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

A. 510(k) Number:

k151771

B. Purpose for Submission:

New device

C. Measurand:

Benzodiazepines

D. Type of Test:

Qualitative and semi-quantitative homogeneous enzyme immunoassay

E. Applicant:

Immunalysis Corporation

F. Proprietary and Established Names:

Immunalysis Benzodiazepines Urine Enzyme Immunoassay
Immunalysis Multi-Drug Calibrators

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
JXM – Benzodiazepine test system	Class II	21 CFR §862.3170	91 – Toxicology
DKB – Clinical Toxicology Calibrator	Class II	21 CFR §862.3200	91 – Toxicology

H. Intended Use:

1. Intended use(s):

   See Indication(s) for use below.

2. Indication(s) for use:

Immunalysis Benzodiazepine Urine Enzyme Immunoassay

The Immunalysis Benzodiazepine 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 Benzodiazepine in human urine with automated clinical chemistry analyzers. This assay is calibrated against Oxazepam. This in-vitro 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 Gas Chromatography/ Mass Spectrometry (GC-MS) or permitting laboratories to establish quality control procedures.

The Immunalysis Benzodiazepine 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. GC-MS or Liquid Chromatography / Mass Spectrometry (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 Multi-Drug Calibrators

The Immunalysis Multi-Drug Calibrators are intended for in vitro diagnostic use for the calibration of assays for the analytes currently listed in the package insert: Benzoylecgonine, Morphine and Oxazepam. The calibrators are designed for prescription use with immunoassays.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   Performance data was obtained using the Beckman AU400e clinical chemistry analyzer.

I. Device Description:

The Immunalysis Benzodiazepine Urine Enzyme Immunoassay Kit contains two reagents, which are provided as ready-to-use:

- Antibody/Substrate Reagent (RA) – This reagent contains monoclonal antibodies to Benzodiazepines, glucose-6-phosphate (G6P) and nicotinamide adenine dinucleotide (NAD) in Tris buffer with Sodium Azide as a preservative.
- Enzyme Conjugate Reagent (RE) – This reagent contains benzodiazepines derivative labeled with glucose-6-phosphate dehydrogenase (G6PDH) in HEPES buffer with Sodium Azide as a preservative.

The Immunalysis Multi-Drug Calibrators set is liquid and ready-to-use. The negative calibrator is a processed, drug-free synthetic urine matrix with sodium azide as a preservative. Each calibrator level contains a known concentration of oxazepam spiked into the negative calibrator matrix.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   DRI® Benzodiazepines Assay
   LZI Multiple Analyte Drugs of Abuse Calibrators and Controls

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

   k930529
   k051088

3. Comparison with predicate:

Similarities		
Item	Candidate Device	Predicate – k930529
Intended Use	Same	For the qualitative and semiquantitative determination of the presence of Benzodiazepines in human urine at a cutoff of 200 ng/mL
Test Principle	Same	Homogeneous Enzyme Immunoassay
Cutoff	Same	200 ng/mL
Reagent Form	Same	Liquid Ready-to-Use Two Reagent Assay
Reagent Storage	Same	2 – 8° C

Differences		
Item	Candidate Device	Predicate – k930529
Antibody	Monoclonal antibodies to Benzodiazepines	Sheep polyclonal antibody to Benzodiazepine

Similarities		
Item	Candidate Device	Predicate – k051088
Analyte	Benzoylecgonine, morphine, oxazepam	Benzoylecgonine, d-methamphetamine, methadone, morphine, oxazepam, secobarbital, phencyclidine and propoxyphene
Matrix	Same	Urine
Calibrator Levels	Same	5 levels
Storage	Same	2 – 8 °C

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

- CLSI EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition
- CLSI EP7-A2, Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition

L. Test Principle:

The assay is based on the competition of Oxazepam 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:
   A precision/cutoff characterization study was performed for 20 days, 2 runs per day in duplicate (N=80) on drug-free negative urine samples spiked with oxazepam to concentrations of ±25%, ±50%, ±75%, and ±100% of each cutoff. The spiked concentrations were confirmed by mass spectrometry (MS). The results of the study are summarized below:

Qualitative Analysis			
Concentration (ng/mL)	% of cutoff	# of determinations	Result
0	-100%	80	80 Neg / 0 Pos
50	-75%	80	80 Neg / 0 Pos
100	-50%	80	80 Neg / 0 Pos
150	-25%	80	80 Neg / 0 Pos
200	Cutoff	80	37 Neg / 43 Pos
250	+25%	80	80 Pos / 0 Neg
300	+50%	80	80 Pos / 0 Neg
350	+75%	80	80 Pos / 0 Neg
400	+100%	80	80 Pos / 0 Neg

Semi-quantitative Analysis			
Concentration (ng/mL)	% of cutoff	# of determinations	Result
0	-100%	80	80 Neg / 0 Pos
50	-75%	80	80 Neg / 0 Pos
100	-50%	80	80 Neg / 0 Pos
150	-25%	80	80 Neg / 0 Pos
200	Cutoff	80	34 Neg / 46 Pos
150	+25%	80	80 Pos / 0 Neg
300	+50%	80	80 Pos / 0 Neg
350	+75%	80	80 Pos / 0 Neg
400	+100%	80	80 Pos / 0 Neg

b. Linearity/assay reportable range:

A linearity study in the semi-quantitative mode was conducted by spiking a drug-free urine pool with a high concentration of oxazepam and generating serial dilutions to achieve concentrations ranging from 100 ng/mL to 1100 ng/mL. Each concentration was tested in triplicate and drug recovery calculated using the mean concentration of the replicates. The results are summarized below:

Linearity/Recovery		
Expected Concentration (ng/mL)	Mean Concentration (ng/mL)	Recovery (%)
100	94.2	94.2
200	213.7	106.8
300	313.9	104.6
400	389.7	97.4
500	511.6	102.3
600	637.2	106.2
700	693.2	99.0
800	820.7	102.6

900	907.6	100.8
1000	1025.3	102.5
1100	1061.1	96.5

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

Traceability

The analyte in the calibrator is traceable to a commercially available oxazepam solution. This standard is diluted with synthetic negative urine to make the calibrator s to the desired concentrations. The concentrations are confirmed by Gas Chromatography/Mass Spectrometry Analysis (GC/MS) and/or Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).

Value Assignment/Expected Values

The Negative Calibrator is a processed, drug free urine matrix. The standard is compared to a reference negative standard to ensure that it is free of analyte. A value is assigned when the test is within the acceptable range.

The non-zero calibrators are prepared by spiking known concentrations of oxazepam into the negative calibrator matrix. The concentrations are confirmed by GC/MS or LC/MS/MS. If any of the analytes are out of the acceptable range, then the calibrator is adjusted and re-tested. Values are assigned to the calibrator once the GC/MS or LC/MS/MS results are within the acceptable range.

Calibrator Stability

Closed-vial accelerated stability and open vial stability studies were conducted for the calibrators. Real-time stability studies are ongoing. All stability protocols were reviewed and found to be acceptable. These studies support the closed vial stability claim of 12 months and the opened vial stability claim of 60 days when calibrators are stored at 2-8°C.

d. Detection limit:

Not applicable.

e. Analytical specificity:

Structurally related compounds

The sponsor performed cross-reactivity studies in both qualitative and semi-quantitative modes by spiking various benzodiazepines or structurally related

compounds into drug free urine at levels that will yield a result that is equivalent to the assay cutoff (200 ng/mL). The results were the same for the qualitative and semi-quantitative modes and are summarized below:

Structurally Related Compounds Qualitative & Semi-quantitative		
Compound	**Lowest Concentration Producing a Positive Result (ng/mL)**	**% Cross-reactivity**
Oxazepam	200	100.0
Alpha-hydroxyalprazolam	110	181.8
Alprazolam	120	166.7
7-Aminoclonazepam	100,000	< 0.002
7-Aminoflunitrazepam	3,500	5.7
7-Aminonitrazepam	35,000	0.6
Bromazepam	750	26.7
Chlordiazepoxide	1,600	12.5
Clorazepate	200	100.0
Clobazam	650	30.8
Clonazepam	180	111.1
Demoxepam	5,500	3.6
Desalkyflurazepam	75	266.7
Diazepam	100	200.0
Estazolam	225	88.9
Flunitrazepam	125	160.0
Flurazepam	110	181.8
Lorazepam	60	333.3
Lorazepam glucuronide	180	111.1
Lormetazepam	50	400.0
Medazepam	500	40.0
Midazolam	40	500.0
Nitrazepam	700	28.6
Norchlordiazepoxide	2,200	9.1
Nordiazepam	180	111.1
Oxazepam glucuronide	1,300	15.4
Prazepam	95	210.5
Temazepam	110	181.8
Temazepam glucuronide	700	28.6
Triazolam	50	400.0

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 into drug free urine containing oxazepam at ± 25% of the 200 ng/mL cutoff (150 ng/mL and 250 ng/mL respectively). The results were the same for the qualitative and semi-quantitative modes and are summarized below:

Structurally Unrelated Compounds			
Compound	**Concentration Tested (ng/mL)**	**-25% Cutoff (150 ng/mL)**	**+25% Cutoff (250 ng/mL)**
4-Bromo-2,5,Dimethoxyphenethylamine	100,000	Negative	Positive
6-Acetylcodeine	100,000	Negative	Positive
6-Acetylmorphine	100,000	Negative	Positive
Acetaminophen	500,000	Negative	Positive
Acetylsalicylic Acid	500,000	Negative	Positive
Amitriptyline	100,000	Negative	Positive
Amobarbital	100,000	Negative	Positive
S-(+) Amphetamine	100,000	Negative	Positive
Benzoylecgonine	500,000	Negative	Positive
Benzylpiperazine	100,000	Negative	Positive
Buprenorphine	100,000	Negative	Positive
Bupropion	100,000	Negative	Positive
Butabarbital	100,000	Negative	Positive
Caffeine	500,000	Negative	Positive
Carbamazepine	100,000	Negative	Positive
Chlorpromazine	100,000	Negative	Positive
cis-Tramadol	100,000	Negative	Positive
Clomipramine	100,000	Negative	Positive
Cannabidiol	100,000	Negative	Positive
Cannabinol	100,000	Negative	Positive
Carisoprodol	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
Desipramine	100,000	Negative	Positive
N-desmethyltapentadol	100,000	Negative	Positive

Structurally Unrelated Compounds			
Compound	**Concentration Tested (ng/mL)**	**-25% Cutoff (150 ng/mL)**	**+25% Cutoff (250 ng/mL)**
Dextromethorphan	100,000	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
Ethyl β-D-glucuronide	100,000	Negative	Positive
Ethylmorphine	100,000	Negative	Positive
Fenfluramine	100,000	Negative	Positive
Fentanyl	100,000	Negative	Positive
Fluoxetine	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 Tartrate	100,000	Negative	Positive
Lidocaine	100,000	Negative	Positive
LSD	100,000	Negative	Positive
Maprotiline	100,000	Negative	Positive
(+)-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	100,000	Negative	Positive
Methaquolone	100,000	Negative	Positive
Methylphenidate	100,000	Negative	Positive

Structurally Unrelated Compounds			
Compound	**Concentration Tested (ng/mL)**	**-25% Cutoff (150 ng/mL)**	**+25% Cutoff (250 ng/mL)**
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
Norbuprenorphine	100,000	Negative	Positive
Norcodeine	100,000	Negative	Positive
Normorphine	100,000	Negative	Positive
Norpropoxyphene	100,000	Negative	Positive
Norpseudoephedrine	100,000	Negative	Positive
Nortriptyline	100,000	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
Phentermine	100,000	Negative	Positive
Phenobarbital	100,000	Negative	Positive
Phenylephedrine	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
11-nor-9carboxy THC	100,000	Negative	Positive
Theophylline	100,000	Negative	Positive

Structurally Unrelated Compounds			
Compound	**Concentration Tested (ng/mL)**	**-25% Cutoff (150 ng/mL)**	**+25% Cutoff (250 ng/mL)**
Thioridazine	100,000	Negative	Positive
Trifluoromethylphenyl-piperazine	100,000	Negative	Positive
Trimipramine	100,000	Negative	Positive
Trazodone	100,000	Negative	Positive
Venlafaxine	100,000	Negative	Positive
Zolpidem Tartrate	100,000	Negative	Positive

Endogenous compounds

Potential interference from endogenous compounds was evaluated in the qualitative and semi-quantitative modes by spiking these compounds into drug free urine containing oxazepam at ± 25% of the 200 ng/mL cutoff (150 ng/mL and 250 ng/mL, respectively). The results were the same for the qualitative and semi-quantitative modes and are summarized below:

Endogenous Compounds			
Compound	**Concentration Tested (ng/mL)**	**-25% Cutoff (150 ng/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
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	0.115 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 Fluoride	1% w/v	Negative	Positive
Urea	6.0 g/dL	Negative	Positive

Boric Acid. Boric Acid was also evaluated. Boric Acid at a concentration of 1% w/v was found to cause false negative results at +25% and +50% (250 ng/mL and 300 ng/mL, respectively) at the 200 ng/mL cutoff in both the qualitative and semiquantitative modes and the following statement is provided in 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

To evaluate 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 oxazepam at ± 25% of the 200 ng/mL cutoff (150 ng/mL and 250 ng/mL, respectively). No positive or negative interference was observed at urine pH values ranging from 3.0 to 11.0 for each test mode.

To evaluate 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 oxazepam at ± 25% of the 200 ng/mL cutoff (150 ng/mL and 250 ng/mL, respectively). 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 concentrations of 200 ng/mL is described in the precision section, M.1.a. above.

2. Comparison studies:

a. Method comparison with predicate device:

Eighty-six unaltered urine samples from clinical testing laboratories were analyzed for Benzodiazepine by the candidate device on the Beckman Coulter AU400e clinical chemistry analyzer and with LC/MS. The results were identical in both qualitative and semi-quantitative modes and are summarized below:

Candidate Device Results	**<50% cutoff (< 100 ng/mL)**	**-50% to cutoff (100 ~ 199 ng/mL)**	**cutoff to +50% (200 ~ 300 ng/mL)**	**>50% cutoff (> 300 ng/mL)**
Positive	0	0	4	38
Negative	36	7	1*	0

*sample contained 187 ng/mL Nordiazepam, which is equivalent to 207.8 ng/mL of Oxazepam (the calibrator drug).

% agreement among positives is 98%
%agreement among negatives is 100%

b. Matrix comparison:

Not applicable.

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):

Not applicable.

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.