



TRA One Step Tramadol Test Strip (Urine) Package Insert

Cat: TRA-101
Version: Z

Specimens: Urine
Effective Date: 2020-9

For professional *in vitro* diagnostic use only.

INTENDED USE

The TRA One Step Tramadol Test Strip (Urine) is a lateral flow chromatographic immunoassay for the detection of Tramadol in urine at a cut-off concentration of 100 ng/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.

INTRODUCTION

Tramadol 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 the urine 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.

PRINCIPLE

The TRA One Step Tramadol Test Strip (Urine) is an immunoassay based on the principle of competitive binding. Drugs that 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. Tramadol, if present in the urine 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 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 drug-bovine protein antigen conjugate on the membrane and the conjugate pad of each test contains monoclonal anti-drug antibody.

KIT COMPONENTS

Individually packed Test Strips
Package insert

Each Strip contains a strip with colored conjugates and reactive reagents pre-spread at the corresponding regions. For operation instruction.

Materials Required but Not provided

Specimen collection container
Timer

For specimens collection use.
For timing use.

PRECAUTIONS

- For professional *in vitro* diagnostic use only.

- Do not use after expiration date indicated on the package. Do not use the test if its foil pouch is damaged. Do not reuse tests.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore, recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest or inhale).
- Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
- Read the entire procedure carefully prior to performing any tests.
- Do not eat, drink or smoke in the area where the specimens and kits are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure 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 assayed.
- Humidity and temperature can adversely affect results.
- The used testing materials should be discarded in accordance with local, state and/or federal regulations.

STORAGE AND STABILITY

- The kit should be stored at 2-30°C until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- Do not freeze.**
- Cares should be taken to protect components in this kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

SPECIMEN COLLECTION AND STORAGE

- 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.
- Collected urine specimens must be put in clear and dry containers.
- Perform the testing immediately after the specimen collection. Do not leave the specimens at room temperature for prolonged periods. Specimens may be stored at 2-8°C for up to 48 hours. For long term storage, specimens should be kept below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Avoid repeated freezing and thawing of specimens.
- Pack the specimens in compliance with applicable regulations for transportation of etiological agents, in case they need to be shipped.

PROCEDURE

Bring tests, specimens and/or controls to room temperature (15-30°C) before use.

- Bring the pouch to room temperature before opening it. Remove the test Strip from the sealed pouch and use it as soon as possible.
- Prepare a container with appropriate amount of urine. **Dip the test strip vertically in the urine specimen for at least 8-10 seconds.** then start the timer, and Place the test Strip on a clean and level surface. Avoid let the urine exceed the max line.
- Wait for the colored line(s) to appear. **Read results at 5 minutes.** Do not interpret the result after 10 minutes.

INTERPRETATION OF RESULTS

**POSITIVE
RESULT:**

C
T

Only one colored band appears in the control region (C). No apparent colored band appears in the test region (T).

**NEGATIVE
RESULT:**



Two colored bands appear on the membrane. One band appears in the control region (C) and another band appears in the test region (T).

**INVALID
RESULT:**



Control band fails to appear. Results from any test which has not produced a control band at the specified reading time must be disregarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

- The intensity of the color in test region (T) may vary depending on the concentration of aimed substances present in the specimen. Therefore, any shade of color in the test region should be considered negative. Besides, the concentration level can not be determined by this qualitative test.
- Insufficient specimen volume, incorrect operation procedure, or performing expired tests are the most likely reasons for control band failure.

QUALITY CONTROL

- Internal procedural controls are included in the test. A colored band appearing in the control region (C) is considered an internal positive procedural control. It confirms sufficient specimen volume and correct procedural technique.
- External controls are not supplied with this kit. 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 OF THE TEST

- The TRA One Step Tramadol Test Strip (Urine) 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.^{1,2}
- It is possible that technical or procedural errors, as well as other interfering substances in the urine specimen may cause erroneous results.
- 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.
- A positive result indicates presence of the drug or its metabolites but does not indicate level of intoxication, administration route or concentration in urine.
- A negative result may not necessarily indicate drug-free urine. Negative results can be obtained when drug is present but below the cut-off level of the test.
- Test does not distinguish between drugs of abuse and certain medications.

PERFORMANCE CHARACTERISTICS

A. Accuracy

129 clinical urine specimens were analyzed by GC-MS and by the TRA One Step Tramadol Test Strip (Urine). Each test was performed by three operators. Samples were divided by concentration into five categories: negative, less than half the cutoff, near cutoff negative, near cutoff positive, and high positive. Results were as follows:

Method		GC/MS					
The TRA One Step Tramadol Test Strip		Neg.	Neg.($< -50\%$ cutoff)	Near cutoff neg.(-50% cutoff to cutoff)	Near cutoff pos.(cutoff to $+50\%$ cutoff)	Pos.($> +50\%$ cutoff)	% agreement with GC/MS
TRA 100	Positive	0	0	1	20	24	97.78%
	Negative	55	15	13	1	0	98.81%

B. Precision

A study was conducted at three physician offices for Tramadol (100 ng/mL) 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 drugs at the concentration of $\pm 50\%$ and $\pm 25\%$ cut-off level, was labeled as a blind and tested at each site. The results are given below:

Drug Conc.	n per site	Site A		Site B		Site C	
		-	+	-	+	-	+
Negative	10	10	0	10	0	10	0
-50% Cut-off	10	10	0	10	0	10	0
-25% Cut-off	10	9	1	10	0	9	1
+25% Cut-off	10	1	9	1	9	0	10
+50% Cut-off	10	0	10	0	10	0	10

C. Effect of Urinary Specific Gravity

Fifteen (15) urine samples of normal, high, and low specific gravity ranges (1.000-1.037) were spiked with drugs at 50% below and 50% above cut-off levels respectively. The TRA One Step Tramadol Test Strip (Urine) was tested in duplicate using fifteen drug free urine and spiked urine samples. The results demonstrate that varying ranges of urinary specific gravity do not affect the test results.

D. Effect of 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 drugs at 50% below and 50% above cut-off levels. The spiked, pH adjusted urine was tested with the TRA One Step Tramadol Test Strip (Urine). The results demonstrate that varying ranges of pH do not interfere with the performance of the test.

E. Cross-Reactivity

The following tables list the concentrations of compounds (ng/mL) above which the TRA One Step Tramadol Test Strip (Urine) identified positive results at 5 minutes.

Tramadol related Compound	Concentration (ng/mL)
Tramadol	100
N-desmethyl-tramadol	62.5
O-desmethyl-tramadol	750

F. Non Cross-Reacting Compounds












The following compounds yielded negative results up to a concentration of 100 $\mu\text{g/mL}$:

4-Acetamidophenol	D-Leucyl-L-tyrosine Hydrate	Loratadine	Procaine
Acetaminophen	Dopamine	L-Thyroxine sodium	Promethazine hydrochlorine
Acetylsalicylic Acid	Droperidol	Maprotiline	6-Propyl-2-thiouracil
Albumin	Enalapril Maleate	Meprobamate	Pyridoxine
Amoxicillin	Erythromycin	Minocycline	Pyrilamine Maleate
Ampicillin	Estradiol	Mosapride Citrate	Pyrogalllic
Ampicillin trihydrate	Estrone	Nalidixic acid	Quetiapine Fumarate
Aspartame	Ethyl 4-aminobenzoate	Naloxone HCl	Quinine
Atropine	Fluoxetine	Naltrexone HCl	Quinolinic acid
Baclofen	Fotemustine	Naproxen	R,R(-)-Pseudoephedrine

Benzoic Acid	Furosemide	Nicotinamide	Ranitidine base
Berberine Chloride Hydrate	Gabapentin	Nicotinic acid	Ranitidine
Bilirubin	Gatifloxacin	Nifedipine	Riboflavin
Caffeine	Gemfibrozil	Nimodipine	Rifampicin
Cephalexin	Gentisic Acid	Norethisterone Acetate	Risperidone
Cephradine	Gliclazide	Norfloxacin Nicotinic	Salicylic acid
Chloral hydrate	Glipizide	Noscapine	Sertraline HCl
Chloramphenicol	Glyburide	(±)-Octopamine	Simvastatin
Chlorpheniramine Maleate	Guaiacol	Ofloxacin	Sodium 2-Propylvalerate
Chlorpromazine	Guaifenesin	Olanzapine	Sulfamethazine
Cholesterol	Hemoglobin	Oxalic acid, anhydrous	Sulindac
Ciprofloxacin hydrate	Hydralazine HCl	Oxolinic acid	Tetracycline
Clarithromycin	Hydrochlorothiazide	Paliperidone	Tetrahydrozoline
Clonidine solution	Hydrocortisone	Pantoprazole sodium	Theophylline
Creatinine	Ibuprofen	Penfluridol	Thiamine
D(-)-Norgestrel	Isoprenaline	Penicillin G potassium salt	Thioridazine solution
d,l-Propranolol	Ketoconazole	Penicillin G sodium salt	Tolbutamide
Deoxycorticosterone	Ketoprofen	Perphenazine	Topiramate
Dextromethorphan solution	Lamotrigine	Phenacetin	2,4,7-Triamino-6-Phenylpteridine
Diclofenac	L-Ascorbic acid	Phenelzine Sulfate	Trimethoprim
Diflunisal	Levofloxacin	Phenothiazine	Tryptamine
Digoxin	Lidocaine	2-Phenylethylamine	Tyramine
4-Dimethyl-aminoantipyrine	Lidocaine Monohydrate	Pioglitazone	Uric acid
Diphenhydramine	Lisinopril Dihydrate	Piracetam	(±)-Verapamil
5,5-Diphenylhydantoin	Lithium carbonate	Pravastatin sodium	Vitamin B1
D-Lactose monohydrate	Loperamide	Prednisone	Zomepirac

LITERATURE REFERENCES

- 1.Baselt RC. Disposition of Toxic Drugs and Chemicals in Man. 2nd Ed. Biomedical Publ., Davis, CA. 1982; 488
- 2.Hawks RL, CN Chiang. *Urine Testing for Drugs of Abuse*. National Institute for Drug Abuse (NIDA), Research Monograph 73, 1986

Index of Symbols			
	Consult Instruction for use		Tests per kit
	For in vitro diagnostic use only		Use by date
	Store between 2-30°C		Lot Number
	Keep away from sunlight		Keep dry
	Caution		Date of manufacture
			Authorized Representative



Hangzhou Sejoy Electronics& Instruments Co.,Ltd.
Area C, Building 2, No.365, Wuzhou Road,Yuhang Economic
Development Zone,Hangzhou City 311100 Zhejiang China



Shanghai International Holding Corporation GmbH (Europe)
Eiffestrasse 80, 20537 Hamburg, Germany

