TRA One Step Tramadol Test Device (Urine)
Package Insert

Cat: TRA-102 **Specimens:** Urine

Version: Z **Effective Date:** 2020-9

For professional in vitro diagnostic use only.

INTENDED USE

The TRA One Step Tramadol Test Device (Urine) is a rapid visual immunoassay for the qualitative, presumptive detection of Tramadol in human urine specimens at the cut-off concentrations listed below:

Parameter	Calibrator	Cut-off (ng/mL)
TML (Tramadol)	Cis-Tramadol	100

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, glucoronidation or sulfation in the liver.

PRINCIPLE

The TRA One Step Tramadol Test Device (Urine) detects Tramadol through visual interpretation of color development on the strip. Drug conjugates are immobilized on the test region of the membrane. During testing, the specimen reacts with antibodies conjugated to colored particles and precoated on the sample pad. The mixture then migrates through the membrane by capillary action, and interacts with reagents on the membrane. If there are insufficient drug molecules in the specimen, the antibody-colored particle conjugate will bind to the drug conjugates, forming a colored band at the test region of the membrane. Therefore, a colored band appears in the test region when the urine is negative for the drug. If drug molecules are present in the urine above the cut-off concentration of the test, they compete with the immobilized drug conjugate on the test region for limited antibody binding sites. This will prevent attachment of the antibody-colored particle conjugate to the test region. Therefore, the absence of a colored band at the test region indicates a positive result. The appearance of a colored band at the control region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

REAGENTS

Each test consists of a reagent strip. The amount of each antigen and/or antibody coated on the strip is less than 0.001 mg for antigen conjugates and goat anti-rabbit IgG antibodies, and less than 0.0015 mg for antibody components.

The control zone of each test contains goat anti-rabbit IgG antibody. The test zone of each test contains drug-bovine protein antigen conjugate, and the conjugate pad of each test contains monoclonal anti-drug antibody and rabbit antibody-colored particle complex

MATERIALS

Materials Provided

- Individually pouched test devices
- Disposable pipettes
- Package insert

Materials Required but Not provided

Specimen collection container	For specimens collection use.
Timer	For timing use.

PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- Do not use after the expiration date indicated on the package. Do not use the test if the foil pouch or canister 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 completely guarantee the absence of transmissible pathogenic agents. It is therefore, recommended that these products be treated as potentially infectious, and handled by observing usual safety precautions (e.g., 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 testing.
- Do not eat, drink or smoke in the area where 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 standard procedures for the 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.
- Used testing materials should be discarded in accordance with local 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 or closed canister until use.
- **Do not freeze.**
- Kits should be kept out of direct sunlight.
- Care should be taken to protect the components of the 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 TRA One Step Tramadol Test Device (Urine) is intended for use with human urine specimens only.
- Urine collected at any time of the day may be used.
- Urine specimens must be collected in clean, dry containers.
- Turbid specimens should be centrifuged, filtered, or allowed to settle and only the clear supernatant should be used for testing.
- Perform testing immediately after specimen collection. Do not leave specimens at room temperature for prolonged periods. Urine specimens may be stored at 2-8°C for up to 2 days. 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.
- If specimens are to be shipped, pack them in compliance with all applicable regulations for transportation of etiological agents.

PROCEDURE

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

1. Bring the pouch to room temperature before opening it. Remove the test device from the sealed pouch and use it as soon as possible.

2. Place the test device on a clean and level surface. Hold the dropper vertically and **transfer 3 full drops of urine** (approx. 100 μL) to the specimen well (S) of the test device, and then start the timer. Avoid trapping air bubbles in the specimen well (S).
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

POSITIVE RESULT:

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 disgarded. 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:

1. The intensity of color in the test region (T) may vary depending on the concentration of analytes present in the specimen. Therefore, any shade of color in the test region should be considered negative. Note that this is a qualitative test only, and cannot determine the concentration of analytes in the specimen.
2. Insufficient specimen volume, incorrect operating procedure or 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, confirming 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

1. The TRA One Step Tramadol Test Device (Urine) is for professional in vitro diagnostic use, and should be only used for the qualitative detection of Tramadol.
2. This assay provides a preliminary analytical test result only. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) has been established as the preferred confirmatory method by the National Institute on Drug Abuse (NIDA). Clinical consideration and professional judgment should be applied to any test result, particularly when preliminary positive results are indicated.
3. There is a possibility that technical or procedural errors as well as other substances and factors may interfere with the test and cause false results.
4. Adulterants, such as bleach and/or alum, in urine specimens may produce erroneous results regardless of the analytical method used. Therefore, please preclude the possibility of urine adulteration prior to testing.
5. A positive result indicates the presence of a Tramadol only, and does not indicate or measure intoxication.
6. A negative result does not at any time rule out the presence of Tramadol in urine, as they may be present below the minimum detection level of the test.
7. This test does not distinguish between Tramadol 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 ± 50% and ± 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	10	0	10	0	10	0
+25% Cut-off	10	0	10	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 µg/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	Pyrogallic
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
Diciofenac	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

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4. McBay AJ. Drug-analysis technology--pitfalls and problems of drug testing. Clin Chem. 1987 Oct; 33 (11 Suppl): 33B-40B.
5. Gilman AG, Goodman LS, Gilman A, eds. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 6th ed. New York: Macmillan; 1980.

Index of Symbols

	Consult Instruction for use		Tests per kit		Do not use if package is damaged
IVD	For in vitro diagnostic use only		Use by date		Do not reuse
2℃ 30℃	Store between 2-30°C	LOT	Lot Number	REF	Catalogue number
	Keep away from sunlight		Keep dry		Manufacturer
	Caution		Date of manufacture	EC REP	Authorized Representative

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