



TRAMADOL

Rapid Test (TML) strip

Introduction

The TML Rapid Test Dipstick (Urine) is a Rapid chromatographic immunoassay for qualitative detection of Tramadol in human urine at a cut-off concentration of 100 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

Tramadol is an opioid prescribed to relieve severe to moderate pain. Its mechanism of action is in two ways: firstly, like other opioids (opium derivatives), it is an agonist of Mu (μ) receptors. Secondly, it prevents the reabsorption of noradrenaline and serotonin, and increases the concentration of these two in the synapse; this creates a property like that of antidepressants as well as alertness, and unlike morphine, makes the user even more active. Frequent, prolonged use of Tramadol causes a tolerance to the drug and physiological dependence and eventually could lead to addiction. Tramadol is mainly metabolized after oral administration. 30% of it will be unchanged and 60% will be excreted through urine in the form of metabolites broken down in the liver. Tramadol's half-life in the serum is around 6 to 7 hours with a concentration of 0.1 to 0.3. Studies show that its concentration in the urine is more than in the serum, therefore urine specimens are more suitable to prove the consumption of the drug.

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Function Method Basis

Rapid Tramadol 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 Tramadol in the urine is below 100 ng/mL, it does not bind to the binding sites of the antibodies coated with nanoparticles. Consequently, the antibody conjugated with nanoparticles will be absorbed by the Tramadol on the surface of the membrane and will leave a colored line in the test area (T). Should the amount be greater than 100 ng/mL, no colored line will appear in the T area, since all the binding sites of the Tramadol antibodies are occupied. So, if the presence of Tramadol 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 Tramadol 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-Tramadol monoclonal antibody particles and a combination of conjugated Protein-Tramadol. 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.

+ **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.

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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

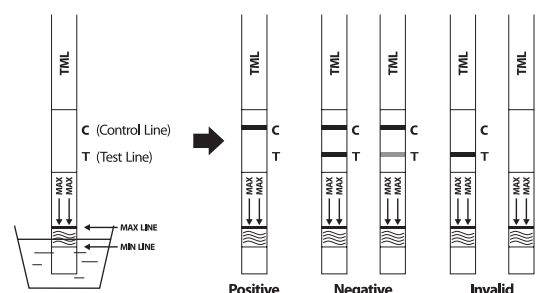
Before testing, let the test strip and the urine specimen reach room temperature (18-30 °C).

- 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.



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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 Tramadol concentration is below the detectable level of 100 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 Tramadol is above the detectable level of 100 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. Tramadol 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.

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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 Tramadol concentration is less than 100 ng/mL, the result will be negative and if the concentration is greater than 100 ng/mL, the result will be positive. The cut-off value of the test is 100 ng/mL.

Executive Feature

Accuracy: In order to compare the results of Hana Gene's Tramadol Rapid test kit, using GC/MS method, 250 clinical specimens (93 positive; 157 negative) were collected, and Hana Gene's kit detected 82 positive and 145 of the specimen's negative. The results are shown in the table below:

Method		GC/MS		Total Result
The TML Rapid Test Dipstick	Results	Positive	Negative	
	Positive	82	12	
	Negative	11	145	
Total Result		93	157	250
Sensitivity: 88.1% (79.8 % ~ 93.0%)		Specificity: 92.3% (87.0% ~ 95.9%)		
Accuracy: 90.8% (86.5% ~ 94.0%)		95% Confidence Intervals		

Analytical Sensitivity:

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

0 ng/mL, 50 ng/mL, 75 ng/mL, 100 ng/mL, 125 ng/mL, 150 ng/mL and 300 ng/mL. The results show more than 99% accuracy in 50% of specimens greater than the cut-off level, and 50% below cut-off value. This information is summarized in the table below:

TML Concentration (ng/mL)	Percent of Cut-off	n	Visual Result	
			Negative	Positive
0	0	30	30	0
50	-50%	30	30	0
75	-25%	30	27	3
100	Cut-off	30	15	15
125	+25%	30	4	26
150	+50%	30	0	30
300	3X	30	0	30

Analytical Features:

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

Compound	Concentration (ng/mL)	Compound	Concentration (ng/mL)
n-Desmethyl-cis-tramadol	200	o-Desmethyl-cis-tramadol	10,000
Cis-tramadol	100	Phencyclidine	100,000
Procyclidine	100,000	d,l-O-Desmethyl venlafaxine	50,000

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 Tramadol 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 100 ng/mL.

Tramadol Concentration (ng/mL)	n per site	Site A		Site B		Site C	
		-	+	-	+	-	+
0	10	10	0	10	0	10	0
50	10	10	0	10	0	10	0
75	10	9	1	9	1	8	2
125	10	1	9	1	9	2	8
150	10	0	10	0	10	0	10

Effect of Urinary Specific Gravity

Tramadol with concentrations of 50 ng/mL and 150 ng/mL was added to 15 urine specimens with low, normal, and high specific gravity. Then pure specimens and specimens with certain Tramadol 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 Tramadol concentrations of 50 ng/mL and 150 ng/mL. The adjusted pH of the urine was done in a duplicate rapid Tramadol 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 Tramadol test results, a study was carried out. The following compounds did not show any interference in the test results with 100 µg/ml Tramadol concentration:

4-Acetaminophenol	Acetone	Acetophenetidin	N-Acetylprocainamide
Acetylsalicylic acid	Albumin	Amitriptyline	Amobarbital
Amoxapine	Amoxicillin	Ampicillin	Ascorbic acid
Aminopyrine	Apomorphine	Aspartame	Atropine
Benzilic 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	(-) Deoxyephedrine	R (-)Deprenyl	Dextromethorphan
Diazepam	Diclofenac	Diflunisal	Digoxin
4-Dimethylaminoantipyrine	Diphenhydramine	Dicyclomine	5,5-Diphenylhydantoin
Disopyramide	Doxylamine	Ecgonine	Ecgonine Methylster
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
Genitisc acid	d-Glucose	Guaiacol Glyceryl Ether	Hydrochlorothiazide
Hemoglobin	Hydralazine	Hydromorphone	Hydrocodone
Hydrocortisone	3-Hydroxytyramine (Dopamine)	o-Hydroxyhippuric acid	p-Hydroxymetham-phetamine
Imipramine	Hydroxyzine	Ibuprofen	Isosuprine
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	Medethadone	Naproxen	Naltrexone
Methyprylon	Metoprolol	Niacinamide	Nifedipine
Nalidixic acid	(+)-3,4-Methylenedioxy-methamphetamine	Perphenazine	Trimipramine
α-Naphthaleneacetic acid	Nimesulide	d/l-Octopamine	Tryptamine
Norethindrone	Morphine-3-β-D Glucuronide	Oxazepam	Orphenadrine
d-Norpropoxyphene	Nalorphine	Oxolinic acid	Oxycodone
Oxalic acid	Norcodeine	Pemoline	Pentobarbital
Oxymorphone	Normorphine	Phenelzine	Perphenazine
Penicillin-G	Noscapine	Pheniramine	Phenobarbital
Prednisolone	Oxymetazoline	l-Phenylephrine	Promazine
Prednisone	Papaverine	d/l-Propranolol	Promethazine
l-Propoxyphene	Pentazocine	d-Pseudoephedrine	Quinacrine
Riboflavin	Phenothiazine	Ranitidine	Salicylic acid
Sulindac	Phentermine	Secobarbital	Sodium Chloride
Tetracycline	Procaine	Sustiva (Efavirenz)	Temazepam
Tolbutamide	Quinidine	Tetrahydrocortisolone	Thiamine
Trimethobenzamide	Quinine	Thebaine	Theophylline
Trimipramine	Serotonin (5-Hydroxytyramine)	l-Thyroxine	Trazodone
d/l-Tyrosine	Sulfamethazine	Tryptamine	Trifluoperazine
Zomepirac	Tetrahydrozoline	d/l-Tryptophan	Trimethoprim
Uric acid	Tetrahydrocortisone, 3-acetate	Verapamil	Tyramine
	Trans-2-phenylcyclopropylamine	Thioridazine	Triamterene

References

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