DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

February 12, 2015

IMMUNALYSIS CORPORATION
JOSEPH GINETE
REGULATORY AFFAIRS SPECIALIST
829 TOWNE CENTER DR.
POMONA CA 91767

Re: K141803
Trade/Device Name: Immunalysis Tramadol Urine Enzyme Immunoassay,
Immunalysis Tramadol Urine Controls,
Immunalysis Tramadol Urine Calibrators
Regulation Number: 21 CFR 862.3650
Regulation Name: Opiate test system
Regulatory Class: II
Product Code: DJG, DLJ, LAS
Dated: December 30, 2014
Received: January 2, 2015

Dear Mr. Joseph Ginete:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the

electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Katherine Serrano -A

For: Courtney H. Lias, Ph.D.
Director
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K141803

Device Name
Immunalysis Tramadol Urine Enzyme Immunoassay, Immunalysis Tramadol Urine Controls and Calibrators

Indications for Use *(Describe)*

Immunalysis Tramadol Urine Enzyme Immunoassay:
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.

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

Immunalysis Tramadol Urine Calibrators:
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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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IMMUNALYSIS

510(k) SUMMARY

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CFR 807.92(c).

A. Contact Information

1. Manufacturer:	Immunalysis Corporation
2. Contact Name:	Joseph Ginete
3. Contact Title:	Regulatory Affairs Specialist
4. Address:	829 Towne Center Drive Pomona, CA 91767
5. Phone:	(909) 482-0840
6. Fax:	(909) 482-0850
7. Email:	jginete@immunalysis.com
8. Summary prepared on:	February 02, 2015

B. Device Information

1. Trade Name:	Immunalysis Tramadol Urine Enzyme Immunoassay
	Immunalysis Tramadol Urine Controls
	Immunalysis Tramadol Urine Calibrators
2. Common Name:	Immunalysis Tramadol Urine Enzyme Immunoassay
	Immunalysis Tramadol Urine Controls
	Immunalysis Tramadol Urine Calibrators

C. Regulatory Information

1. Device Classification:	Class II
	Class I, reserved
2. Regulation Number:	CFR 862.3650 Opiate Test System
	CFR 862.3200 Clinical Toxicology Calibrator
	CFR 862.3280 Clinical Toxicology Control Materials
3. Panel:	Toxicology(91)
4. Product Code:	DJG
	DLJ
	LAS

D. Legally Marketed Device to Which We are Claiming Equivalence (807.92(A)(3))

1. Predicate Device:	LZI Opiate 2000 Enzyme Immunoassay
	LZI Opiate 2000 Enzyme Controls
	LZI Opiate 2000 Enzyme Calibrators
2. Predicate Company:	Lin-Zhi International Inc.
3. Predicate K Number:	K120761

E. Device Description

The assay consists of antibody/ substrate reagent and enzyme conjugate reagent. The 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. The 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. Reagents are liquid, ready to use

The tramadol calibrator and controls consists of a single calibrator at 200ng/mL, a control set containing a LOW control at 150ng/mL and a HIGH control at 250ng/mL and a calibrator set containing 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.

F. Intended Use

Immunalysis Tramadol Urine Enzyme Immunoassay:

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.

Immunalysis Tramadol Urine Controls:

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

Immunalysis Tramadol Urine Calibrators:

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.

G. Comparison of the new device with the predicate device

Item	Predicate Device (K120761)	Test Device
Intended Use	For the qualitative and semi-quantitative determination of the presence of opiates in human urine at a cutoff of 2000ng/ml	For the qualitative and semi-quantitative determination of the presence of tramadol in human urine at a cutoff of 200ng/ml
Type of Product	Analytical Reagents	Analytical Reagents
Measured Analytes	Opiates	Tramadol
Test Matrix	Urine	Urine
Cutoff Levels	2000ng/mL of Opiates	200ng/mL of Tramadol
Test System	Enzyme Immunoassay	Homogenous Enzyme Immunoassay
Materials	R1 antibody reagent and R2 enzyme reagent	Antibody/ Substrate Reagents and Enzyme Labeled Conjugate
Mass Spectroscopy Confirmation	Required for preliminary positive analytical results	Required for preliminary positive analytical results
Antibody	Mouse monoclonal anti-morphine derivative	Goat Polyclonal Antibody to Tramadol
Storage	2 – 8°C until expiration date	2 – 8°C until expiration date
Calibrator Form	Liquid	Liquid
Calibrator Levels	One (1) Level (2000ng/mL)	One (1) Level (200ng/mL)
Control Set Levels	Two (2) Levels (1500ng/mL and 2500ng/mL)	Two (2) Levels (150ng/mL and 250ng/mL)
Calibrator Set Levels	Five (5) Levels (0, 1000, 2000, 4000 and 6000 ng/mL)	Five (5) Levels (0, 100, 200, 500 and 1000 ng/mL)

H. Test Principle

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

I. The following laboratory performance studies were performed to determine substantial equivalence of the Immunalysis Tramadol Urine Enzyme Immunoassay to the predicate

1. Precision/ Cutoff Characterization – Study was performed for 20 days, 2 runs per day in duplicate (N=80) on concentration of ±25%, ±50%, ±75% and ±100% of the cutoff. The study verified that the cutoff serves as a boundary between a negative and positive interpretation of a qualitative result. In addition, it also verified the product performance relative to the ability of the device to produce the same value during repeated measurements. The instrument used for this test was a Beckman Coulter AU 400e.

a. The following is a summary table of the Qualitative Analysis for the 200ng/mL cutoff test data results.

Qualitative Analysis (for 200ng/mL cutoff)			
Concentration (ng/mL)	% of cutoff	# of determinations	Result
0	-100%	80	80 Negative
50	-75%	80	80 Negative
100	-50%	80	80 Negative
150	-25%	80	80 Negative
200	Cutoff	80	44 Negative/36 Positive
250	+25%	80	80 Positive
300	+50%	80	80 Positive
350	+75%	80	80 Positive
400	+100%	80	80 Positive

b. The following is a summary table of the Semi-Quantitative Analysis for the 200ng/mL cutoff test data results.

Semi-Quantitative Analysis (for 200ng/mL cutoff)			
Concentration (ng/mL)	% of cutoff	# of determinations	Result
0	-100%	80	80 Negative
50	-75%	80	80 Negative
100	-50%	80	80 Negative
150	-25%	80	80 Negative
200	Cutoff	80	47 Negative/ 33 Positive
250	+25%	80	80 Positive
300	+50%	80	80 Positive
350	+75%	80	80 Positive
400	+100%	80	80 Positive

2. Specificity and Cross-Reactivity – Structurally similar compounds were spiked into drug free urine at levels that will yield a result that is equivalent to the cutoff. The study verified assay performance relative to the ability of the device to exclusively determine certain drugs. The instrument used for this test was a Beckman Coulter AU 400e.
   a. The qualitative result summary table is outlined below:

Structurally Related Compounds – Qualitative			
Compound	Concentration Tested (ng/mL)	Result	Cross-Reactivity (%)
Tramadol	200	N/A	100.00
n-Desmethyl Tramadol	450	POS	44.4
o-Desmethyl Tramadol	25,000	POS	0.8
Venlafaxine	100,000	NEG	N.D.
o-Desmethyl Venlafaxine	100,000	NEG	N.D.

N.D. = Not Detected (<0.05%)

   b. The semi-quantitative result summary table is outlined below:

Structurally Related Compounds – Semi-Quantitative		
Compound	Concentration Tested (ng/mL)	Cross-Reactivity (%)
Tramadol	200	100.00
n-Desmethyl Tramadol	450	44.4
o-Desmethyl Tramadol	25,000	0.8
Venlafaxine	100,000	N.D.
o-Desmethyl Venlafaxine	100,000	N.D.

3. Interference – Structurally non-similar compounds, endogenous compounds, the effect of pH and the effect of specific gravity was evaluated by spiking the potential interferent into drug free urine containing the target analyte at ±25% of the cutoff. Boric Acid caused a false negative response at the concentration

tested. All other potential interferents analyzed verified that assay performance is unaffected by externally ingested compounds or an internally existing physiological condition. The instrument used for this test was a Beckman Coulter AU 400e.

a. The following is a summary table of the structurally non-similar compounds for the 200ng/mL cutoff

Structurally Non-Similar Compounds (for 200ng/mL cutoff)					
Compound	Concentration Tested (ng/mL)	**-25% Cutoff (150ng/mL)**		**+25% Cutoff (250ng/mL)**	
		Result	Interference?	Result	Interference?
6-Acetylcodeine	100,000	Negative	No	Positive	No
6-Acetylmorphine	100,000	Negative	No	Positive	No
7-Aminoclonazepam	100,000	Negative	No	Positive	No
7-Aminoflunitrazepam	100,000	Negative	No	Positive	No
7-Aminonitrazepam	100,000	Negative	No	Positive	No
Acetaminophen	500,000	Negative	No	Positive	No
Acetylsalicyclic Acid	500,000	Negative	No	Positive	No
Alprazolam	50,000	Negative	No	Positive	No
Amitriptyline	100,000	Negative	No	Positive	No
Amobarbital	100,000	Negative	No	Positive	No
S-(+) Amphetamine	100,000	Negative	No	Positive	No
Benzoylecgonine	500,000	Negative	No	Positive	No
Benzylpiperazine	100,000	Negative	No	Positive	No
Bromazepam	100,000	Negative	No	Positive	No
4-Bromo-2,5,Dimethoxyphenethylamine	100,000	Negative	No	Positive	No
Buprenorphine	100,000	Negative	No	Positive	No
Bupropion	25,000	Negative	No	Positive	No
Butabarbital	100,000	Negative	No	Positive	No
Caffeine	500,000	Negative	No	Positive	No
Cannabidiol	100,000	Negative	No	Positive	No
Cannabinol	100,000	Negative	No	Positive	No
Carbamazeprine	100,000	Negative	No	Positive	No
Carisoprodol	100,000	Negative	No	Positive	No
Chlordiazepoxide	100,000	Negative	No	Positive	No
Chlorpromazine	100,000	Negative	No	Positive	No
Clobazam	100,000	Negative	No	Positive	No
Clomipramine	100,000	Negative	No	Positive	No
Clonazepam	100,000	Negative	No	Positive	No
Cocaine	100,000	Negative	No	Positive	No
Codeine	100,000	Negative	No	Positive	No
Cotinine	100,000	Negative	No	Positive	No
Cyclobenzaprine	100,000	Negative	No	Positive	No
Delta-9-THC	100,000	Negative	No	Positive	No
Demoxepam	100,000	Negative	No	Positive	No
Desakylflurazepam	100,000	Negative	No	Positive	No
Desipramine	100,000	Negative	No	Positive	No
Dextromethorphan	100,000	Negative	No	Positive	No
Diazepam	50,000	Negative	No	Positive	No
Dihydrocodeine	100,000	Negative	No	Positive	No
Diphenhydramine	500,000	Negative	No	Positive	No

Structurally Non-Similar Compounds (for 200ng/mL cutoff)					
Compound	Concentration Tested (ng/mL)	**-25% Cutoff (150ng/mL)**		**+25% Cutoff (250ng/mL)**	
		Result	Interference?	Result	Interference?
Doxepin	100,000	Negative	No	Positive	No
Ecgonine	100,000	Negative	No	Positive	No
Ecgonine methyl ester	100,000	Negative	No	Positive	No
EDDP	100,000	Negative	No	Positive	No
1R,2S(-)-Ephedrine	100,000	Negative	No	Positive	No
1S,2R(+)-Ephedrine	100,000	Negative	No	Positive	No
EtG	100,000	Negative	No	Positive	No
Ethylmorphine	100,000	Negative	No	Positive	No
Fenfluramine	100,000	Negative	No	Positive	No
Fentanyl	100,000	Negative	No	Positive	No
Flunitrazepam	100,000	Negative	No	Positive	No
Fluoxetine	100,000	Negative	No	Positive	No
Flurazepam	100,000	Negative	No	Positive	No
Heroin	100,000	Negative	No	Positive	No
Hexobarbital	100,000	Negative	No	Positive	No
Hydrocodone	100,000	Negative	No	Positive	No
Hydromorphone	100,000	Negative	No	Positive	No
11-hydroxy-delta-9-THC	100,000	Negative	No	Positive	No
Ibuprofen	100,000	Negative	No	Positive	No
Imipramine	100,000	Negative	No	Positive	No
Ketamine	100,000	Negative	No	Positive	No
Lamotrigine	100,000	Negative	No	Positive	No
Levorphanol	100,000	Negative	No	Positive	No
Lidocaine	100,000	Negative	No	Positive	No
Lorazepam	100,000	Negative	No	Positive	No
Lorazepam Glucuronide	50,000	Negative	No	Positive	No
Lormetazepam	100,000	Negative	No	Positive	No
LSD	100,000	Negative	No	Positive	No
Maprotiline	100,000	Negative	No	Positive	No
S(+)-MDA	100,000	Negative	No	Positive	No
MDEA	100,000	Negative	No	Positive	No
MDMA	100,000	Negative	No	Positive	No
Meperidine	100,000	Negative	No	Positive	No
Meprobamate	100,000	Negative	No	Positive	No
Methadone	500,000	Negative	No	Positive	No
S(+)-Methamphetamine	500,000	Negative	No	Positive	No
Methaquolone	100,000	Negative	No	Positive	No
Methylphenidate	100,000	Negative	No	Positive	No
Midazolam	100,000	Negative	No	Positive	No
Morphine	100,000	Negative	No	Positive	No
Morphine-3 -glucuronide	100,000	Negative	No	Positive	No
Morphine-6 -glucuronide	100,000	Negative	No	Positive	No
Nalorphine	100,000	Negative	No	Positive	No
Naloxone	100,000	Negative	No	Positive	No
Naltrexone	100,000	Negative	No	Positive	No
Naproxen	100,000	Negative	No	Positive	No

Structurally Non-Similar Compounds (for 200ng/mL cutoff)					
Compound	Concentration Tested (ng/mL)	**-25% Cutoff (150ng/mL)**		**+25% Cutoff (250ng/mL)**	
		Result	Interference?	Result	Interference?
N-desmethyltapentadol	100,000	Negative	No	Positive	No
Nitrazepam	100,000	Negative	No	Positive	No
Norbuprenorphine	100,000	Negative	No	Positive	No
Norcodeine	100,000	Negative	No	Positive	No
Nordiazepam	100,000	Negative	No	Positive	No
Normorphine	100,000	Negative	No	Positive	No
Norpropoxyphene	100,000	Negative	No	Positive	No
Norpseudoephedrine	100,000	Negative	No	Positive	No
Nortriptyline	100,000	Negative	No	Positive	No
Oxazepam	100,000	Negative	No	Positive	No
Oxazepam Glucuronide	10,000	Negative	No	Positive	No
Oxycodone	100,000	Negative	No	Positive	No
Oxymorphone	100,000	Negative	No	Positive	No
PCP	100,000	Negative	No	Positive	No
Pentazocine	100,000	Negative	No	Positive	No
Pentobarbital	100,000	Negative	No	Positive	No
Phenobarbital	100,000	Negative	No	Positive	No
Phentermine	100,000	Negative	No	Positive	No
Phenylephrine	100,000	Negative	No	Positive	No
Phenylpropanolamine	100,000	Negative	No	Positive	No
Phenytoin	100,000	Negative	No	Positive	No
PMA	100,000	Negative	No	Positive	No
Propoxyphene	100,000	Negative	No	Positive	No
Propranolol	100,000	Negative	No	Positive	No
Protriptyline	100,000	Negative	No	Positive	No
R,R(-)-Pseudoephedrine	100,000	Negative	No	Positive	No
S,S(+)-Pseudoephedrine	100,000	Negative	No	Positive	No
Ranitidine	100,000	Negative	No	Positive	No
Ritalinic Acid	100,000	Negative	No	Positive	No
Salicylic Acid	100,000	Negative	No	Positive	No
Secobarbital	100,000	Negative	No	Positive	No
Sertraline	100,000	Negative	No	Positive	No
Sufentanil Citrate	100,000	Negative	No	Positive	No
Temazepam	100,000	Negative	No	Positive	No
11-nor-9 carboxy THC	100,000	Negative	No	Positive	No
Theophylline	100,000	Negative	No	Positive	No
Thioridazine	100,000	Negative	No	Positive	No
Trazodone	100,000	Negative	No	Positive	No
Triazolam	100,000	Negative	No	Positive	No
Trifluoromethylphenyl-piperazine	100,000	Negative	No	Positive	No
Trimipramine	100,000	Negative	No	Positive	No
Zolpidem Tartrate	100,000	Negative	No	Positive	No

b. The following is a summary table of the endogenous compounds results for the 200ng/mL cutoff

Endogenous Compounds (for 200ng/mL cutoff)					
Compound	Concentration Tested (ng/mL)	**-25% Cutoff (150ng/mL)**		**+25% Cutoff (250ng/mL)**	
		Result	Interference?	Result	Interference?
Acetone	1.0 g/dL	Negative	No	Positive	No
Ascorbic Acid	1.5 g/dL	Negative	No	Positive	No
Bilirubin	0.002 g/dL	Negative	No	Positive	No
Boric Acid	**1% w/v**	Negative	No	**Negative**	**Yes**
Creatinine	0.5 g/dL	Negative	No	Positive	No
Ethanol	1.0 g/dL	Negative	No	Positive	No
Galactose	0.01 g/dL	Negative	No	Positive	No
γ-Globulin	0.5 g/dL	Negative	No	Positive	No
Glucose	2.0 g/dL	Negative	No	Positive	No
Hemoglobin	0.300 g/dL	Negative	No	Positive	No
Human Serum Albumin	0.5 g/dL	Negative	No	Positive	No
Oxalic Acid	0.1 g/dL	Negative	No	Positive	No
Riboflavin	0.0075 g/dL	Negative	No	Positive	No
Sodium Azide	1% w/v	Negative	No	Positive	No
Sodium Chloride	6.0 g/dL	Negative	No	Positive	No
Sodium Flouride	1% w/v	Negative	No	Positive	No
Urea	6.0 g/dL	Negative	No	Positive	No

c. Boric Acid interferes with the assay and the limitation has been added to the labeling regarding this compound.

d. The following is a summary table of the effect of pH results for the 200ng/mL cutoff

Effect of pH (for 200ng/mL cutoff)					
Test Parameter	Value	**-25% Cutoff (150ng/mL)**		**+25% Cutoff (250ng/mL)**	
		Result	Interference?	Result	Interference?
pH	3.0	Negative	No	Positive	No
pH	4.0	Negative	No	Positive	No
pH	5.0	Negative	No	Positive	No
pH	6.0	Negative	No	Positive	No
pH	7.0	Negative	No	Positive	No
pH	8.0	Negative	No	Positive	No
pH	9.0	Negative	No	Positive	No
pH	10.0	Negative	No	Positive	No
pH	11.0	Negative	No	Positive	No

e. The following is a summary table of the effect of specific gravity result for the 200ng/mL cutoff:

Effect of Specific Gravity (for 200ng/mL cutoff)					
Test Parameter	Value	**-25% Cutoff (150ng/mL)**		**+25% Cutoff (250ng/mL)**	
		Result	Interference?	Result	Interference?
Specific Gravity	1.000	Negative	No	Positive	No
Specific Gravity	1.002	Negative	No	Positive	No
Specific Gravity	1.005	Negative	No	Positive	No
Specific Gravity	1.010	Negative	No	Positive	No
Specific Gravity	1.015	Negative	No	Positive	No
Specific Gravity	1.020	Negative	No	Positive	No
Specific Gravity	1.025	Negative	No	Positive	No
Specific Gravity	1.030	Negative	No	Positive	No

4. Linearity/ Recovery– A drug free urine pool was spiked with a high concentration of the target analyte as a high value specimen. Additional pools were made by serially diluting the high value specimen. The study verified assay linearity in the semi-quantitative mode. The instrument used for this test was a Beckman Coulter AU 400e.
    a. Summary results are listed in the following table:

Linearity/ Recovery		
Expected Concentration (ng/mL)	Mean Concentration (ng/mL)	Recovery (%)
0	1	N/A
50	51	101
100	95	95
200	201	100
300	330	110
400	426	107
500	512	102
600	647	108
700	770	110
800	853	107
900	911	101
1000	944	94
1100	1059	96

5. Method Comparison – Unaltered, anonymous and discarded clinical urine samples obtained from clinical testing laboratories were analyzed with the test device. The study verified that the product performance can be verified by Mass Spectrometry. The instrument used for this test was a Beckman Coulter AU 400e and an Agilent 6430 Liquid Chromatography Tandem Mass Spectrometry.
    a. The following is a comparison table of qualitative assay performance for the 200ng/mL cutoff

		LC/MS Confirmation	
		(+)	(-)
Test Device	(+)	100	0
	(-)	0	50

    b. The following is a summary table of qualitative assay performance for the 200ng/mL cutoff

Assay Performance verified by LC/MS – 200ng/mL Cutoff					
Type	Tramadol Concentration				Agreement (%)
	< 100ng/mL	100 ~ 199 ng/mL	200 ~ 300 ng/mL	> 300 ng/mL	
Qualitative/ Positive	0	0	10	90	100%
Qualitative/ Negative	45	5	0	0	100%

    c. The following is a comparison table of semi-quantitative assay performance for the 200ng/mL cutoff

		LC/MS Confirmation	
		(+)	(-)
Test Device	(+)	100	0
	(-)	0	50

    d. The following is a summary table of semi-quantitative assay performance for the 200ng/mL cutoff

Assay Performance verified by LC/MS – 200ng/mL Cutoff					
Type	Tramadol Concentration				Agreement (%)
	< 100ng/mL	100 ~ 199 ng/mL	200 ~ 300 ng/mL	> 300 ng/mL	
Semi-Quantitative/ Positive	0	0	10	90	100%
Semi-Quantitative / Negative	45	5	0	0	100%

6. Stability –
    a. A closed accelerated stability study was performed on reagents, calibrators and controls at 25°C to establish the initial expiration dating. The stability study supported an initial expiration date of 1 year for

reagents. This stability study supported an initial expiration date of 12 months for calibrators and controls. The instrument used for this test was a Beckman Coulter AU 400e.

1. The following is a summary of the qualitative stability data. The 0 and 150ng/mL levels were negative in comparison to the 200ng/mL cutoff for Day 0, 2, 8, 16, 24, 32 and 40. The 250ng/mL level was positive in comparison to the 200ng/mL cutoff for Day 0, 2, 8, 16, 24, 32 and 40. This accelerated stability study was performed to establish initial expiration dating. Real time stability studies are ongoing.
2. The following is a summary of the semi-quantitative stabililty data for the 200ng/mL cutoff. The 150ng/mL level was negative in comparison to the 200ng/mL cutoff for Day 0, 2, 8, 16, 24, 32 and 40. The 250ng/mL level was positive in comparison to the 200ng/mL cutoff for Day 0, 2, 8, 16, 24, 32 and 40. This accelerated stability study was performed to establish initial expiration dating. Real time stability studies are ongoing.

b. An open/ on-board stability study was performed on reagents to establish expiration dating when reagents are opened and stored on board the instrument at 2°C to 8°C. The stability study supported an initial open vial expiration date of 28 days. The instrument used for this test was a Beckman Coulter AU 400e.

1. The following is a summary of the qualitative open/ on-board stability data for the 200ng/mL cutoff. All replicates for the 150ng/mL level were negative in comparison to the 200ng/mL cutoff for Day 0, 7, 14, 21 and 28. All replicates of the 250ng/mL level were positive in comparison to the 200ng/mL cutoff for Day 0, 7, 14, 21 and 28.
2. The following is a summary of the semi-quantitative open/ on-board stability data for the 200ng/mL cutoff. The mean of the replicates for the 150ng/mL level were negative in comparison to the 200ng/mL cutoff for Day 0, 7, 14, 21 and 28. The mean of the replicates of the 250ng/mL level were positive in comparison to the 200ng/mL cutoff for Day 0, 7, 14, 21 and 28.

c. A Specimen and Storage Handling study was performed on a specimen below the cutoff near the bracketing control and a specimen above the cutoff near the bracketing control to establish specimen storage and handling stability at 2 – 8°C. The stability study supported a 1 month specimen storage and handling recommendation at 2 – 8°C.

1. The following is a summary of the Mass Spectrometry data. The specimen below the cutoff was negative in comparison to the 200ng/mL cutoff for Day 0, Week 1, 2, 3 and 4. The specimen above the cutoff was positive in comparison to the 200ng/mL cutoff for Day 0, Week 1, 2, 3 and 4.

7. Calibrator and Control Traceability – all components of the calibrator and controls have been traced to a commercially available standard solution from Cerilliant Chemicals.
8. Calibrator and Control Stability - An open accelerated stability study was performed at 37°C to establish the initial open vial expiration dating. The stability study supported an initial open vial expiration date of 6 months. The instrument used for this test was a Beckman Coulter AU 400e. All calibrator levels (100, 200, 500 and 1000ng/mL) and control levels (150 and 250ng/mL) were within specifications for Day 0, 3, 7, 10 and 13. This accelerated stability study was

performed to establish initial expiration dating. Real time stability studies are ongoing.

9. Calibrator and Control Value Assignment – calibrators and controls are manufactured and are tested by mass spectrometry. If any of the analytes are out of the acceptable range, then the calibrator or control is adjusted and re-tested. Values are assigned to the calibrator and controls once the Mass spectrometry results are within the acceptable ranges.

J. Conclusion

The information provided in this pre-market notification demonstrates that the Immunalysis Tramadol Urine Enzyme Immunoassay is substantially equivalent to the legally marketed predicate device for its general intended use.