

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k141803

B. Purpose for Submission:

New device

C. Measurand:

Tramadol

D. Type of Test:

Homogenous Enzyme Immunoassay, Qualitative and Semi-quantitative.

E. Applicant:

Immunoanalysis Corporation

F. Proprietary and Established Names:

Immunoanalysis Tramadol Urine Enzyme Immunoassay

Immunoanalysis Tramadol Urine Controls

Immunoanalysis Tramadol Urine Calibrators

G. Regulatory Information:

1. Regulation section:

21 CFR 862.3650, Opiate Test System

21 CFR 862.3200, Clinical Toxicology Calibrator

21 CFR 862.3280, Clinical Toxicology Control Materials

2. Classification:

Class II

Class I, reserved.

3. Product code:

DJG

DLJ

LAS

4. Panel:

Toxicology (91)

H. Intended Use:

1. Intended use(s):

See Indications for use below.

2. Indication(s) for use:

The Immunalysis Tramadol Urine Enzyme Immunoassay is a homogeneous enzyme immunoassay with a cutoff of 200ng/mL. The assay is intended for use in laboratories for the qualitative and semi-quantitative analysis of Tramadol in human urine with automated clinical chemistry analyzers. This assay is calibrated against Tramadol. This *in-vitro* diagnostic device is for prescription use only.

The semi-quantitative mode is for purposes of enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as GC-MS or permitting laboratories to establish quality control procedures. The test is not intended to differentiate between drugs of abuse and prescription use of Tramadol. There are no uniformly recognized drug levels for Tramadol in urine.

The Immunalysis Tramadol Urine Enzyme Immunoassay Kit provides only a 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) or Liquid Chromatography / Mass Spectroscopy (LC/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.

The Immunalysis Tramadol Urine Controls are used as control materials in the Immunalysis Tramadol Urine Enzyme Immunoassay.

The Immunalysis Tramadol Urine Calibrators are used as calibrators in the Immunalysis Tramadol Urine Enzyme Immunoassay for the qualitative and semi-quantitative determination of Tramadol in urine on automated clinical chemistry analyzers.

3. Special conditions for use statement(s):

For prescription use only.

Not for use in Point of Care settings.

4. Special instrument requirements:

Beckman Coulter AU 400e chemistry analyzer was used to generate data for this submission.

Automated clinical chemistry analyzers capable of maintaining a constant reaction temperature, pipetting samples and reagents, mixing reagents, timing reactions and measuring enzyme rates at 340nm can be used for the assay.

I. Device Description:

Immunalysis Tramadol Urine Enzyme Immunoassay (EIA) Kit includes antibody/ substrate reagent and enzyme conjugate reagent.

- Antibody/ substrate reagent includes goat antibodies to Tramadol, glucose-6-phosphate (G6P) and nicotinamide adenine dinucleotide (NAD) in Tris buffer with Sodium Azide as a preservative.
- Enzyme conjugate reagent includes tramadol derivative labeled with glucose-6-phosphate dehydrogenase (G6PDH) in Tris buffer with Sodium Azide as a preservative.

Calibrators and controls are sold separately. The tramadol calibrators and controls consist of:

- A single calibrator - 200ng/mL Tramadol.
- A control set - contains a LOW control at 150ng/mL and a HIGH control at 250ng/mL.
- A calibrator set - contains a negative calibrator, a Level 1 calibrator at 100ng/mL, a Level 2 calibrator at 200ng/mL, a Level 3 calibrator at 500ng/mL and a Level 4 calibrator at 1000ng/mL tramadol.

All reagents are in liquid form and ready to use.

J. Substantial Equivalence Information:

1. Predicate device name(s):

LZI Opiate 2000 Enzyme Immunoassay
 LZI Opiate 2000 Enzyme Controls
 LZI Opiate 2000 Enzyme Calibrators

2. Predicate 510(k) number(s):

k120761

3. Comparison with predicate:

Item	Immunalysis Tramadol Urine Enzyme Immunoassay (Candidate Device)	LZI Opiate 2000 Enzyme Immunoassay, k120761 (Predicate Device)
Intended use	For the qualitative and semi-quantitative determination of the presence of drugs of abuse in human urine. For in vitro diagnostic use.	Same
Measured analyte(s)	Tramadol	Opiates
Assay cutoff	200 ng/mL of Tramadol	2000 ng/mL of Opiates
Assay calibrated against	Tramadol	Morphine
Test system type	Homogenous enzyme immunoassay	Enzyme immunoassay
Storage conditions	2 - 8°C until expiration date	Same
Calibrator form	Liquid	Same
Calibrator levels	One level (200 ng/mL)	One level (2000 ng/mL)
Control set levels	Two levels (150 ng/mL and 250ng/mL)	Two levels (1500 ng/mL and 2500 ng/mL)

Item	Immunoanalysis Tramadol Urine Enzyme Immunoassay (Candidate Device)	LZI Opiate 2000 Enzyme Immunoassay, k120761 (Predicate Device)
Calibrator set levels	Five levels (0, 100, 200, 500 and 1000 ng/mL)	Five levels (0, 1000, 2000, 4000 and 6000 ng/mL)

K. Standard/Guidance Document Referenced (if applicable):

- CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods.
- CLSI EP7-A2: Interference Testing in Clinical Chemistry

L. Test Principle:

The assay is based on the competition of Tramadol labeled enzyme glucose-6-phosphate dehydrogenase (G6PDH) and the free Tramadol in the urine sample for the fixed amount of antibody binding sites. In the absence of the free Tramadol in the urine sample, the antibody (goat-polyclonal) binds the Tramadol enzyme conjugate and enzyme activity is inhibited. This creates a dose response relationship between Tramadol concentration in the urine and enzyme activity. The enzyme G6PDH activity is determined at 340 nm spectrophotometrically by the conversion of NAD to NADH.

M. Performance Characteristics (if/when applicable):

All the performance studies were performed on the Beckman Coulter AU 400e chemistry analyzer.

1. Analytical performance:

a. Precision/Reproducibility:

A precision study was performed by one experienced operator following the CLSI (EP5-A2) precision guidelines. Negative urine sample pools were spiked with Tramadol at 0, 50, 100, 150, 200, 250, 300, 350, and 400ng/mL, representing 0, 25, 50, 75, 100, 125, 150, 175 and 200% of the device cutoff (200 ng/mL). Each level sample was tested in duplicate per run, two runs per day for twenty consecutive days (total N= 80/level) on the Beckman Coulter AU 400e chemistry analyzer. The results are summarized in the table below:

Qualitative Precision Result:

Concentration as % of the Cutoff Level	Target Tramadol concentration (ng/mL)	Immunoanalysis Tramadol Urine EIA # Neg / # Pos
0	0	80 Neg / 0 Pos
25	50	80 Neg / 0 Pos
50	100	80 Neg / 0 Pos

75	150	80 Neg / 0 Pos
100	200	44 Neg / 36 Pos
125	250	0 Neg / 80 Pos
150	300	0 Neg / 80 Pos
175	350	0 Neg / 80 Pos
200	400	0 Neg / 80 Pos

Semi-Quantitative Precision Result:

Concentration as % of the Cutoff Level	Target Tramadol concentration (ng/mL)	Immunoanalysis Tramadol Urine EIA # Neg / # Pos
0	0.00	80 Neg / 0 Pos
25	50	80 Neg / 0 Pos
50	100	80 Neg / 0 Pos
75	150	80 Neg / 0 Pos
100	200	47 Neg / 33 Pos
125	250	0 Neg / 80 Pos
150	300	0 Neg / 80 Pos
175	350	0 Neg / 80 Pos
200	400	0 Neg / 80 Pos

b. Linearity/assay reportable range:

Linearity study in the semi-quantitative mode was conducted by spiking drug free urine pool with tramadol (serial dilutions of a high concentration tramadol in urine in increments of 10%) to achieve concentrations ranging from 100ng/mL to 1100ng/mL, and testing each level in duplicate on the Beckman Coulter AU 400e clinical chemistry analyzer. The results are summarized below:

Expected Concentration (ng/mL)	Mean Concentration (ng/mL)	Recovery (%)
0	1	N/A
100	102	102
200	196	98
300	323	108

Expected Concentration (ng/mL)	Mean Concentration (ng/mL)	Recovery (%)
400	416	104
500	506	101
600	558	93
700	637	91
800	756	95
900	872	97
1000	956	96
1100	1012	92

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Traceability

Calibrators and controls are prepared from a standard solution of tramadol purchased from a commercial vendor. This standard solution is diluted with a BSA buffer to make the cutoff calibrator, control set of low and high controls, and calibrator set (of 5 level calibrators). The concentrations of the prepared solutions are confirmed by GC/MS or LC/MS/MS.

Value Assignment – Calibrators and Controls

A commercially available standard solution of Tramadol is mixed with a commercially available BSA buffer to the desired calculated concentrations for the LOW control, HIGH control and Calibrators. The calibrators and controls are tested by GC/MS or LC/MS/MS. Values are assigned to the controls once the GC/MS or LC/MS/MS results are within acceptable ranges. The negative standard is prepared with the BSA buffer. The negative standard is compared to a reference negative standard to ensure that it is free of tramadol. Value is assigned when the test result is within the acceptable range. Acceptance criteria for value assignment were reviewed and deemed acceptable.

Calibrators and Controls Stability Studies

Accelerated and real-time stability studies in the qualitative and semi-quantitative modes were conducted on multiple lots of Immunoanalysis Tramadol Urine Controls and Immunoanalysis Tramadol Urine Calibrators. The stability protocols and acceptance criteria for open and closed vial were reviewed and found acceptable. The open vial

and closed vial study results support the open vial stability claim of six months and closed vial stability claim of twelve months when stored at 2 to 8 °C for the Immunoanalysis Tramadol Urine Controls and Immunoanalysis Tramadol Urine Calibrators.

Sample Storage - Stability Studies

A specimen storage study was performed using urine specimens at tramadol concentration of 147 ng/mL (below the cutoff) and 245 ng/mL (above the cutoff) to establish specimen storage stability at 2 – 8°C. The urine specimen at tramadol concentration below the cutoff was negative in comparison to the 200ng/mL cutoff for day 0, week 1, 2, 3 and 4. The urine specimen at tramadol concentration above the cutoff was positive in comparison to the 200ng/mL cutoff for Day 0, Week 1, 2, 3 and 4. The stability study supported a one month urine specimen containing tramadol stability when stored at 2 – 8°C.

d. Detection limit:

Not applicable.

e. Analytical specificity:

An analytical specificity study to evaluate interference from non-structurally and structurally related compounds was performed in the qualitative and semi-quantitative mode. The study design and results are described below. Results were the same for each mode (qualitative and semi-quantitative modes).

Structurally Related Compounds

Compounds sharing structural or conformational similarity to tramadol were tested for cross-reactivity with the candidate device. The structurally related compounds that exhibited cross-reactivity with the candidate device in urine were titrated to determine the percent cross-reactivity. The concentration (ng/mL) of cross-reactant that gives a response equivalent to the cutoff, and the percent cross-reactivity are presented in the table below.

Compound	Concentration of the compound tested (ng/mL)	Immunoassay Result	Percent Cross-reactivity
Tramadol	200	Positive	100.00
n-Desmethyl Tramadol	450	Positive	44.40
o-Desmethyl Tramadol	25,000	Positive	0.80
Venlafaxine	100,000	Negative	Not detected (< 0.05%)
o-Desmethyl Venlafaxine	100,000	Negative	Not detected (< 0.05%)

Non-Structurally Related Compounds

Potential interference from non-structurally related drugs and metabolites was

evaluated in the qualitative and semi-quantitative modes, by spiking these compounds at high concentrations in pooled urine spiked with tramadol at $\pm 25\%$ of the cutoff (150 and 250 ng/mL).

Compounds	Concentration tested (ng/mL)	-25% Cutoff (150ng/mL)	+25% Cutoff (250ng/mL)
6-Acetylcodeine	100,000	Negative	Positive
6-Acetylmorphine	100,000	Negative	Positive
7-Aminoclonazepam	100,000	Negative	Positive
7-Aminoflunitrazepam	100,000	Negative	Positive
7-Aminonitrazepam	100,000	Negative	Positive
Acetaminophen	500,000	Negative	Positive
Acetylsalicylic Acid	500,000	Negative	Positive
Alprazolam	50,000	Negative	Positive
Amitriptyline	100,000	Negative	Positive
Amobarbital	100,000	Negative	Positive
S-(+) Amphetamine	100,000	Negative	Positive
Benzoyllecgonine	500,000	Negative	Positive
Benzylpiperazine	100,000	Negative	Positive
Bromazepam	100,000	Negative	Positive
4-Bromo-2,5-Dimethoxyphenethylamine	100,000	Negative	Positive
Buprenorphine	100,000	Negative	Positive
Bupropion	25,000	Negative	Positive
Butabarbital	100,000	Negative	Positive
Caffeine	500,000	Negative	Positive
Cannabidiol	100,000	Negative	Positive
Cannabinol	100,000	Negative	Positive
Carbamazepine	100,000	Negative	Positive
Carisoprodol	100,000	Negative	Positive
Chlordiazepoxide	100,000	Negative	Positive
Chlorpromazine	100,000	Negative	Positive
Clobazam	100,000	Negative	Positive
Clomipramine	100,000	Negative	Positive
Clonazepam	100,000	Negative	Positive
Cocaine	100,000	Negative	Positive
Codeine	100,000	Negative	Positive
Cotinine	100,000	Negative	Positive
Cyclobenzaprine	100,000	Negative	Positive
Delta-9-THC	100,000	Negative	Positive
Demoxepam	100,000	Negative	Positive
Desakylflurazepam	100,000	Negative	Positive
Desipramine	100,000	Negative	Positive
Dextromethorphan	100,000	Negative	Positive
Diazepam	50,000	Negative	Positive

Dihydrocodeine	100,000	Negative	Positive
Diphenhydramine	500,000	Negative	Positive
Doxepin	100,000	Negative	Positive
Ecgonine	100,000	Negative	Positive
Ecgonine methyl ester	100,000	Negative	Positive
EDDP	100,000	Negative	Positive
1R,2S(-)-Ephedrine	100,000	Negative	Positive
1S,2R(+)-Ephedrine	100,000	Negative	Positive
EtG	100,000	Negative	Positive
Ethylmorphine	100,000	Negative	Positive
Fenfluramine	100,000	Negative	Positive
Fentanyl	100,000	Negative	Positive
Flunitrazepam	100,000	Negative	Positive
Fluoxetine	100,000	Negative	Positive
Flurazepam	100,000	Negative	Positive
Heroin	100,000	Negative	Positive
Hexobarbital	100,000	Negative	Positive
Hydrocodone	100,000	Negative	Positive
Hydromorphone	100,000	Negative	Positive
11-hydroxy-delta-9-THC	100,000	Negative	Positive
Ibuprofen	100,000	Negative	Positive
Imipramine	100,000	Negative	Positive
Ketamine	100,000	Negative	Positive
Lamotrigine	100,000	Negative	Positive
Levorphanol	100,000	Negative	Positive
Lidocaine	100,000	Negative	Positive
Lorazepam	100,000	Negative	Positive
Lorazepam Glucuronide	50,000	Negative	Positive
Lormetazepam	100,000	Negative	Positive
LSD	100,000	Negative	Positive
Maprotiline	100,000	Negative	Positive
S(+)-MDA	100,000	Negative	Positive
MDEA	100,000	Negative	Positive
MDMA	100,000	Negative	Positive
Meperidine	100,000	Negative	Positive
Meprobamate	100,000	Negative	Positive
Methadone	500,000	Negative	Positive
S(+)-Methamphetamine	500,000	Negative	Positive
Methaqualone	100,000	Negative	Positive
Methylphenidate	100,000	Negative	Positive
Midazolam	100,000	Negative	Positive
Morphine	100,000	Negative	Positive
Morphine-3 -glucuronide	100,000	Negative	Positive
Morphine-6 -glucuronide	100,000	Negative	Positive
Nalorphine	100,000	Negative	Positive

Naloxone	100,000	Negative	Positive
Naltrexone	100,000	Negative	Positive
Naproxen	100,000	Negative	Positive
N-desmethylnaltrexone	100,000	Negative	Positive
Nitrazepam	100,000	Negative	Positive
Norbuprenorphine	100,000	Negative	Positive
Norcodeine	100,000	Negative	Positive
Nordiazepam	100,000	Negative	Positive
Normorphine	100,000	Negative	Positive
Norpropoxyphene	100,000	Negative	Positive
Norpseudoephedrine	100,000	Negative	Positive
Nortriptyline	100,000	Negative	Positive
Oxazepam	100,000	Negative	Positive
Oxazepam Glucuronide	10,000	Negative	Positive
Oxycodone	100,000	Negative	Positive
Oxymorphone	100,000	Negative	Positive
PCP	100,000	Negative	Positive
Pentazocine	100,000	Negative	Positive
Pentobarbital	100,000	Negative	Positive
Phenobarbital	100,000	Negative	Positive
Phentermine	100,000	Negative	Positive
Phenylephrine	100,000	Negative	Positive
Phenylpropanolamine	100,000	Negative	Positive
Phenytoin	100,000	Negative	Positive
PMA	100,000	Negative	Positive
Propoxyphene	100,000	Negative	Positive
Propranolol	100,000	Negative	Positive
Protriptyline	100,000	Negative	Positive
R,R(-)-Pseudoephedrine	100,000	Negative	Positive
S,S(+)-Pseudoephedrine	100,000	Negative	Positive
Ranitidine	100,000	Negative	Positive
Ritalinic Acid	100,000	Negative	Positive
Salicylic Acid	100,000	Negative	Positive
Secobarbital	100,000	Negative	Positive
Sertraline	100,000	Negative	Positive
Sufentanil Citrate	100,000	Negative	Positive
Temazepam	100,000	Negative	Positive
11-nor-9 carboxy THC	100,000	Negative	Positive
Theophylline	100,000	Negative	Positive
Thioridazine	100,000	Negative	Positive
Trazodone	100,000	Negative	Positive
Triazolam	100,000	Negative	Positive
Trifluoromethylphenyl-piperazine	100,000	Negative	Positive
Trimipramine	100,000	Negative	Positive

Zolpidem Tartrate	100,000	Negative	Positive
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Endogenous Compounds

Potential interference from endogenous compounds was evaluated in the qualitative and semi-quantitative modes, by spiking these compounds at high concentrations in pooled negative urine and in pooled urine spiked with tramadol at $\pm 25\%$ of the cutoff (150 and 250 ng/mL).

Compounds	Concentration tested	-25% Cutoff (150ng/mL)	+25% Cutoff (250ng/mL)
Acetone	1.0 g/dL	Negative	Positive
Ascorbic Acid	1.5 g/dL	Negative	Positive
Bilirubin	0.002 g/dL	Negative	Positive
Boric Acid	1% w/v	Negative	Negative
Creatinine	0.5 g/dL	Negative	Positive
Ethanol	1.0 g/dL	Negative	Positive
Galactose	0.01 g/dL	Negative	Positive
γ -Globulin	0.5 g/dL	Negative	Positive
Glucose	2.0 g/dL	Negative	Positive
Hemoglobin	0.300 g/dL	Negative	Positive
Human Serum Albumin	0.5 g/dL	Negative	Positive
Oxalic Acid	0.1 g/dL	Negative	Positive
Riboflavin	0.0075 g/dL	Negative	Positive
Sodium Azide	1% w/v	Negative	Positive
Sodium Chloride	6.0 g/dL	Negative	Positive
Sodium Flouride	1% w/v	Negative	Positive
Urea	6.0 g/dL	Negative	Positive

Compounds that showed interference was further evaluated by spiking it in pooled urine spiked with tramadol at $\pm 50\%$ of the cutoff (100 and 300 ng/mL). Boric Acid at 1% w/v caused a false negative response for tramadol at +25% and also at +50% of the cutoff.

The following statement is listed under the Limitations section of the labeling, “*Boric Acid at 1% w/v may cause false negative results. Boric Acid is not recommended as a preservative for urine.*”

pH and Specific Gravity

For potential interference from the pH of urine, device performance in the qualitative and semi-quantitative modes was tested using a range of urine pH values (3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0). All test samples were prepared in drug free urine containing Tramadol at $\pm 25\%$ of the cutoff (150 ng/mL and 250 ng/mL tramadol concentrations). No positive or negative interference was observed at urine pH values ranging from 3.0 to 11.0 for each test mode.

For potential interference from the specific gravity of urine, device performance in

the qualitative and semi-quantitative modes was tested using a range of urine specific gravity values (1.000, 1.002, 1.005, 1.010, 1.015, 1.020, 1.025 and 1.030). All test samples were prepared in drug free urine containing Tramadol at $\pm 25\%$ of the cutoff (150 ng/mL and 250 ng/mL tramadol concentrations). No positive or negative interference was observed at urine specific gravity values ranging from 1.000 to 1.030 for each test mode.

f. Assay cut-off:

Characterization of how the device performs analytically around the claimed cutoff concentration of 200 ng/mL tramadol is described in the precision section, M.1.a. above.

2. Comparison studies:

a. Method comparison with predicate device:

A total 150 unaltered urine samples from clinical testing laboratories were analyzed by the candidate device in the qualitative and semi-quantitative modes on the Beckman Coulter AU 400e clinical chemistry analyzer and the comparative mass spectrometry based quantitative method (LC/MS). The results from the study are summarized below:

Qualitative Mode

Candidate Device Results	<50% of cutoff concentration by LC/MS (< 100ng/mL)	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration by LC/MS) (100 ~ 199 ng/mL)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration by LC/MS) (200 ~ 300 ng/mL)	High Positive (Greater than 50% above the cutoff concentration by LC/MS) > 300 ng/mL
Positive	0	0	10	90
Negative	45	5	0	0

% Agreement among positives is 100%.

% Agreement among negatives is 100%.

Semi-quantitative Mode

Candidate Device Results	<50% of cutoff concentration by LC/MS (< 100ng/mL)	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration by LC/MS) (100 ~ 199 ng/mL)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration by LC/MS) (200 ~ 300 ng/mL)	High Positive (Greater than 50% above the cutoff concentration by LC/MS) > 300 ng/mL
Positive	0	0	10	90

Negative	45	5	0	0
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% Agreement among positives is 100%.

% Agreement among negatives is 100%.

b. Matrix comparison:

Not applicable. Urine is the only claimed matrix for the candidate device.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable.

b. Clinical specificity:

Not applicable.

c. Other clinical supportive data (when a. and b. are not applicable):

Literature was provided to support a screening cut-off of 200 ng/mL for tramadol in urine.

Melanson, S.E.F., Ptolemy, A.S., and Wasan, A.D. Optimizing Urine Drug Testing for Monitoring Medication Compliance in Pain Management. Pain Medicine 2013; 14: 1813 -1820.

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

Not applicable.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.