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

A. 510(k) Number:

k161714

B. Purpose for Submission:

New device

C. Measurand:

Barbiturates

D. Type of Test:

Homogenous Enzyme Immunoassay, Qualitative and Semi-quantitative.

E. Applicant:

Immunalysis Corporation

F. Proprietary and Established Names:

Immunalysis Barbiturates Urine Enzyme Immunoassay
Immunalysis Multi-Drug Calibrators

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.3150 Barbiturate test system
   21 CFR 862.3200, Clinical toxicology calibrator

2. Classification:

   Class II

3. Product code:

   DIS
   DKB

4. Panel:

   Toxicology (91)

H. Intended Use:

1. Intended use(s):

   Refer to Indications for Use below

2. Indication(s) for use:

   Immunalysis Barbiturates Urine Enzyme Immunoassay

   The Immunalysis Barbiturates Urine Enzyme Immunoassay is a homogeneous enzyme immunoassay with a cutoff of 200 ng/mL. The assay is intended for use in laboratories for the qualitative and semi-quantitative analysis of Barbiturates in human urine with automated clinical chemistry analyzers. This assay is calibrated against Secobarbital. 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 Gas Chromatography/ Mass Spectrometry (GC-MS) or Liquid Chromatography/ Tandem Mass Spectrometry (LC-MS/MS) or permitting laboratories to establish quality control procedures.

   The Immunalysis Barbiturates Urine Enzyme Immunoassay 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 LC-MS/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.

   lmmunalysis Multi-Drug Calibrators:

   The lmmunalysis Multi-Drug Calibrators are intended for in vitro diagnostic use for the calibration of assays for the following analytes: Benzoylecgonine, Methamphetamine, Morphine, PCP, Secobarbital 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 Barbiturates Urine Enzyme Immunoassay Kit reagents are provided as ready to use liquids and consist of the following reagents:

- The Antibody/ Substrate Reagent (Reagent A; 25, 100, or 500 mL) contains a recombinant antibody to Secobarbital, a mouse monoclonal antibody to Secobarbital, glucose-6-phosphate (G6P) and nicotinamide adenine dinucleotide (NAD) in HEPES buffer with Sodium Azide as a preservative.
- The Enzyme Conjugate Reagent (Reagent E; 25, 100, or 500 mL) contains glucose-6-phosphate dehydrogenase (G6PDH) labeled with Barbiturates in HEPES buffer with Sodium Azide as a preservative.

Immunalysis Multi-Drug Calibrators are provided separately, as five individual, liquid ready-to use drug levels (15 mL or 25 mL).

J. Substantial Equivalence Information:

1. Predicate device name(s):

   DRI Barbiturates EIA Assay

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

   k955928

3. Comparison with predicate:

Similarities - Reagent		
Item	Candidate Device – Immunalysis Barbiturates Urine Enzyme Immunoassay	Predicate Device – DRI Barbiturates EIA Assay k955928
Intended Use	Same	For the qualitative and semiquantitative determination of the presence of Barbiturates in human urine
Assay cutoff	Same	200 ng/mL
Calibrator compound	Same	Secobarbital
Assay type	Same	Homogeneous enzyme immunoassay, qualitative and semi-quantitative
Storage conditions	Same	2° - 8° C

Differences - Reagent		
Item	Candidate Device – Immunalysis Barbiturates Urine Enzyme Immunoassay	Predicate Device – DRI Barbiturates EIA Assay k955928
Calibrators	Provided separately	Provided with assay
Controls	Provided separately	Provided with assay
Antibody type	Recombinant and mouse monoclonal	Mouse monoclonal

Similarities - Calibrators		
Item	Candidate Device – Immunalysis Multi-Drug Calibrators (MDC)	Predicate Device – Calibrators included with DRI Barbiturates EIA Assay k955928
Calibrator drug	Same	Secobarbital

Differences - Calibrators		
Item	Candidate Device – Immunalysis Negative and Multi-Drug Calibrators (MDC)	Predicate Device – Calibrators included with DRI Barbiturates EIA Assay k955928
Calibrator Levels	Calibrator levels at 0 100, 200, 500 and 1000 ng/mL	Calibrator levels at 0, 200, and 1000 ng/mL

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

- CLSI EP5-A3: Evaluation of Precision Performance of Quantitative Measurement Methods
- CLSI EP 7-A3: Interference Testing in Clinical Chemistry.
- ISO 14971 Second edition 2007-03-01, Medical devices – application of risk management to medical devices
- EN ISO 14971:2012 Medical devices. Application of risk management to medical devices

L. Test Principle:

This assay uses a recombinant and a mouse monoclonal antibody, both directed against Secobarbital. The assay is based on the competition of Barbiturates 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 and enzyme activity. The enzyme G6PDH activity is determined at 340 nm spectrophotometrically by the conversion of Nicotinamide Adenine Dinucleotide (NAD) to NADH.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

A precision/cutoff characterization study was performed over 20 days, with two runs per day in duplicate (n=80) on calibrators (100 and 200 ng/mL), controls (150 and 250 ng/mL) and drug-free negative urine samples spiked with secobarbital to concentrations of 50, 300, 350, and 400 ng/mL. The spiked concentrations were confirmed by mass spectrometry (MS). The concentrations and 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	33 neg / 47 pos
250	+25%	80	0 neg / 80 pos
300	+50%	80	0 neg / 80 pos
350	+75%	80	0 neg / 80 pos
400	+100%	80	0 neg / 80 pos

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	23 neg / 57 pos
250	+25%	80	0 neg / 80 pos
300	+50%	80	0 neg / 80 pos
350	+75%	80	0 neg / 80 pos
400	+100%	80	0 neg / 80 pos

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 Secobarbital and generating serial dilutions to

achieve concentrations ranging from 0 ng/mL to 1100 ng/mL. The 0 ng/mL sample utilized drug free urine and was not achieved through serial dilution. 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 (%)
0	-0.6	n/a
100	93.2	93.2
200	196.0	98.0
300	314.7	104.9
400	418.1	104.5
500	487.9	97.6
600	604.1	100.7
700	724.2	103.5
800	819.3	102.4
900	891.2	99.0
1000	968.7	96.9
1100	981.8	89.3

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

Traceability

The secobarbital in the calibrator is traceable to a commercially available secobarbital solution. This standard is diluted with synthetic negative urine to make the calibrators at 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

Calibrators are manufactured and tested by mass spectrometry. The negative calibrator is a processed, drug free urine matrix. The negative calibrator is compared to a reference negative standard to ensure that it is free of analyte. The non-zero calibrators are prepared by spiking a known concentration of secobarbital into a negative calibrator matrix. If any of the analytes are not within the acceptable range, as measured by mass spectrometry,then the calibrator is adjusted and re-tested. Values are assigned to the calibrators once the mass spectrometry results are within the acceptable ranges.

Calibrator Stability

Stability protocols and acceptance criteria for the calibrators were reviewed and found to be acceptable. The sponsor claims that when stored at 2 – 8^{o} C calibrators

and controls are stable for one year. The sponsor claims that once opened, the calibrators and controls are stable for 60 days when 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 barbiturates 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:

Compound	Concentration Tested (ng/mL)	Cross-Reactivity (%)
Secobarbital	200	100
Allobarbital	690	29.0
Alphenal	190	105.3
Amobarbital	200	100.0
Aprobarbital	700	28.6
Barbital	9,000	2.2
Butabarbital	510	39.2
Butalbital	290	69.0
Butobarbital	190	105.3
Cyclopentobarbital	200	100.0
Hexobarbital	70,000	0.3
Mephobarbital	65,000	0.3
Pentobarbital	420	47.6
Phenobarbital	460	43.5
Phenytoin	100,000	<0.2
Talbutal	220	90.9
Thiopental	3,700	5.4

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 secobarbital 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 (for 200 ng/mL cutoff)			
Compound	Concentration Tested (ng/mL)	**-25% Cutoff (150 ng/mL)**	**+25% Cutoff (250 ng/mL)**
		Result	Result
4-Bromo-2,5,Dimethoxyphenethylamine	100,000	Negative	Positive
Acetaminophen	500,000	Negative	Positive
Acetylsalicyclic Acid	100,000	Negative	Positive
6-Acetylcodeine	100,000	Negative	Positive
6-Acetylmorphine	100,000	Negative	Positive
Alprazolam	100,000	Negative	Positive
7-Aminoclonazepam	100,000	Negative	Positive
7-Aminoflunitrazepam	100,000	Negative	Positive
7-Aminonitrazepam	100,000	Negative	Positive
Amitriptyline	100,000	Negative	Positive
S-(+) Amphetamine	100,000	Negative	Positive
Benzylpiperazine	100,000	Negative	Positive
Bromazepam	100,000	Negative	Positive
Buprenorphrine	100,000	Negative	Positive
Bupropion	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
cis-Tramadol	100,000	Negative	Positive
Clobazam	100,000	Negative	Positive
Clomipramine	100,000	Negative	Positive
Clonazepam	100,000	Negative	Positive
Clozapine	100,000	Negative	Positive
Codeine	100,000	Negative	Positive
Cotinine	100,000	Negative	Positive
Cyclobenzaprine	100,000	Negative	Positive
Demoxepam	100,000	Negative	Positive
Desalkyflurazepam	100,000	Negative	Positive

Structurally Unrelated Compounds (for 200 ng/mL cutoff)			
Compound	Concentration Tested (ng/mL)	**-25% Cutoff (150 ng/mL)**	**+25% Cutoff (250 ng/mL)**
		Result	Result
Desipramine	100,000	Negative	Positive
Dextromethorphan	100,000	Negative	Positive
Diazepam	100,000	Negative	Positive
Digoxin	100,000	Negative	Positive
Dihydrocodeine	100,000	Negative	Positive
Diphenhydramine	500,000	Negative	Positive
Dehydronorketamine	25,000	Negative	Positive
Delta-9-THC	100,000	Negative	Positive
Doxepin	100,000	Negative	Positive
EDDP	100,000	Negative	Positive
EMDP	100,000	Negative	Positive
1R,2S(-)-Ephedrine	100,000	Negative	Positive
1S,2R(+)-Ephedrine	100,000	Negative	Positive
Ethyl glucuronide	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
Haloperidol	100,000	Negative	Positive
Heroin	100,000	Negative	Positive
Hydrocodone	100,000	Negative	Positive
Hydromorphone	100,000	Negative	Positive
11-hydroxy-delta-9-THC	100,000	Negative	Positive
Ibuprofen	500,000	Negative	Positive
Imipramine	100,000	Negative	Positive
Ketamine	100,000	Negative	Positive
Lamotrignine	100,000	Negative	Positive
Levorphanol tartrate	100,000	Negative	Positive
Lidocaine	100,000	Negative	Positive
Lorazepam	100,000	Negative	Positive

Structurally Unrelated Compounds (for 200 ng/mL cutoff)			
Compound	Concentration Tested (ng/mL)	**-25% Cutoff (150 ng/mL)**	**+25% Cutoff (250 ng/mL)**
		Result	Result
Lorazepam Glucuronide	50,000	Negative	Positive
Lormetazepam	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
S(+)-Methamphetamine	100,000	Negative	Positive
Methadone	500,000	Negative	Positive
Methaquolone	100,000	Negative	Positive
Methoxetamine	100,000	Negative	Positive
Methylone	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	50,000	Negative	Positive
N-desmethyltapentadol	100,000	Negative	Positive
Nalorphine	100,000	Negative	Positive
Naloxone	100,000	Negative	Positive
Naltrexone	100,000	Negative	Positive
Naproxen	100,000	Negative	Positive
Nitrazepam	100,000	Negative	Positive
11-nor-9 carboxy THC	100,000	Negative	Positive
Norbuprenorphine	50,000	Negative	Positive
Norcodeine	100,000	Negative	Positive

Structurally Unrelated Compounds (for 200 ng/mL cutoff)			
Compound	Concentration Tested (ng/mL)	**-25% Cutoff (150 ng/mL)**	**+25% Cutoff (250 ng/mL)**
		Result	Result
Nordiazepam	100,000	Negative	Positive
Norketamine	100,000	Negative	Positive
Normorphine	100,000	Negative	Positive
Norpropoxyphene	100,000	Negative	Positive
Norpseudoephedrine	100,000	Negative	Positive
Nortriptyline	100,000	Negative	Positive
Olanzapine	100,000	Negative	Positive
Secobarbital	100,000	Negative	Positive
Oxycodone	100,000	Negative	Positive
Oxymorphone	100,000	Negative	Positive
PCP	100,000	Negative	Positive
Pentazocine	100,000	Negative	Positive
Phentermine	100,000	Negative	Positive
Phenylephedrine	100,000	Negative	Positive
Phenylpropanolamine	100,000	Negative	Positive
PMA	100,000	Negative	Positive
Prazepam	100,000	Negative	Positive
Proproxyphene	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	500,000	Negative	Positive
Sertraline	100,000	Negative	Positive
Sufentanil Citrate	50,000	Negative	Positive
Tapentadol	100,000	Negative	Positive
Temazepam	100,000	Negative	Positive
Theophylline	100,000	Negative	Positive
Thioridazine	100,000	Negative	Positive
Triazolam	100,000	Negative	Positive

Structurally Unrelated Compounds (for 200 ng/mL cutoff)			
Compound	Concentration Tested (ng/mL)	**-25% Cutoff (150 ng/mL)**	**+25% Cutoff (250 ng/mL)**
		Result	Result
Trifluoromethylphenyl-piperazine	100,000	Negative	Positive
Trimipramine	100,000	Negative	Positive
Trazodone	100,000	Negative	Positive
Venlafaxine	100,000	Negative	Positive
Verapamil	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 secobarbital 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:

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, and at a concentration of 1% w/v was found to cause false negative results at both the +25% and +50% (250 ng/mL and 300

ng/mL, respectively) at the 200 ng/mL cutoff in both the qualitative and semiquantitative modes. 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 secobarbital 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 the qualitative and semi-quantitative modes.

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 secobarbital 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 the qualitative and semi-quantitative modes.

f. Assay cut-off:

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

2. Comparison studies:

a. Method comparison with predicate device:

The method comparison study was performed in-house using unaltered, clinical urine samples obtained from clinical testing laboratories. A total of 96 samples were analyzed. Each sample was run in singlicate on a Beckman Coulter AU400e Chemistry Analyzer and the result was compared to that obtained by liquid chromatography/mass spectroscopy (LC/MS-MS). The results were the same for the qualitative and semi-quantitative modes and are summarized below.

Candidate Device Results vs. stratified LC/MS-MS Values

Candidate Device Results	**Negative** by the predicate device or less than half the cutoff concentration by GC/MS analysis	**Near Cutoff Negative** (Between 50% below the cutoff and the cutoff concentration)	**Near Cutoff Positive** (Between the cutoff and 50% above the cutoff concentration)	**High Positive** (greater than 50% above the cutoff concentration)
Positive	0	0	8	44
Negative	36	8	0	0

% Agreement among positives is 52/52 = 100%
% Agreement among negatives is 44/44 = 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):

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.