ROJAN® TCA

One Step
Tricyclic Antidepressants Test Strip
Package Insert

REF: R-1109	English

A rapid, one step test for the qualitative detection of Tricyclic Antidepressants in human urine. For healthcare professionals including professionals at point of care sites. For in vitro diagnostic use only.

INTENDED USE

The TCA One Step Tricyclic Antidepressants Test Strip (Urine) is a lateral flow chromatographic immunoassay for the detection of Nortriptyline at a cut-off concentration of 1000 ng/mL. This test will detect other Tricyclic Antidepressants.

Please refer to the Analytical Specificity table in the package insert.

This assay provides only a qualitative, preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) or high performance liquid chromatography (HPLC) is the preferred confirmatory method. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

SUMMARY

TCA (Tricyclic Antidepressants) are commonly used for the treatment of depressive disorders. TCA overdoses can result in profound central nervous system depression, cardiotoxicity and anticholinergic effects. TCA overdose is the most common cause of death from prescription drugs. TCAs are taken orally or sometimes by injection.

TCAs are metabolized in the liver. Both TCAs and their metabolites are excreted in urine mostly in the form of metabolites for up to ten days. The TCA One Step Tricyclic Antidepressants Test Strip is a rapid urine-screening test that can be performed without the use of an instrument. The test utilizes the antibody to selectively detect elevated levels of Tricyclic Antidepressants in urine. The TCA One Step Tricyclic Antidepressants Test Strip yields a positive result when the Tricyclic Antidepressant in urine exceeds cut-off concentration.

PRINCIPLE

The TCA One Step Tricyclic Antidepressants Test Strip (Urine) is a rapid chromatographic immunoassay based on the principle of competitive binding. Drugs which may be present in the urine specimen compete against the drug conjugate for binding sites on the antibody. During testing, a urine specimen migrates upward by capillary action. Tricyclic Antidepressants, if present in the urine specimen below the cutoff level, will not saturate the binding sites of the antibody in the test strip. The antibodies will then be captured by immobilized Tricyclic Antidepressants conjugate and a visible colored line will show up in the test line region. The colored line will not form in the test line region if the Tricyclic Antidepressants level exceeds the cutoff level because it will saturate all the binding sites of anti-Tricyclic Antidepressants antibodies.

A drug-positive urine specimen will not generate a colored line in the test line region because of drug competition, while a drug-negative urine specimen or a specimen containing a drug concentration less than the cut-off will generate a line in the test line region. To serve as a procedural control, a colored line will always appear at the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

REAGENTS

The test contains a membrane strip coated with drug-protein conjugate on the test line, goat anti-rabbit IgG antibody on the control line, a dye pad which contains colloidal gold particles coated with mouse monoclonal antibody specific to Tricyclic Antidepressants.

PRECAUTIONS

- For healthcare professionals including professionals at point of care sites.
- For in vitro diagnostic use only. Do not use after the expiration date.
- The test strip should remain in the sealed pouch until use.
- All specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The used test strip should be discarded according to federal, state and local regulations.

STORAGE AND STABILITY

Store as packaged in the sealed pouch at 2-30°C. The test strip is stable through the expiration date printed on the sealed pouch. The test strips must remain in the sealed pouch until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

Urine Assay

The urine specimen must be collected in a clean and dry container. Urine collected at any time of the day may be used. Urine specimens exhibiting visible particles should be centrifuged, filtered, or allowed to settle to obtain clear specimen for testing.

Specimen Storage

Urine specimens may be stored at 2-8°C for up to 48 hours prior to testing. For long-term storage, specimens may be frozen and stored below -20°C. Frozen specimens should be thawed and mixed before testing.

MATERIALS

Materials Provided

- Test strips
- Package insert

Materials Required But Not Provided

- Specimen collection container
- Timer
- External controls

DIRECTIONS FOR USE

Allow the test strip, urine specimen, and/or controls to reach room temperature (15-30°C) prior to testing.

1. Bring the pouch to room temperature before opening it. Remove the test strip from the sealed pouch and use it as soon as possible.
2. With arrows pointing toward the urine specimen, immerse the test strip vertically in the urine specimen for at least 10-15 seconds. Do not pass the maximum line (MAX) on the test strip when immersing the strip. See the illustration below.
3. Place the test strip on a non-absorbent flat surface, start the timer and wait for the red line(s) to appear. The result should be read at 5 minutes. Do not interpret the result after 10 minutes.

INTERPRETATION OF RESULTS

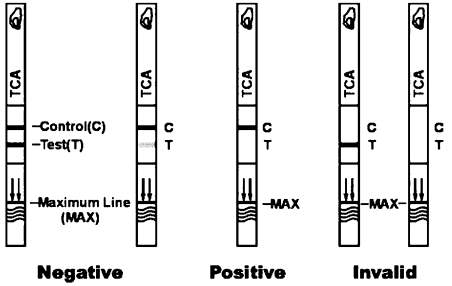
(Please refer to the illustration below)

NEGATIVE:* Two lines appear. One red line should be in the control region (C), and another apparent red or pink line should be in the test region (T). This negative result indicates that the Tricyclic Antidepressants concentration is below the detectable level.

*** NOTE:** The shade of red in the test line region (T) will vary, but it should always be considered as negative whenever there is even a faint pink line.

POSITIVE: One red line appears in the control region (C). No line appears in the test region (T). This positive result indicates that the Tricyclic Antidepressants concentration exceeds the detectable level.

INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test using a new test strip. If the problem persists, discontinue using the lot immediately and contact your local distributor.



QUALITY CONTROL

A procedural control is included in the test. A red line appearing in the control region (C) is considered an internal positive procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

Control standards are not supplied with this kit; however it is recommended that positive and negative controls be tested as good laboratory testing practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS

1. The TCA One Step Tricyclic Antidepressant Test Strip provides only a qualitative, preliminary analytical result. A secondary analytical method must be used to obtain a confirmed result. Gas chromatography/mass spectrometry (GC/MS) or high performance liquid chromatography (HPLC) are the preferred confirmatory methods.[1,2]
2. It is possible that technical or procedural errors, as well as other interfering substances in the urine specimen may cause erroneous results.
3. Adulterants, such as bleach and/or alum, in urine specimens may produce erroneous results regardless of the analytical method used. If adulteration is suspected, the test should be repeated with another urine specimen.
4. A Positive Result indicates presence of the drug or its metabolites

but does not indicate level or intoxication, administration route or concentration in urine.

5. A Negative Result may not necessarily indicate drug-free urine. Negative results can be obtained when drug is present but below the cutoff level of the Test.

6. Test does not distinguish between drugs of abuse and certain medications.

PERFORMANCE CHARACTERISTICS

Accuracy

A side-by-side comparison was conducted using the TCA One Step Tricyclic Antidepressants Test Strip (Urine) and a leading commercially available TCA rapid test. Testing was performed on 222 clinical specimens. Ten percent of the specimens employed were either at –25% or +25% level of the cut-off concentration of Tricyclic Antidepressants. Presumptive positive results were confirmed by HPLC. The following compounds were quantified by HPLC and contributed to the total amount of drugs in presumptive positive urine samples tested: Amitriptyline, Nortriptyline, Imipramine, Desipramine, Doxepine, Desmethyldoxepine.The following results were tabulated:

Method		Other TCA Rapid Test		Total Result
TCA One step test strip	Result	Positive	Negative	
	Positive	55	0	55
	Negative	3	164	167
Total result		58	164	222
%Agreement whit this Rapid kit		95%	>99%	99%

When compared with HPLC at a cut-off of 1,000ng/ml, the following results were tabulated:

HPLC						
TCA Test Strip	Drug-Free Urine	< -25% of Cut-off	-25% Cut-off to Cut-off	Cut-off to +25% Cut-off	>+25 % Cut-off	% Agreement
Negative	150	17	0	0	0	89%
Positive	0	12	8	15	20	>99%

Analytical Sensitivity

A drug-free urine pool was spiked with Tricyclic Antidepressants at the following concentrations: 0 ng/mL, 500 ng/mL, 750 ng/mL, 1,000 ng/mL, 1,250 ng/mL and 1,500 ng/mL. The result demonstrates >99% accuracy at 50% above and 50% below the cutoff concentration. The data are summarized below:

Nortriptyline Conc. (ng/ml)	Percent of cutoff	n	Visual Result	
			Negative	Positive
0	0	30	30	0
500	-50%	30	30	0
750	-25%	30	22	8
1000	Cutoff	30	12	18
1250	25%	30	7	23
1500	50%	30	0	30

Analytical Specificity

The following table lists compounds that are positively detected in urine by the TCA One Step Tricyclic Antidepressants Test Strip (Urine) at 5 minutes.

Compound	Concentration (ng/mL)
Notriptyline	1,000
Nordoxepine	1,000
Trimipramine	3,000
Amitriptyline	1,500
Promazine	1,500
Desipramine	200
Imipramine	400
Clomipramine	12,500
Doxepine	2,000
Maprotiline	2,000
Promethazine	2,500

Precision

A study was conducted at 3 physician's offices by untrained operators using 3 different lots of product to demonstrate the within run, between run and between operator precision. An identical panel of coded specimens containing no Nortriptyline, 25% Nortriptyline above and below the cut-off and 50% Nortriptyline above and below the 1,000 ng/mL cut-off was provided to each site. The results were tabulated:

Nortriptyline Conc. (ng/ml)	n per site	Site A			Site B			Site C		
		-	+	Invalid	-	+	Invalid	-	+	Invalid
0	15	15	0	0	15	0	0	15	0	0
500	15	15	0	0	15	0	0	15	0	0
750	15	14	1	0	11	4	0	14	1	0
1000	15	8	7	0	2	13	0	6	9	0
1500	15	1	14	0	0	15	0	1	14	0

Effect of Urinary Specific Gravity

Fifteen (15) urine specimens with specific gravity range from 1.001 to 1.032 were spiked with 500 ng/mL and 1,500 ng/mL of Nortriptyline respectively. The TCA One Step Tricyclic Antidepressants Test Strip (Urine) was tested in duplicate using the fifteen neat and spiked urine specimens. The results demonstrate that varying ranges of urinary specific gravity does not affect the test results.

Effect of the Urinary pH

The pH of an aliquoted negative urine pool was adjusted to a pH range of 5 to 9 in 1 pH unit increments and spiked with Nortriptyline to 500 ng/mL and 1,500 ng/mL.

The spiked, pH-adjusted urine was tested with the TCA One Step Tricyclic Antidepressants Test Strip (Urine) in duplicate. The results demonstrate that varying ranges of pH does not interfere with the performance of the test.

Cross-Reactivity

A study was conducted to determine the cross-reactivity of the test with compounds in either drug-free urine or Nortriptyline-positive urine. The following compounds show no cross-reactivity when tested with the TCA One Step Tricyclic Antidepressants Test Strip (Urine) at a concentration of 100 µg/mL.

Non Cross-Reacting Compounds

4-Acetamidophenol
Acetophenetidin
N-Acetylprocainamide
Acetylsalicylic acid
Aminopyrine
Amobarbital
Amoxicillin
Ampicillin
L-ascorbic acid
Estrone-3-sulfate
Ethyl-p-aminobenzoate
Fenoprofen
Furosemide
Gentisic acid
Hemoglobin
Hydralazine
Hydrochlorothiazide
Hydrocodone
Oxolinic acid
Oxycodone
Oxymetazoline
Papaverine
Penicillin-G
Pentazocine hydrochloride
Pentobarbital
Perphenazine
Phencyclidine
DL-Amphetamine sulfate
Apomorphine
Aspartame
Atropine
Benzilic acid
Benzoic acid
Benzoylecgonine
Benzphetamine
Bilirubin
(±) - Brompheniramine
Caffeine
Cannabidiol
Cannabinol
Chloralhydrate
Chloramphenicol
Chlorothiazide
(+) Chlorpheniramine
Chlorpromazine
Chlorquine
Cholesterol
Clonidine
Cocaethylene
Cocaine hydrochloride
Codeine
Cortisone
(-) Cotinine
Creatinine
Deoxycorticosterone
Dextromethorphan
Diclofenac
Diflunisal
Digoxin
Diphenhydramine
Doxylamine
Ecgonine hydrochloride
Ecgonine methylester
(-) –Ψ-Ephedrine
[IR,2S] (-) Ephedrine
(L) - Epinephrine
Erythromycin
β-Estradiol
Hydrocortisone
O-Hydroxyhippuric acid
p-Hydroxy-amphetamine
p-Hydroxy-methamphetamine
3-Hydroxytyramine
Ibuprofen
Iproniazid
(±) - Isoproterenol
Isoxsuprine
Ketamine
Ketoprofen
Labetalol
Loperamide
MDE
Meperidine
Meprobamate
Methadone
(L) Methamphetamine
Methoxyphenamine
(±) -3,4-Methylenedioxy-amphetamine-hydrochloride
(+) 3,4-Methylenedioxy-methamphetamine hydrochloride
Morphine-3-β-D-glucuronide
Morphine sulfate
Nalidixic acid
Naloxone
Naltrexone
Naproxen
Niacinamide
Nifedipine
Norcodein
Norethindrone
D-Norpropoxyphene
Noscapine
DL-Octopamine
Oxalic acid
Oxazepam
Phenelzine
Phenobarbital
Phentermine
Trans-2-phenylcyclopropylamine - hydrochloride
L-Phenylephrine
β-Phenylethlamine
Phenylpropanolamine
Prednisolone
Prednisone
Procaine
Promethazine
DL-Propanolol
D-Propoxyphene
D-Pseudoephedrine
Quinacrine
Quinidine
Quinine
Ranitidine
Salicylic acid
Secobarbital
Serotonin
Sulfamethazine
Sulindac
Tetracycline
Tetrahydrocortisone, 3-acetate
Tetrahydrocortisone 3-(β-D-glucuronide)
Tetrahydrozoline
Thiamine
Thioridazine
DL-Tyrosine
Tolbutamide
Triamterene
Trifluoperazine
Trimethoprim
Tryptamine
DL-Tryptophan
Tyramine
Uric acid
Verapamil
Zomepirac

BIBLIOGRAPHY

1. Rose,J.B.,Tricyclic antidepressants toxicity. J. Toxicity Clin. Toxicol., 11,381-402,1977.
2. *Urine* Testing *for Drugs of Abuse*, National Institute on Drug-Abuse (NIDA), Research Monogragh 73 ,1986.
3. Fed. Register, Department of Health and Human Services, Mandatory Guidelines for Fedaral Workplace Drug Testing Programs,53,69,11979,1988.
4. McBay, *A.* J.Clin.Chem.,33,33B-40B,1987.

Index of Symbols

	Attention, see instructions for use		Tests per kit		Manufacturer
IVD	For *in vitro* diagnostic use only		Use by		Do not reuse
2°C 30°C	Store between 2-30℃	LOT	Lot Number		

ISO, CE, GMP

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