2. Open the foil pouch at the notch and remove the test device.
3. Dip the device in the specimen for 10 seconds. Keep the specimen surface at the level indicated by the arrow sign on the device.
4. Remove the device from the specimen, and place it on a flat, dry surface.
5. Read the test result between four (4) to seven (7) minutes after adding the specimen.

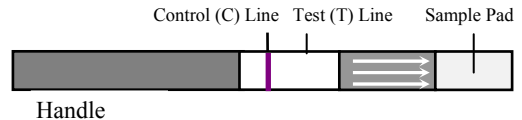
IMPORTANT: Do not read test results after seven (7) minutes.

INTERPRETATION OF RESULTS

Positive:

If only the Control line (C) appears, the test indicates a positive result.

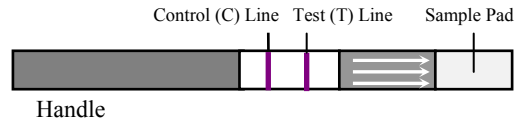
Samples with positive results should be confirmed with a more specific method before a positive determination is made.⁵



Negative:

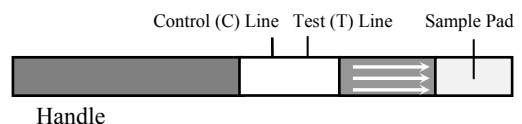
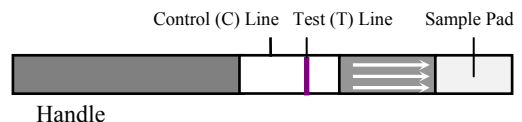
If both the Control (C) line and Test (T) line appear, the test indicates a negative result.

Note: A very faint line in the test region should be considered negative.



Invalid:

If no Control (C) line develops within 5 minutes, the test is invalid. In that case, repeat the assay with a new test device.



QUALITY CONTROL

• Internal Quality Control

This test contains a built-in control feature, the Control (C) line. The presence of this Control line indicates that an adequate sample volume was used and that the reagents migrated properly. If a C line does not form, the test is considered invalid. In this case, review the whole procedure and repeat the testing with a new device.

• External Quality Control

Users should always follow the appropriate federal, state, and local guidelines concerning the running of external quality controls. SAMHSA⁶ recommends that the concentration of drug(s) in positive and negative controls be approximately 25% above and below the cutoff concentration of the assay.

LIMITATIONS

1. This test is for *professional in vitro* diagnostic use only.
2. Results obtained by this device provide only a preliminary qualitative result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result.
3. This product is designed for testing human urine only.
4. Adulterants such as bleach or other strong oxidizing agents may produce erroneous test results. When suspected, collect a fresh specimen and repeat the test with a new device.
5. Samples in which bacterial contamination is suspected should not be used. These samples may interfere with the test and cause false results.

PERFORMANCE CHARACTERISTICS

1. Cutoff

The cutoff concentration of the Cortez Diagnostics, Inc. TCA Urine Test is 1000 ng/ml

2. Accuracy

The accuracy study was conducted by the comparison between the Cortez Diagnostics, Inc TCA Test and the GC/MS method. Eighty (80) clinical urine specimens were used in this study. Among the eighty (80) specimens, forty (40) were known drug-free specimens, and forty (40) contained different levels of TCA, Desipramine or Nortriptyline. The concentration of TCA was determined with GC/MS. Of the forty (40) specimens with TCA, ten (10) were below 75% of the cutoff (< 750 ng/ml), seven (7) out of the ten (10) were altered TCA clinical specimens diluted from original clinical specimens with known drug-free urine; ten (10) were within the range from 75% of the cutoff to the cutoff (750 ~ 1000 ng/ml); eight (8) were within the range from cutoff to 125% of the cutoff (1000 ~ 1250 ng/ml); and twelve (12) were above 125% of the cutoff (> 1250 ng/ml). All specimens were blind labeled.

		Cortez Diagnostics, Inc. Test		Total	Agreement
		Positive	Negative		
GC/MS (ng/ml)	Drug-free	0	40	40	100%
	<75% (0~750)		10	10	100%
	75%~Cutoff (750~1000)	2	8	10	80%
	Cutoff~125% (1000~1250)	8	0	8	100%
	Positive (>1250)	12		12	100%
Total		22	58	80	97.5%

The results from the Cortez Diagnostics, Inc. TCA Urine Test agreed 100% with the TCA GC/MS data at levels below 75% of the cutoff (negative) and above the cutoff (positive). Two (2) discrepancies were observed on the specimens with the GC/MS data between 75% of the cutoff and the cutoff. The overall agreement was 97.5%.

3. Reproducibility

Off-site evaluation

This study was done off-site at three (3) Physician's Office Laboratories (POL) and a clinical reference laboratory by personnel with diverse educational backgrounds and working experiences. One hundred (100) urine samples were divided into six groups, and spiked with nortriptyline at 0 ng/ml (40 members), 764 ng/ml (10 members), 1024 ng/ml (10 members), 1224 ng/ml (10 members), 1425 ng/ml (18 members), and 1825 ng/ml (12 members). All samples were blind labeled and tested with the Cortez Diagnostics, Inc. TCA Urine Test. Same results were obtained for all samples except one with ID =12 at the four evaluation sites. A total of four hundred (400) Cortez Diagnostics, Inc. TCA Urine Test devices were evaluated, and they all yielded the expected results except nine (9) tested with samples at the

concentration of 764 ng/ml. The discrepancies are shown in the following table.

ID	Expected Result	Conc. (ng/ml)	POL #1	POL #2	POL #3	Ref. Lab
12	-	764	+	-	-	-
41	-	764	+	+	+	+
94	-	764	+	+	+	+

The results from the four evaluation sites agreed 97.8% ((400-9)/400) with the concentration of nortriptyline and 99% with each other, indicating a high reproducibility of the Cortez Diagnostics, Inc. TCA Urine Test.

In-house evaluation

This study was conducted with three different lots. Specimens used in this study were the same used for the outside evaluation. The devices were tested for five consecutive days five times each, for a total of 25 assays for each standard.

The results were in 100% agreement among the replicates within each lot. No significant inter-lot or inter-day variation across the three different lots of devices.

4. Cross-Reactivity

A study was conducted with the Cortez Diagnostics, Inc. TCA Urine Test to evaluate the cross-reactivity of compounds structurally related to TCA. The following compounds, when spiked into known drug-free urine pools and then tested with the Cortez Diagnostics, Inc. TCA Urine Test, showed a positive response at the concentration listed.

Description	Concentration (ng/ml)
Amitriptyline	1,000
Clomipramine	5,000
Cyclobenzaprine	1,500
Desipramine	1,000
Doxepine	3,000
Imipramine	1,000
Nordoxepine	1,000
Nortriptyline	1,000
Perphenazine	75,000
Promazine	15,000
Protriptyline	2,000
Trimipramine	2,000

4. Interference

To evaluate the possible interference of structurally unrelated compounds with the Cortez Diagnostics, Inc. TCA Urine Test, the following analytes, usually found in urine and commonly prescribed therapeutic drugs, were spiked in drug-free urine pools, as well as TCA positive (spiked with TCA to the level of 1000 ng/ml) urine pools accordingly, and then tested with the Cortez Diagnostics, Inc. TCA Urine Test. No significant interference with either negative or positive results was observed at the concentrations listed below:

Compounds tested and found not to interfere with the test at 1.0 mg/ml concentration in urine	
Acetylsalicylic Acid	Cortisone
Amikacin	Dextromethorphan
Ampicillin	Methadone
Arterenal	Methanol
Aspirin	Oxalic Acid
Atropine	Penicillin-G (Benzylpenicillin)
Benzoic Acid	Pheniramine
Benzoylcegonine	Phenylpropanolamine
Caffeine	Ranitidine
(+)-Chlorpheniramine	Salicylic Acid
Cocaine	Thioridazine
Codeine	Trifluoperazine

Biological analytes tested and found no interference with the test at the concentrations listed	
Biological Analytes	Concentration
Albumin	2 mg/ml
Bilirubin	1 mg/ml
Creatine	1 mg/ml
Glucose	2 mg/ml
Hemoglobin	1 mg/ml
PH	4.5 - 8.5
Uric Acid	1 mg/ml
Vitamin C (L-Ascorbic Acid)	1 mg/ml

Effect of Specific Gravity: Eight (8) human urine specimens with the specific gravity ranging from 1.002 to 1.035 g/ml were collected in house. Each was spiked with nortriptyline to three levels, 750, 1,500, and 2,000 ng/ml. All those specimens were tested with the Cortez Diagnostics, Inc. TCA Urine Test, separately. The results indicated that the specific gravity of urine, ranging from 1.002 to 1.035, did not affect the performance of the Cortez Diagnostics, Inc. TCA Urine Test.

REFERENCES

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