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

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 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) 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 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, glucoronidation 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[1].

【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 Tramadollevel exceeds the cut-off level because it will saturate all the binding sites of anti-Tramadolantibodies.

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 controlline region indicating that proper volume of specimen has been added and membrane wicking has occurred.

【REAGENTS】

The test contains mouse monoclonal anti-Tramadolantibody coupled particles and Tramadol-protein conjugate. A goat antibody is employed in the controlline 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 if they contain infectious agents. Observe established precautions against microbiological hazards throughout testing and follow the standard procedures for proper disposal ofspecimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection whenspecimens are being tested.
- The used test should be discarded according to local regulations.
- Humidity and temperature can adverselyaffect results.

【STORAGE AND STABILITY】

Store as packaged in the sealed pouch at room temperature or refrigerated (2-30°C). The test is stablethrough the expiration date printed on the sealed pouch. The test must remain in the sealed pouch untiluse. **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**:
  - 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 fingertip 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 area of the test cassette.
- Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. For long term storage, specimens should be kept below -20°C. Whole blood or serum or plasma 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 or serum or plasma specimens. Whole blood or serum or plasma 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

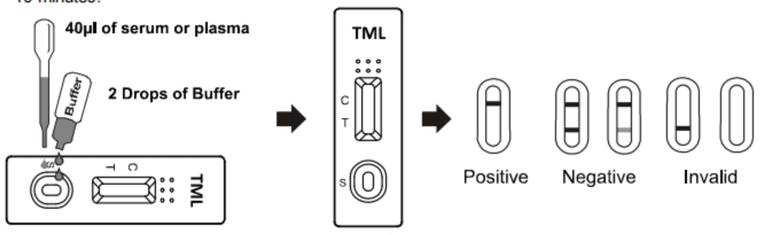
• Specimen collection containers • Centrifuge
•lancets (for fingerstick whole blood only) • Timer
•Heparinized capillary tubes and dispensing bulb (for fingerstick whole blood only)

【DIRECTIONS FOR USE】

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

For serum or plasma specimen:

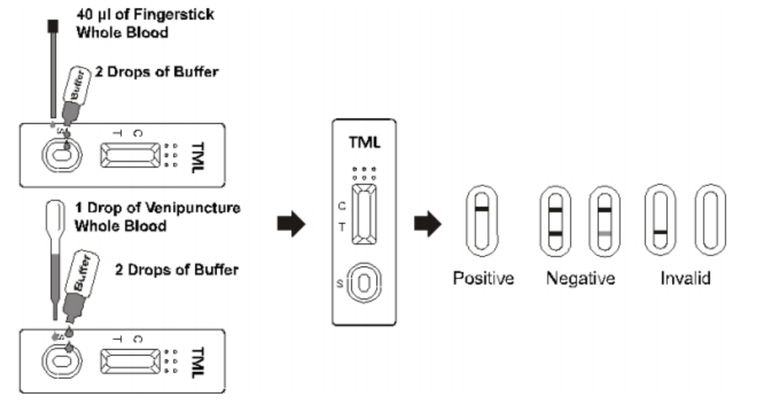
1. Bring the pouch to room temperature (15-30℃) before opening it. Remove the cassette from the sealed pouch and use it within one hour.
2. Place the cassette on a clean and level surface. Hold the dropper vertically and transfer **1 full drop of serum or plasma** (approximately 40ul), then add **2 drops of buffer** (approximately 80 μl) to the specimen well of the cassette, and then start the timer. Avoid trapping air bubbles in the specimen well. See illustration below.
3. Wait for the colored line(s) to appear. **Read the result at 5 minutes**. Do not interpret the result after 10 minutes.



For whole blood specimen:

1. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it as soon as possible.
2. Place the cassette on a clean and level surface.
   For **Venipuncture Whole Blood** specimen:
   - Hold the dropper vertically and transfer **1 drop of whole blood** (approximately 40μl) to the specimen well, 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 40μl of fingerstick whole blood specimen** to the specimen area of test cassette, then add **2 drops of buffer**(approximately 80 μl) and start the timer. See illustration below.
3. Wait for the colored line(s) to appear. **Read results at 5 minutes.** Do not interpret the result after 10 minutes.



【INTERPRETATION OF RESULTS】

(Please refer to the illustration above)

NEGATIVE:* Two lines appear. One colored line should be in the controlline region (C), and another apparent colored line should be in the test line region (T). This negative result indicates that the Tramadolconcentration 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 controlline 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: Controlline fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for controlline failure. 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 analytical 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]
2. It is possible that technical or procedural errors, as well as other interfering substances in thewhole 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.

【EXPECTED VALUES】

This negative result indicates that the Tramadol concentration is below the detectable level of 50ng/ml. Positive result means the concentration of Tramadolis above the level of 50ng/ml. The TML Rapid Test Cassette has a sensitivity of 50ng/ml

【PERFORMANCE CHARACTERISTICS】

Accuracy

A side-by-side comparison was conducted using The TML Rapid Test Cassette and GC/MSat 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
TML Rapid Test Cassette	Results	Positive	Negative	
	Positive	19	1	20
	Negative	2	75	77
Total Results		21	76	97
% Agreement		90.5%	98.7%	96.9%

Clinic Result of Serum or Plasma

Method		GC/MS		Total Results
TML Rapid Test Cassette	Results	Positive	Negative	
	Positive	19	1	20
	Negative	2	75	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 ±50%cutoff and 3x cutoff,The data are summarized below:

For whole blood:

TMLConcentration (ng/ml)	Percent of Cut-off	n	Visual Result	
			Negative	Positive
0	0	30	30	0
25	-50%	30	30	0
50	Cut-off	30	15	15
75	+50%	30	0	30
150	3X	30	0	30

For serum or plasma:

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

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-O-Desmethyl venlafaxine	25,000

Precision

A study was conducted at three hospitals by untrained operators 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	10	0	10	0	10	0
25	10	10	0	10	0	10	0
75	10	0	10	0	10	0	10

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-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	Cyclobarbital	Cyclobenzaprine
Deoxycorticosterone	(-) Deoxyephedrine	R (-)Deprenyl	Dextromethorphan
Diazepam	Diclofenac	Diflunisal	Digoxin
4-Dimethylaminoantipyrine	Diphenhydramine	Dicyclomine	5,5-Diphenylhydantoin
Disopyramide	Doxylamine	Ecgonine	EcgonineMethylester
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	GuaiacolGlyceryl 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-Methylendioxy-methamphetamine	Nimesulide	d/l-Octopamine
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

Interfering Substances

The TML Rapid Test Cassette (whole blood/serum/plasma) has been tested for possible interferencefrom visibly hemolyzed and lipemic specimens. In addition, no interference was observed in specimens containing up to 100 mg/dl hemoglobin;up to 100 mg/dl bilirubin; and up to 200 mg/dl human serum albumin.

【BIBLIOGRAPHY】

1. Tietz NW. Textbook of Clinical Chemistry. W.B. Saunders Company. 1986; 1735
2. Baselt RC. Disposition of Toxic Drugs and Chemicals in Man.2nd Ed. Biomedical Publ., Davis, CA. 1982; 488

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