TRL

One Step
Tramadol Test Strip (Urine)
Package Insert

REF-R1111	English

A rapid, one step test for the qualitative detection of Tramadol (TRL) in urine.

For professional in vitro diagnostic use only.

INTENDED USE

The TRL One Step Tramadol Test Strip (Urine) is a lateral flow, one-step immunoassay for the qualitative detection of tramadol and its metabolites in human urine. The detection limit (cut-off) for tramadol is 100 ng/ml. The test is interpreted visually and provides a qualitative result. The test is a competitive immuno-assay and in case there is no tramadol (non-consumer) in the urine two red lines (test- and control-line) appear at the strips. If there is tramadol in the urine sample (consumer) only one control-line appears at the test strip.

SUMMARY

Tramadol is a centrally acting synthetic analgesic compound that is not derived from natural sources nor is it chemically related to opiates. Although its mode of action is not completely understood at least two complementary mechanisms appear applicable: a low binding affinity to μ-opioid receptors and an inhibition of reuptake of norepinephrine and serotonin. Continuous use of large doses of tramadol can result into tolerance and physiological dependency on the drug 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. The half-life of tramadol in serum is 6-7 hours in healthy individuals. The therapeutic concentration range in serum is normally 0.1-0.3 μg/ml. Studies show that tramadol concentration in urine were considerably higher than in serum. Therefore urine samples are very suitable for proof of tramadol intake in therapeutic drug monitoring or forensic toxicology.

PRINCIPLE

The One Step Tramadol Test Strip (Urine) is a competitive immunoassay in which drug conjugate from the test competes with free drug which may be present in urine for limited antibody binding sites. The membrane strips are pre-coated with immobilized tramadol conjugate as antigen in the test result line region (T-region). Red gold-colloid-labelled anti-tramadol-antibodies are placed in the conjugate pad at the left ending of the membrane. After dipping it into urine the antibodies move upwards by capillary action and get to the T-region. If there is no tramadol in the urine the antibody attaches to the immobilized tramadol conjugate and a visible line is formed.

Therefore, a line in the T-region indicates that no tramadol is present in the urine or that the tramadol concentration is below the cut-off. If tramadol is present in the urine, it competes with the immobilized tramadol conjugate in the T-region for the limited antibody sites. With increasing concentrations of tramadol in the sample the binding of the antibody is more and more inhibited and the colour of test result line becomes weaker. When the amount of drug is equal or more than the cut-off, 100ng/ml, it will prevent the binding of the antibody to the drug conjugate and the line gradually vanishes. Therefore, the absence of a coloured band in the T-region indicates a positive result.

A control line with a different antigen/antibody reaction is also added to the immunochromatographic membrane strip at the control region (C-region) to indicate that the test has been performed properly. The presence of this control lines serves as 1) verification that sufficient volume has been added, and 2) that proper flow was obtained. The control line should always appear, regardless of the presence of tramadol. This means that negative urine will produce two coloured bands (non-consumer), where as positive urine will produce only one coloured line in the reaction zone (consumer).

REAGENTS

The test strip contains anti-TRL particles and TRL coated on the membrane.

PRECAUTIONS

- For professional *in vitro* diagnostic use only. Do not use after the expiration date.
- The test strip should remain in the sealed pouch until use.
- All specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The test strip should be discarded in a proper biohazard container after testing.

STORAGE AND STABILITY

Store as packaged in the sealed pouch at 4-30°C. The test strip is stable through the expiration date printed on the sealed pouch. The test strip must remain in the sealed pouch until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

Urine Assay

A urine specimen must be collected in a clean and dry container. Fresh urine does not require any special handling or pretreatment. Urine samples should be collected such that testing can be performed as soon as possible after the specimen collection, preferably during the same day. Urine specimens exhibiting visible precipitates should be centrifuged, filtered, or allowed to settle to obtain a clear specimen for testing.

Specimen Storage

Urine specimens may be stored at 2-8°C for up to 48 hours prior to testing. For prolonged storage, specimens may be frozen and stored below -20°C. Frozen specimens should be thawed and mixed before testing.

PROCEDURE

Materials Provided

- Test strips
- Package insert

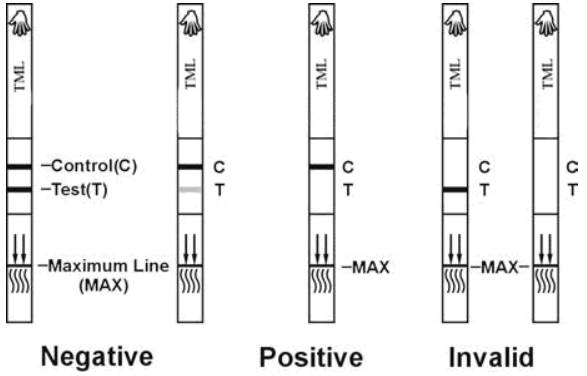
Materials Required But Not Provided

- Specimen collection container
- Timer

DIRECTIONS FOR USE

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

1. Bring the pouch to room temperature before opening it. Remove the test strip from the sealed pouch and use it as soon as possible.
2. With arrows pointing toward the urine specimen, immerse the test strip vertically in the urine specimen for at least 5 seconds. Do not pass the maximum line (MAX) on the test strip when immersing the strip. See the illustration below.
3. Place the test strip on a non-absorbent flat surface, start the timer and wait for the red line(s) to appear. The result should be read at 5 minutes. Do not interpret the test result after more than 15 minutes.



INTERPRETATION OF RESULTS

(Please refer to the illustration above)

Positive:

One colored band forms. One colored band appears in control line zone. No colored band is found in test line zone. This is an

indication the level of tested drug(s) in the specimen is above the cut-off level.

Negative:

Two colored bands form. The appearance of two colored bands, one in test line zone and the other in control line zone, indicates negative result for that particular test(s). The negative result does not indicate the absence of drug in the specimen. It only indicates the level of tested drug in the specimen is less than cut-off level.

Invalid:

If there are no colored bands or control line fails, the test result is invalid. Retest the sample with a new Strip.

QUALITY CONTROL

Good laboratory practice recommends the use of control materials to ensure proper kit performance. Quality control specimens are available from commercial sources. When testing the positive and negative controls, use the same assay procedure as with a urine specimen. The tested positive controls should be close to the cut-off to ensure a correct performance of the assay.

LIMITATIONS

1· The assay is designed for use with human urine only. Due to absence of ions and other components in pure water the usage or pure water for test could lead to false or invalid results.

2· A positive result with any of the tests indicates the presence of a drug/metabolite only, and does not indicate or measure intoxication.

3· There is a possibility that technical or procedural errors as well as other substances and factors not listed may interfere with the test and cause false results. See SPECIFICITY for lists of substances that will produce positive results, or that do not interfere with test performance.

4· If it is suspected that the samples have been mislabelled or tampered with, a new specimen should be collected and the test should be repeated.

EXPECTED VALUES

The TRL One Step Tramadol Test Strip (Urine) is a qualitative assay. It identifies the drug(s) in human urine at its cut-off concentration or higher. The concentration of the drug(s) can not be determined by this assay. The test is intended to distinguish negative result from presumptive positive result. All positive results must be confirmed using an alternate method, preferably GC/MS.

PERFORMANCE CHARACTERISTICS

Accuracy

A side-by-side comparison was conducted using the TRL one step tramadol test strip and leading commercially available TRL rapid test. Testing was performed one specimens previously collected from subjects presenting for Drug Screen Testing. Presumptive positive results was confirmed by GC/MS.

Analytical Sensitivity

The cut-off concentration (sensitivity level) of tramadol test is determined to be 100 ng/ml of tramadol. The cut-off is defined as the drug concentration at which the test result lines starts to disappear.

Precision

Test precision was determined by blind tests with control solutions. Controls with a tramadol concentration of 0.5x cut-off (50 ng/ml) yielded reliable negative results.

Controls with a tramadol concentration of 1.5x cut-off (150 ng/ml) provided positive results.

Specificity

The specificity of the TRL One Step Tramadol Test Strip (Urine) was tested with the substances listed below, all of which can be found in a normal urine specimen. The following compounds with a similar chemical structure yield a positive result with the TRL test at the specified concentration:

Drug	Concentration (ng/ml)
Tramadol	100
N-desmethyl-tramadol	250
O-desmethyl-tramadol	10,000

Cross-Reactivity

A study was conducted to determine the cross- reactivity of the test with compounds in either drug-free urine or Tramadol positive urine. The following compounds show no interference when tested with the TRL One Step Tramadol Test Strip at a concentration of 100µg/mL.

Non Cross-Reacting Compounds

Acetaminophen	Ecgonine	Morphine
4-Acetamidophenol	Ecgoninemethyl	Neomycin
Acetylsalicylic acid	ester	Niacinamide
Amikacin	Ephedrine	Oxazepam
Amitriptyline	Epinephrine	Perphenazine
Amobarbital	Gentisic	Penicillin G
Amphetamine	Guaiacolglycer	Phencyclidine
Arterenol	ester	Phenylethylamine-α
Aspartame	Histamine	Phenylpropanolamine
Ascorbic acid	Hydrochlorothiazide	Promethazine
Atrophine	Homatrophine	Pseudoephedrin
Caffeine	Imipramine	Quinine antidine
Camphor	Ibuprofen	Salicylic acid
Chloroquine	Isoproterenol	Tetracycline
Chlopheniramine	Ketamine	Tetrahydrozolin
Codeine	Lidocaine	Theophyline
Cortisone	Meperidine	11-nor-Δ8 –THC-9-COOH
Deoxyephedrine	Methadone	11-nor-Δ8 –THC-9-COOH
Dextromethorphan	Methamphetamione	Thioridazine
Digitoxin	3,4±MDMA	Trifluoperazine
Digoxin	Methaqualone	Tryptophan
Diphenhydramine	Methylphenidate	Tyramine

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Index of Symbols

Attention, see instructions for use	Tests per kit	EC REP	Authorized Representative
For *in vitro* diagnostic use only	Use by	2	Do not reuse
Store between 2-30℃	Lot Number		

Manufacturer

ISO

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