IMMUNOSPEC CORPORATION
7018 Owensmouth Ave. Suite 103
Canoga Park, CA 91303
Tel.:(818)-710-1281
Fax :(818)936-0121
Email:Info@immunospec.com
www.immunospec.com

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TRAMADOL TEST CASSETTE

FOR THE QUALITATIVE ASSESSMENT OF TRAMADOL IN HUMAN URINE

REF

Catalog No.R13-470

IVD

For in vitro diagnostic use only

INTENDED USE

Immunospec Tramadol test cassette is an immunochromatography based one step in vitro test. It is designed for qualitative determination of the tramadol and its metabolites in human urine specimens at cut-off level of 200 ng/ml. This assay has not been evaluated in the point of care location and is for use by Healthcare Professionals only.

This assay provides only a preliminary analytical test result. 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 Substance Abuse Mental Health Services Administration (SAMHSA). Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are indicated.

SUMMARY AND EXPLANATION

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.

TEST PRINCIPLE

Tramadol test cassette is based on the principle of specific immunochemical reaction between antibodies and antigens to analyze particular compounds in human urine specimen. The assay relies on the competition for binding antibody between drug conjugate and free drug which may be present in the urine specimen being tested. When drug is present in the urine specimen, it competes with drug conjugate for the limited amount of antibody-dye conjugate. When the amount of drug is equal or more than the cut-off, 200ng/ml, it will prevent the binding of drug conjugate to the antibody. Therefore, a positive urine specimen will not show a colored band on the test line zone, indicating a positive result, while the presence of a colored band indicates a negative result.

A control line is present in the test window to work as procedural control. This colored band should always appear on the control line zone if the test device is stored in good condition and the test is performed appropriately.

MATERIALS PROVIDED

1. Instructions for use.
2. Tramadol test device. The amount of each coated antigen and/or antibody on the strip is less than 1.0 mg for antigen conjugate and is less than 1.0 mg for goat anti-mouse IgG antibody.
   Test zone: contains tramadol bovine protein antigen conjugates.
   Control zone: contains Goat anti-mouse IgG antibody.
3. Conjugate pad: contains mice monoclonal anti-drug antibody.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Urine collection container.
2. Timer or clock.

STORAGE AND STABILITY

The test device should be stored at 2 to 30ºC and will be effective until the expiration date stated on the package. The product is humidity-sensitive and should be used immediately after being open. Any improperly sealed product should be discarded.

PRECAUTIONS

1. For in vitro diagnostic and forensic use only.
2. Do not use the product beyond the expiration date.
3. Handle all specimens as potentially infectious.
4. Humidity sensitive product, do not open foil pouch until it is ready to be tested.
5. Use a new urine specimen cup for each sample to avoid cross contamination.

SPECIMEN COLLECTION AND PREPARATION

It is required that approximately 150 µl of sample for each test. Fresh urine specimens do not need any special handling or treatment. Specimens should be collected in a clean, dry, plastic or glass container. If the assay is not performed immediately, urine specimen may be refrigerated at 2-8ºC or frozen up to 7 days. Specimens should be thawed and brought to room temperature before testing. Urine specimens exhibiting a large amount of precipitate or turbidity should be centrifuged or allowed to settle before testing. Avoid contact with skin by wearing gloves and proper laboratory attire.

QUALITY CONTROL

Good Laboratory Practice recommends the daily use of control materials to validate the reliability of device. Control materials should be assayed as clinical specimen and challenging to the assay cutoff concentration, e.g., 25% above and below cutoff concentration. If control values do not fall within established range, assay results are invalid. Control materials which are not provided with this test kit are commercially available.

Tramadol test provides a built-in process control with a different antigen/antibody reaction at the control region (C). This control line should always appear regardless the presence of drug or metabolite. If the control line does not appear, the test device should be discarded and the obtained result is invalid. The presence of this control band in the control region serve as 1) verification that sufficient volume is added, 2) that proper flow is obtained.

PROCEDURE

1. Bring all materials and specimens to room temperature.
2. Remove the test cassette from the sealed foil pouch.
3. Place the transfer pipette in the specimen and depress the bulb to withdraw a sample.
4. Hold the pipette in a vertical position over the sample well of the test cassette and deliver 3 drops (120-150 µl) of sample in to the sample well.
5. Read the results at 5 minutes after adding the sample.

INTERPRETATION OF RESULTS

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 results. The negative result indicates that the tramadol concentration in the specimen is either zero or less than cut-off level.

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 that the tramadol level in the specimen is above the cut-off level.

Invalid:

If there is no colored band in control line zone, the test result is invalid. Retest the sample with a new device.

Note: A borderline (+/-) in test line zone should be considered negative result.

LIMITATION OF PROCEDURE

The assay is designed for use with human urine only. A positive result with any of the tests indicates only the presence of a drug/metabolite and does not indicate or measure intoxication. There is a possibility that technical or procedural error as well other substances in certain foods and medicines may interfere with the test and cause false results. Please refer "SPECIFICITY" section for lists of substances that will produce either positive results, or that do not interfere with test performance. If a drug/metabolite is found present in the urine specimen, the assay does not indicate frequency of drug use or distinguish between drug of abuse and certain foods and medicines.

EXPECTED RESULTS

Immunospec Tramadol Test is a qualitative assay. It identifies tramadol in human urine at a concentration of 200 ng/ml or higher. The concentration of the tramadol cannot 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.

PERRFOMANCE CHARACTERISTICS

A. Sensitivity

The cut-off concentration (sensitivity level) of tramadol test is determined to be 200 ng/ml of tramadol.

B. Precision

The precision study was performed by three individuals observing the test result to determine the random error of visual interpretation. The test results were found to have no significant differences between the three observers.

C. Specificity

The specificity for tramadol test was tested by adding various drugs, drug metabolites, and other compounds that are likely to be present in urine. All compounds were prepared in drug-free normal human urine.

1. Interference testing

Tramadol test performance at cut-off level is not affected when pH and Specific Gravity ranges of urine specimen are at 4.5 to 8.0 and 1.005 to 1.035.

The following substances were tested and confirmed not to interfere with tramadol test at the listed concentrations.

Glucose	2000 mg/dl,
Human albumin	2000 mg/dl
Human hemoglobin	10 mg/dl,
Urea	4000 mg/dl
Uric acid	10 mg/dl

2. Specificity

The following table lists compounds that are detected by tramadol test which produced positive results when tested at levels equal or greater than the concentrations listed below:

Compounds	Concentration	reactivity
Tramadol	200 ng/ml	100%
N-desmethyl-tramadol	500 ng/ml	40%
O-desmethyl-tramadol	20,000 ng/ml	1%

Each listed substance that commonly found in the urine was evaluated and indicated negative result at concentration of 100 µg/ml.

Acetaminophen	4-Acetamidophenol	Acetylsalicylic acid	Amikacin
Amitriptyline	Amobarbital	Amphetamine	Arterenol
Aspartame	Ascorbic acid	Atrophine	Caffeine
Camphor	Chloroquine	Chlopheniramine	Codeine Cortisone
	Deoxyephedrine	Dextromethorphan	Digitoxin
Digoxin	Diphenhydramine	Ecgonine	Ecgonine methyl ester
Ephedrine	Epinephrine	Gentisic	Guaiacol glycer ester
Histamine	Hydrochlorothiazide	Homatrophine	Imipramine
Ibuprofen	Isoproterenol	Ketamine	Lidocaine
Meperidine	Methadone	Methamphetamione	3,4±MDMA
Methaqualone	Methylphenidate	Morphine	Neomycin
Niacinamide	Oxazepam	Perphenazine	Penicillin G
Phencyclidine	Phenylethylamine-α	Phenylpropanolamine	Promethazine
Pseudoephedri	Quinine antidine	Salicylic acid	Tetracycline
Tetrahydrozolin	Theophyline	11-nor-Δ^8–THC-9-COOH	11-nor-Δ^8–THC-9-COOH
Thioridazine	Trifluoperazine	Tryptophan	Tyramine

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EC REP **European Authorized Representative:**

CEpartner4U , Esdoornlaan 13, 3951DB Maarn . The Netherlands. Tel.: +31 (0)6.516.536.26

Manufacturer:

IMMUNOSPEC CORPORATION
7018 Owensmouth Ave. Suite 103
Canoga Park, CA 91303
Tel.:(818)-710-1281

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