

**TML Rapid Test Cassette
(Whole Blood/Serum/Plasma)**
Package Insert

REF DTM-402 English

A rapid test for the qualitative detection of TML in human whole blood or serum or plasma. For medical and other professional in vitro diagnostic use only.

INTENDED USE

The TML Rapid Test Cassette (Whole Blood/Serum/Plasma) is a lateral flow chromatographic immunoassay for the detection of Tramadol in whole blood or serum or plasma at a cut-off concentration of 50ng/mL. This test will detect other related compounds, please refer to the analytical Specificity table in this package insert.

This assay provides only a qualitative, preliminary 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) 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]

Tramadol (TML) is a quasi-narcotic analgesic used in the treatment of moderate to severe pain. It is a synthetic analog of codeine, but has a low binding affinity to the mu-opioid receptors. Large doses of tramadol can develop tolerance and physiological dependency and can lead to its abuse. Tramadol is extensively metabolized after oral administration. Approximately 30% of the dose is excreted in whole blood or serum or plasma as unchanged drug, whereas 60% is excreted as metabolites. The major pathways appear to be N- and O-demethylation, glucuronidation or sulfation in the liver.

The TML Rapid Test Cassette (Whole Blood/Serum/Plasma) is a rapid whole blood screening test that can be performed without the use of an instrument. The test utilizes a monoclonal antibody to selectively detect elevated levels of Tramadol in whole blood/serum/plasma. The TML Rapid Test Cassette yields a positive result when Tramadol in whole blood exceed 50 ng/mL.

[PRINCIPLE]

The TML Rapid Test Cassette (Whole Blood/Serum/Plasma) is an immunoassay based on the principle of competitive binding. Drugs that may be present in the whole blood/serum/plasma specimen compete against the drug conjugate for binding sites on the antibody.

During testing, a whole blood/serum/plasma specimen migrates upward by capillary action. Tramadol, if present in the whole blood/serum/plasma specimen below the cut-off level, will not saturate the binding sites of the antibody in the test. The antibody coated particles will then be captured by immobilized Tramadol-protein 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 Tramadol level exceeds the cut-off level because it will saturate all the binding sites of anti-Tramadol antibodies.

A drug-positive whole blood/serum/plasma specimen will not generate a colored line in the test line region because of drug competition, while a drug-negative whole blood/serum/plasma 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 mouse monoclonal anti-Tramadol antibody coupled particles and Tramadol-protein conjugate. A goat antibody is employed in the control line system.

[PRECAUTIONS]

• For professional in vitro diagnostic use only. Do not use after the expiration date.
• Do not eat, drink or smoke in the area where the specimens or kits are handled.
• Do not use test if pouch is damaged.
• Handle all specimens as they contain infectious agents. Observe established precautions against microbiological hazards throughout testing and follow the standard procedures for proper disposal of specimens.
• Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested.
• The test should be discarded according to local regulations.
• Humidity and temperature can adversely affect results.

[STORAGE AND STABILITY]

Store as packaged in the sealed pouch at room temperature or refrigerated (2-30°C). The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch. DO NOT FREEZE. Do not use beyond the expiration date.

[SPECIMEN COLLECTION AND PREPARATION]

• The TML Rapid Test Cassette can be performed using whole blood (from venipuncture or fingerstick)/serum/plasma.

• To collect **Fingerstick Whole Blood specimens**:

Analytical Specificity

The following table lists compounds that are positively detected in whole blood by the TML Rapid Test Cassette (Whole Blood/Serum/Plasma) at 5 minutes.

Compound	Concentration (ng/mL)
n-Desmethyl-cis-tramadol	100
Cis-tramadol	50
Procyclidine	50
o-Desmethyl-cis-tramadol	5,000
Phencyclidine	50,000
d,l-Desmethyl venlafaxine	25,000

Precision

A study was conducted at three hospitals using three different lots of product to demonstrate the within run, between run and between operator precision. An identical panel of coded specimens, containing no TML and 50% TML above and below the 50ng/mL cut-off was provided to each site. The following results were tabulated:

TML Concentration (ng/mL)	n per Site	Site A	Site B	Site C
0	10	-	+	-
25	10	0	0	0
75	10	0	0	0

Cross-Reactivity

A study was conducted to determine the cross-reactivity of the test with compounds in either drug-free whole blood/serum/plasma or determine positive whole blood. The following compounds show no cross-reactivity when tested with the TML Rapid Test Cassette (Whole Blood/Serum/Plasma) at a concentration of 100 µg/mL.

Non Cross-Reacting Compounds

4-Acetaminophen	Acetone	Acetophenetidin	N-Acetylprocainamide
Acetylsalicylic acid	Albumin	Amphetamine	Amobarbital
Amoxapine	Amoxapinol	Ampicillin	Ascorbic acid
Benzphetamine	Apomorphine	Aspartame	Atropine
Brompheniramine	Benzocaine	Benzphetamine	Bilirubin
Cannabidiol	Bisuprone	Caffeine	Cannabidiol
(+/-)-Chlorazepoxide	Cimetine	Chlorhydrate	Chloramphenicol
(+/-)-Chlorpheniramine	Chloroguanidine	Chlorothiazide	(+)-Chlorpheniramine
Clomipramine	Chlorpromazine	Chlorophenoxis	Cholesterol
(-)-Cotinine	Clonidine	Codine	Cortisone
Deoxycorticosterone	Crestinoline	Cyclobutabital	Cyberenazapine
Diazepam	(-)-Desoxyephedrine	R (-)-Dexeprenyl	Dextromethorphan
4-Dimethylaminoypyridine	Diphenhydramine	Dicyclomine	5,5-Diphenylhydantoin
Disopyramide	Doxylamine	Ergotamine	Ergonine/Methylergot
EDDP	EMDP	Ephedrine	l-Ephedrine
(-)-Ephedrine	(1R,2S)-(-)-Ephedrine	l-Epinephrine	(-)-Epinephrine
Erythromycin	B-Estradiol	Estone-3-sulfate	Ethanol (Ethyl alcohol)
Ethyl- <i>p</i> -aminobenzoate	Etodolac	Famotidine	Fenfluramine
Fenoprofen	Fentanyl	Fluoxetin	Furosemide
Gentisic acid	d-Glucose	Guaiacol/Glyceryl	Hydrochlorothiazide
Hemoglobin	Hydralazine	Hydromorphone	Ivermectin
Hydrocortisone	3-Hydroxytyramine	o-Hydroxyhippuric acid	p-Hydroxymethamphetamine
Imipramine	Hydroxyizine	Ibuprofen	Isoxsuprime
Iproniazid	(-)-Isoproterenol	Ketoprofen	Kanamycin
Ketamine	Lidocaine	Labetalol	Levorphanol
Loperamide	Lithium Carbonate	Meperidine	Methamphetamine
Meprobamate	Lindane	Methylphenidate	Mephentermine
l-Methamphetamine	Maprotiline	Morphine sulfate	Naloxone
Methoxyphenamine	Methadone	Naproxen	Naltrexone
Methylprylon	Metoprolol	Niacinamide	Nifedipine
Nalidixic acid	(+/-)-3,4-Methylendioxy-methamphetamine	Nimesulide	dl-Oxodopamine
o-Naphthaleneacetic acid	Morphine-3- <i>D</i> -Glucuronide	Oxazepam	Orphenadrine
Norethindrone	Nalorphine	Oxolinic acid	Oxycodone
d-Norpseudoephedrine	Norcodeine	Pemoline	Pentobarbital
Oxalic acid	Normorphine	Phenelzine	Perphenazine
Oxymorphone	Noscapine	Phenamine	Phenobarbital

Interfering Substances

The TML Rapid Test Cassette (Whole Blood/Serum/Plasma) has been tested for possible

- Wash the patient's hand with soap and warm water or clean with an alcohol swab. Allow to dry.
- Massage the hand without touching the puncture site by rubbing down the hand towards the fingers of the middle or ring finger.
- Puncture the skin with a sterile lancet. Wipe away the first sign of blood.
- Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site.
- Add the Fingerstick Whole Blood specimen to the test by using a **capillary tube**
 - Touch the end of the capillary tube to the blood until filled to approximately 40 µL. Avoid air bubbles.
 - Place the bulb onto the top end of the capillary tube, then squeeze the bulb to dispense the whole blood to the specimen well of the test cassette.
- Testing should be performed immediately after specimen collection. Do not leave the specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 3 days, for long-term storage, specimens should be kept below -20°C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

[MATERIALS]

Materials Provided

- Test cassettes
- Droppers
- Buffer
- Package insert

Materials Required But Not Provided

- Specimen collection containers
- Lancets (for fingerstick whole blood only)
- Heparinized capillary tubes and dispensing bulb (for fingerstick whole blood only)
- Centrifuge
- Timer

[DIRECTIONS FOR USE]

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

- Bring the pouch to room temperature before opening it. Remove the cassette from the sealed pouch and use it within one hour.
- Place the cassette on a clean and level surface.

For serum or plasma specimen:

- Hold the dropper vertically and transfer **1 full drop of serum or plasma** (approximately 40 µL), then add **2 drops of buffer** (approximately 80 µL) to the specimen well(S) of the cassette, and then start the timer. Avoid trapping air bubbles in the specimen well. See illustration below.

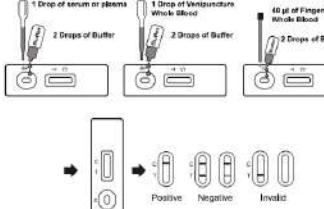
For Venipuncture Whole blood specimen:

- Hold the dropper vertically and transfer **1 drop of whole blood** (approximately 40 µL) to the specimen well(S), then add **2 drops of buffer** (approximately 80 µL), and start the timer. See illustration below.

For Fingerstick Whole blood specimen:

- To use a capillary tube: Fill the capillary tube and **transfer approximately 40 µL of fingerstick whole blood specimen** to the specimen well(S) of test cassette, then add **2 drops of buffer** (approximately 80 µL) and start the timer. See illustration below.

Wait for the colored lines to appear. **Read the result at 5 minutes.** Do not interpret the result after 10 minutes.

**[INTERPRETATION OF RESULTS]**

(Please refer to the illustration above)

NEGATIVE: Two colored lines appear. One colored line should be in the control line region (C) and another colored line should be in the test line region (T). This negative result indicates

that the Tramadol concentration is below the detectable cut-off level.

***NOTE:** The shade of color in the test line region (T) may vary, but it should be considered negative whenever there is even a faint colored line.

POSITIVE: One colored line appears in the control line region (C). No line appears in the test line region (T). This positive result indicates that the Tramadol concentration exceeds the detectable cut-off level.

INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

[QUALITY CONTROL]

A procedural control is included in the test. A colored line appearing in the control region (C) is the internal procedural control. It confirms sufficient specimen volume and correct procedural technique. Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

[LIMITATIONS]

1. The TML Rapid Test Cassette (Whole blood/Serum/Plasma) provides only a qualitative, preliminary result. A secondary analytical method must be used to obtain a confirmed result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.

2. It is possible that technical or procedural errors, as well as other interfering substances in the whole blood or serum or plasma specimen may cause erroneous results.

3. A positive result indicates presence of the drug or its metabolites but does not indicate level of intoxication, administration route or concentration in whole blood or serum or plasma.

4. A negative result may not necessarily indicate drug-free Whole blood/serum/plasma. Negative results can be obtained when drug is present but below the cut-off level of the test.

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

[PERFORMANCE CHARACTERISTICS]**Accuracy**

A side-by-side comparison was conducted using the TML Rapid Test Cassette and GC/MS at the cut-off of 50ng/mL. Testing was performed on 97 clinical specimens previously collected from subjects present for Drug Screen Testing. The following results were tabulated:

Clinic Result of Whole Blood

Method	GC/MS		Total Results
	Results	Positive	
TML Rapid Test Cassette	Positive	19	20
	Negative	2	77
Total Results	21	76	97
% Agreement	90.5%	98.7%	96.9%

Clinic Result of Serum or Plasma

Method	GC/MS		Total Results
	Results	Positive	
TML Rapid Test Cassette	Positive	19	20
	Negative	2	77
Total Results	21	76	97
% Agreement	90.5%	98.7%	96.9%

Analytical Sensitivity

A drug-free whole blood/serum/plasma pool was spiked with TML at the following concentrations of $\pm 50\%$ cutoff and 3x cutoff, the data are summarized below:

For whole blood:

ML Concentration (ng/mL)	Percent of Cut-off	n	Visual Result
0	0	30	0
25	-50%	30	0
50	Cut-off	30	15
75	+50%	30	30
150	3X	30	30

For serum or plasma:

ML Concentration (ng/mL)	Percent of Cut-off	n	Visual Result
0	0	30	0
25	-50%	30	0
50	Cut-off	30	15
75	+50%	30	30
150	3X	30	30

Hangzhou AllTest Biotech Co.,Ltd.

1530 Yihua Street
Hangzhou Economic & Technological Development Area
Hangzhou, 310019 P.R. China
Web: www.alltest.com.cn Email: info@alltest.com.cn



Medical EC-REP GmbH

Borkenstrasse 10,
48163 Muenster,
Germany

Number: 145321502

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Interfering Substances

The TML Rapid Test Cassette (Whole Blood/Serum/Plasma) has been tested for possible