



ONE STEP Tricyclic Antidepressants (TCA) TEST

(Urine)

FOR *IN VITRO* DIAGNOSTIC USE ONLY

INTENDED USE

THE ADVANCED QUALITY™ ONE STEP TRICYCLIC ANTIDEPRESSANTS TEST IS A RAPID, QUALITATIVE, COMPETITIVE BINDING IMMUNOASSAY FOR THE DETERMINATION OF TRICYCLIC ANTIDEPRESSANTS(TCA) AND ITS METABOLITES IN HUMAN URINE. THE TEST IS USED TO SCREEN URINE FOR THE PRESENCE OF TRICYCLIC ANTIDEPRESSANTS AND THEIR METABOLITES AT A CUTOFF CONCENTRATION OF 1000 NG/ML. THE TEST IS FOR USE BY HEALTH CARE PROFESSIONALS 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 few hours to 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.

The test is a qualitative, visual screening immunoassay. The method employs unique antibodies to selectively identify the drug in the test urine with a high degree of sensitivity and specificity.

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 (1,000 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.

A **negative** specimen produces two (2) distinct color bands, one in the test area and one in the control region.

A **positive** specimen produces only one (1) color band in the control region.

REAGENTS AND MATERIALS SUPPLIED

Each Kit contains:

FOR STRIP TEST

1. Fifty (50) foil pouches; each pouch contains one test strip and one desiccant.
2. 50 urine cups
3. Instruction for use

FOR CARD TEST

1. Forty (40) foil pouches; each pouch contains one test card, one plastic dropper and one desiccant.
2. Instruction for use

MATERIALS REQUIRED BUT NOT PROVIDED

1. Urine collection containers (for card test)
2. Clock or Timer
3. Positive and negative urine controls available from commercial distributors.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. Avoid cross contamination of urine samples by using a new urine specimen cup for each sample.
3. Do not use the kit beyond the expiration date printed on the outside of the foil pouch.
4. Do not open the foil pouch until urine specimen is collected and ready to be tested.
5. Urine specimens may be infectious. Handle and dispose of all used specimens and devices in an approved biohazard container.

STORAGE AND STABILITY

The test device can be stored under refrigeration and at room temperature (2 - 30) and will be stable until the expiration date. **Do not** open foil pouch until ready to test.

SAMPLE COLLECTION AND PREPARATION

10 ml of urine must be collected in a clean, dry, plastic or glass container, that does not contain preservative. Some plastics may adsorb drugs. If not tested immediately, urine specimens may be stored refrigerated at 2-8°C for up to 7 days and then frozen(-20° C or colder) prior to assaying. Refrigerated or frozen samples must be warmed to room temperature and gently mixed before testing. Urine samples exhibiting visible precipitates or turbidity should be centrifuged or allowed to settle so a clear aliquot may be sampled for this assay. Collection of samples may require mandatory procedures and custody and control records. Poppy seed ingestion has been associated with positive test results in some samples.

ASSAY PROCEDURES

FOR STRIP TEST:

1. Bring all materials and specimens to room temperature.
2. Remove test strip from the sealed foil pouch.
3. Dip the test strip into the urine sample with the arrows pointing toward the specimen.
4. The urine level should reach the maximum line marked on the strip, but must not exceed the maximum line.
5. Hold the strip in the urine until a reddish color appears at the lower edge of the test membrane (approximately 10 seconds).
6. Withdraw the strip and place it face up on a clean, dry surface.
7. Read the result between 3 - 8 minutes after adding the sample.

FOR CARD TEST:

1. Bring all materials and specimens to room temperature.
2. Remove test card from the sealed foil pouch.
3. Place the test card on a flat dry surface.
4. Using the provided plastic dropper, dispense 2 drops (100µl) of urine sample to the sample well of the test card. Start timing.
5. Read result between 3 - 8 minutes after adding the sample.

READING THE TEST RESULTS

Read test results between 3 - 8 minutes. **Do not interpret results after 8 minutes.**

NEGATIVE Two (2) pink/purple bands form. In addition to the control band, a pink/purple band also appears in the test region.

Note: This immunoassay is a screening test. A negative result indicates the drug level is below the detection sensitivity. It is important to understand that concentrations of the drug below cut off may cause a faint "ghost line" to form in the test region. This "ghost line" should be considered a negative result.

POSITIVE One (1) pink/purple band appears in the control region. No band is found in the test region. This is an indication that the drug level is above the detection sensitivity level.

INVALID If there is no pink/purple band in the control area of the strip, the test result is invalid. Retest the sample using a new device.

Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary results are positive. Positive results should be confirmed by an alternate method such as GC/MS.

QUALITY CONTROL

1. Each test device has a control band to indicate that the sample volume and migration is adequate, and that the colloidal gold is dissolving as expected. An invalid result must be repeated using a new test device.
2. Positive and negative, drug free urine controls can be used to validate reagent performance and establish test reliability. Commercial drug urine controls are available, but not provided with this test. NIDA recommended guidelines for drugs of abuse screening indicate controls should contain the drug at a level at least 20% above the NIDA cutoff value. If control values do not fall within the established limits, assay results are invalid.

EXPECTED RESULTS

The Advanced Quality TCA Test identifies Tricyclic Antidepressants and its metabolites in human urine at a concentration of 1000 ng/mL. The concentration of the drug cannot be determined using this test. The test is intended to screen urine to separate a negative result

from a presumptive positive result. All positive results must be confirmed using an alternate method, preferably GC/MS.

PERFORMANCE CHARACTERISTICS

Cutoff

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

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%.

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.

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

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
Benzoyllecgonine	Phenylpropanalamin
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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