



TRICYCLIC ANTIDEPRESSANT

Rapid Test (TCA) strip

Introduction

The TCA Rapid Test Dipstick (Urine) is a Rapid chromatographic immunoassay for qualitative detection of Tricyclic Antidepressant in human urine at a cut-off concentration of 1000 ng/mL which is only utilized for in vitro diagnostics (IVD). It merely provides a preliminary result, therefore, an alternative method with more specificity is recommended to be used to confirm the initial results, in which case, the most preferred method is gas chromatography-mass spectrometry (GC-MS).

Summary

Tricyclic antidepressants are used to treat depression disorders, phobias, anxiety disorders, obsessive compulsive disorder, eating disorders, premature ejaculation treatment, and some chronic pains. Imipramine, imipramipryline, nortriptyline, etc. are among the derivatives of this drug. The main action of the drugs in this group on the brain is to inhibit the reabsorption of serotonin and norepinephrine by blocking the action of serotonin and norepinephrine transmitters. Therefore, they increase the level of serotonin and norepinephrine in the brain. These drugs have very little effect on dopamine transmission. Tricyclics are used both orally and by injection. High doses of tricyclics can cause a severe decrease in central nervous activity, and lead to cardiotoxicity and anticholinergic effects. Tricyclics are metabolized in the liver and excreted through urine for 10 days. Rapid tricyclic test is a quick screening method that can be done without any laboratory equipment. This test is a monoclonal antibody for the specific detection of the increased level of nortriptyline in the urine, and the result is positive when its concentration exceeds 1000 ng/mL in the urine.

01

Function Method Basis

Rapid Tricyclic urine test employs an immunochromatographic method based on competitive binding. Substances that may be found in urine specimen compete with ones metabolized by the liver to bind to antibodies. During the test, the urine specimen flows upward via capillary action. If the amount of tricyclic in the urine is below 1000 ng/mL, it does not bind to the binding sites of the antibodies coated with nanoparticles. Consequently, the antibody will be absorbed by the tricyclic on the surface of the membrane and will leave a colored line in the test area (T). Should the amount be greater than 1000 ng/mL, no colored line will appear in the T area, since all the binding sites of the tricyclic antibodies are occupied. So, if the presence of tricyclic in the urine specimen is positive, no colored line will be formed in the test area (T) due to drug competition, whereas if the urine specimen contains tricyclic amounts lower than the desired level in the test, a colored line will appear in this area. In order to have a procedural control, a colored line always appears in the area of the control line (C), indicating the appropriate volume of the specimen added to the membrane.

Compounds

The test strip contains anti-tricyclic monoclonal antibody particles and a combination of conjugated protein-tricyclic. The secondary antibody prepared in goat species is also employed for the control line.

+ **Caution:** for in vitro diagnostic use only.

Do not use after the expiration date. The test must remain in its package until use. All specimens must be considered infectious and all precautions and safety measures for handling and use must be taken.

Storage

The package should be stored at room temperature or in refrigerator (2-30°C). The test is valid only until the expiration date printed on the package. Keep from freezing.

Specimen Collection and Preparation

+ **Urine collection:** The urine specimen must be collected in a clean and dry container. It could be collected at any time of the day.

02

+ **Specimen storage:** The urine specimen can be stored at 2-8 °C for more than 48 hours, and frozen for long-term storage at -20 °C. Frozen specimens must be thawed and mixed well before testing.

Contents and Required Supplies

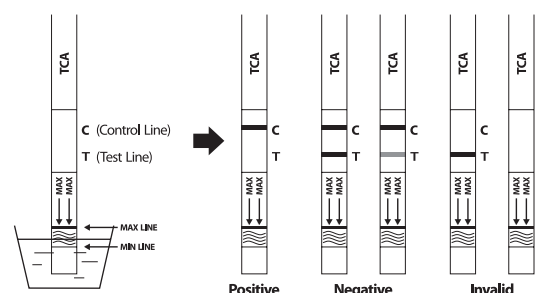
Items found in the kit: + Brochure + Test strip

Required items not included in the kit: + Specimen collection container + Timer

User Manual

Before testing, let the test strip and the urine specimen reach room temperature (18-30 °C).

- Bring the pouch to room temperature, before opening it. Remove the test strip from the sealed pouch, and use it as soon as possible.
 - Dip the test strip vertically into the urine specimen in the direction indicated by the arrows up to the MAX line, and hold it for at least 10-15 seconds (See illustration).
 - Place the test strip on a flat, non-absorbent surface, set the timer and wait for the colored line to appear. Interpret the results within 5 minutes.
- + Do not interpret the results after 10 minutes.



03

Result Interpretation

Negative: Two lines appear, one line should be in the control area (C) and another colored line should appear in the test area (T). This negative result indicates that the tricyclic concentration is below the detectable level of 1000 ng/mL.

+ **Attention:** The color shade in the testing area may vary, but it should be considered negative whenever there is even a faint colored line.

Positive: A colored line should appear in the control area (C) and no line should be seen in the test area (T). This positive result shows that the concentration of tricyclic is above the detectable level of 1000 ng/mL.

Invalid: The control line (C) is not formed. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem is not solved, stop using the strip and contact the after-sales service immediately.

Quality Control

The quality control of the method is included in the test, and the colored line appearing in the control area (C) is considered as an inner controller which confirms the sufficient volume of the specimen and the correct execution of the test. The test strip does not come with control standards. It is recommended to do the test with positive and negative controls in an accredited laboratory to confirm the method and the correctness of the test.

Limitations

1. Tricyclic Rapid Test strip provides only preliminary analytical and qualitative results, and therefore, a secondary analytical method must be employed to confirm the results. For the confirmation of the results of this test, gas chromatography-mass spectrometry (GC-MS) is preferred.
2. There is a possibility of error in this technique. As some interfering substances in the urine specimen may lead to wrong results.
3. Adding substances like bleaches or white alum in the urine specimen may cause a wrong result, despite the analytical method used, and should the substances remain, the test should be performed with another urine specimen.

04

4. A positive result indicates the presence of substances or their metabolites. A negative result, on the other hand, may not necessarily indicate the absence of substances in the urine. The drug level may be lower than the level detectable by the test.

5. The test is not able to detect the abuse of certain therapeutic cases.

+ **Expected values:** if tricyclic concentration is less than 1000 ng/mL, the result will be negative and if the concentration is greater than 1000 ng/mL, the result will be positive. The cut-off value of the test is 1000 ng/mL.

Executive Feature

Accuracy: In order to have a comparison, the results obtained from Hana Gene's TCA Rapid Test strip using GC-MS were collected from 250 clinical specimens (96 positive and 154 negative); Hana Gene's kit detected 91 of the specimens positive and 141 negative. The results are presented in table below:

Method		GC/MS		Total Result
The TCA Rapid Test Dipstick	Results	Positive	Negative	
	Positive	91	13	
	Negative	5	141	
Total Result		96	154	250
Sensitivity: 94.7% (88.2 % ~ 98.2%)		Specificity: 91.5% (86.0% ~ 95.4%)		
Accuracy: 92.8% (88.8% ~ 95.6%)		95% Confidence Intervals		

Analytical Sensitivity:

Some amount of drug-free urine specimens was prepared in the following concentrations of tricyclic:

0 ng/mL, 500 ng/mL, 750 ng/mL, 1000 ng/mL, 1250 ng/mL, 1500 ng/mL and 3000 ng/mL. The results show more than 99% accuracy in 50% of specimens greater than the cut-off level, and 50% below the cut-off value. This information is summarized in the table below:

TCA Concentration (ng/mL)	Percent of Cut-off	n	Visual Result	
			Negative	Positive
0	0	30	30	0
500	-50%	30	30	0
750	-25%	30	25	5
1000	Cut-off	30	15	15
1250	+25%	30	3	27
1500	+50%	30	0	30
3000	3X	30	0	30

Analytical Features:

The following table shows the compounds positively identified by the tricyclic test strip in the urine specimen within 5 minutes.

Compound	Concentration (ng/mL)	Compound	Concentration (ng/mL)
Nortriptyline	1,000	Amitriptyline	1,500
Nordoxepine	400	Promazine	3,000
Trimipramine	3,000	Desipramine	200

Precision

A study was carried out in three different centers by untrained people making use of three different lots of the product in order to evaluate the intra-rater, inter-rater, and inter-individual reliability.

A panel of coded specimens containing certain tricyclic concentrations with GC/MS method was used in each center, at 0 ng/mL, 25% above and below the minimum detection limit, and 50% above and below the minimum detection limit of 1000 ng/mL.

TCA Concentration (ng/mL)	n per site	LOT A		LOT B		LOT C	
		-	+	-	+	-	+
0	10	10	0	10	0	10	0
500	10	10	0	10	0	10	0
750	10	9	1	8	2	8	2
1000	10	1	9	1	9	1	9
1500	10	0	10	0	10	0	10

Effect of Urinary Specific Gravity

Tricyclic with concentrations of 500 ng/mL and 1500 ng/mL was added to 15 urine specimens with low, normal, and high specific gravity. Then pure specimens and specimens with certain tricyclic concentrations were checked by the test strip. The results show that the specific weight and gravity do not affect the test results.

Effect of Urinary pH

The pH of the negative diluted urine specimen was changed from 5 to 9, and the specimens were given benzoylcegonine concentrations of 500 ng/mL and 1500 ng/mL. The adjusted pH of the urine was done in a duplicate rapid tricyclic test whose results show that the pH range does not interfere with the test.

Cross Reactivity

In order to assess the interferences with positive and negative tricyclic test results, a study was carried out. The following compounds did not show any interference in the test results with 100 µg/ml tricyclic concentration:

4-Acetaminophenol	Acetone	Acetophenetidin	N-Acetylprocainamide
Acetylsalicylic acid	Albumin	Amitriptyline	Amobarbital
Amoxapine	Amoxicillin	Ampicillin	Ascorbic acid
Aminopyrine	Apomorphine	Aspartame	Atropine
Benzoic acid	Benzoic acid	Benzphetamine	Bilirubin
Brompheniramine	Buspirone	Caffeine	Cannabidiol
Cannabinol	Cimetidine	Chloralhydrate	Chloramphenicol
Chlordiazepoxide	Chloroquine	Chlorothiazide	(+)-Chlorpheniramine
(+/-)-Chlorpheniramine	Chlorpromazine	Chlorprothixene	Cholesterol
Clomipramine	Clonidine	Codeine	Cortisone
(-) Cotinine	Creatinine	Cyclobarbitol	Cyclobenzaprine
Deoxycorticosterone	R (-) Deoxyephedrine	R (-) Deprenyl	Dextromethorphan
Diazepam	Diclofenac	Diflunisal	Digoxin
4-Dimethylaminoantipyrine	Diphenhydramine	Dicyclomine	5,5-Diphenylhydantoin
Disopyramide	Doxylamine	Ecgonine	Ecgonine Methyl ester
EDDP	EMDP	Ephedrine	l-Ephedrine
(-) -ψ-Ephedrine	[1R,2S] (-) Ephedrine	l-Epinephrine	(+/-)-Epinephrine
Erythromycin	β-Estradiol	Estrone-3-sulfate	Ethanol (Ethyl alcohol)
Ethyl-p-aminobenzoate	Etodolac	Famprofazone	Fenfluramine
Fenoprofen	Fentanyl	Fluoxetine	Furosemide
Gentisic acid	d-Glucose	Guaiacol Glyceryl Ether	Hydrochlorothiazide
Hemoglobin	Hydralazine	Hydromorphone	Hydrocodone
Hydrocortisone	3-Hydroxytyramine (Dopamine)	o-Hydroxyhippuric acid	p-Hydroxymethamphetamine
Imipramine	Hydroxyzine	Ibuprofen	Isoxsuprine
Iproniazide	(-) Isoproterenol	Ketoprofen	Kanamycin
Ketamine	Lidocaine	Labetalol	Levorphanol

Loperamide	Lithium Carbonate	Meperidine	Methamphetamine
Meprobamate	Lindane		
(Hexachlorocyclohexane)	Methylphenidate	Mephentermine	
l-Methamphetamine	Maprotiline	Morphine sulfate	Naloxone
Methoxyphenamine	Methadone	Naproxen	Naltrexone
Methyprylon	Metoprolol	Niacinamide	Nifedipine
Nalidixic acid	(+/-)-3,4-Methylenedioxy-methamphetamine	Perphenazine	Trimipramine
Nimesulide	Nimesulide	d/l-Octopamine	Tryptamine
α-Naphthaleneacetic acid	Morphine-3-β-D Glucuronide	Oxazepam	Orphenadrine
Norethindrone	Nalorphine	Oxolinic acid	Oxycodone
d-Norpropoxyphene	Norcodeine	Pemoline	Pentobarbital
Oxalic acid	Normorphine	Phenelzine	Perphenazine
Oxymorphone	Noscapine	Pheniramine	Phenobarbital
Penicillin-G	Oxymetazoline	l-Phenylephrine	Promazine
Prednisolone	Papaverine	d/l-Propranolol	Promethazine
Prednisone	Pentazocine	d-Pseudoephedrine	Quinacrine
l-Propoxyphene	Phenothiazine	Ranitidine	Salicylic acid
Riboflavin	Phentermine	Secobarbital	Sodium Chloride
Sulindac	Procaine	Sustiva (Efavirenz)	Temazepam
Tetracycline	Quinidine	Tetrahydrocortisolone	Thiamine
Tolbutamide	Quinine	Thebaine	Theophylline
Trimethobenzamide	Serotonin (5-Hydroxytyramine)	l-Thyroxine	Trazodone
Trimipramine	Sulfamethazine	Tryptamine	Trifluoperazine
d/l-Tyrosine	Tetrahydrozoline	d/l-Tryptophan	Trimethoprim
Zomepirac	Tetrahydrocortisone, 3-acetate	Verapamil	Tyramine
Uric acid	Trans-2-phenylcyclopropylamine	Thioridazine	Triamterene

References

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