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

A. 510(k) Number:

k143500

B. Purpose for Submission:

New device

C. Measurand:

Amphetamine

D. Type of Test:

Homogenous enzyme immunoassay

E. Applicant:

Immunalysis Corporation

F. Proprietary and Established Names:

Immunalysis Amphetamine Urine Enzyme Immunoassay
Immunalysis Amphetamine Urine Calibrators
Immunalysis Amphetamine Urine Controls

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
DKZ	Class II	21 CFR 862.3100, Amphetamine test system	Toxicology (91)
DLJ	Class II	21 CFR 862.3200, Clinical toxicology calibrator	Toxicology (91)
LAS	Class 1, reserved	21 CFR 862.3280, Clinical toxicology control material	Toxicology (91)

H. Intended Use:

1. Intended use(s):

Refer to Indications for Use below.

2. Indication(s) for use:

The Immunalysis Amphetamine Urine Enzyme Immunoassay Kit is a homogeneous enzyme immunoassay with dual cutoffs of 500 ng/mL and 1000 ng/mL. The assay is intended for use in laboratories for the qualitative and semi-quantitative analysis of Amphetamine in human urine with automated clinical chemistry analyzers. This assay is calibrated against Amphetamine. 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 GC-MS or permitting laboratories to establish quality control procedures.

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

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

3. Special conditions for use statement(s):

For prescription use only.
For in vitro diagnostic use only.

4. Special instrument requirements:

The Beckman Coulter AU400e Chemistry Analyzer was used to generate the performance data in this submission. Instruments must be capable of maintaining a constant reaction temperature, pipetting samples and reagents, mixing reagents, timing reactions and measuring enzyme rates precisely.

I. Device Description:

The assay consists of antibody/substrate reagent and enzyme conjugate reagent. The

antibody/substrate reagent includes monoclonal mouse antibodies to Amphetamine, glucose-6-phosphate (G6P) and nicotinamide adenine dinucleotide (NAD) in Tris buffer with Sodium Azide as a preservative. The enzyme conjugate reagent contains glucose-6-phosphate dehydrogenase (G6PDH) labeled with Amphetamine in Tris buffer with Sodium Azide as a preservative.

Calibrators and control materials are prepared by mixing a commercially available standard solution of Amphetamine with BSA (Bovine Serum Albumin) to the desired concentrations. The negative standard is prepared with BSA Buffer and is compared to a negative reference standard to ensure that it is free of analyte.

The following calibrators and controls are available:

500 ng/mL Cutoff	
Level	Concentration (ng/mL)
LOW Control	375
Calibrator Level 1	500
HIGH Control	625
Calibrator Level 2	1000
Calibrator Level 3	1500
Calibrator Level 4	2000

1000 ng/mL Cutoff	
Level	Concentration (ng/mL)
LOW Control	750
Calibrator Level 1	500
HIGH Control	1250
Calibrator Level 2	1000
Calibrator Level 3	1500
Calibrator Level 4	2000

All reagents, calibrators, and controls are in a liquid, ready to use form.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Ortho-Clinical Diagnostics VITROS Chemistry Products AMPH Reagent, Calibrators, and Performance Verifiers

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

   k062077

3. Comparison with predicate:

Similarities - Reagent		
Item	Device	Predicate (k062077)
Intended Use	Same	For the qualitative and semiquantitative determination of the presence of amphetamine
Cutoffs	Same	500 and 1000 ng/mL
Matrix	Same	Urine
Methodology	Same	Homogenous Enzyme Immunoassay
Storage	Same	2 – 8° C until expiration date
Reagent Form	Same	Liquid, ready to use

Differences - Reagent		
Item	Device	Predicate
Antibodies	Mouse monoclonal antibody to Amphetamine	Mouse Monoclonal antibodies to Amphetamine and Methamphetamine

Similarities - Calibrators		
Item	Device	Predicate (k062077)
Calibrator Form	Refrigerated liquid ready to use	Frozen liquid ready to use

Differences - Calibrators		
Item	Device	Predicate (k062077)
Calibrator Levels	Five	Six
Storage	2 – 8° C	$\leq$ -18^{o} C

Similarities - Controls		
Item	Device	Predicate (k062077)
Storage	Same	2 – 8° C

Differences - Controls		
Item	Device	Predicate (k062077)
Control Levels	Four	Five

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 Amphetamine labeled enzyme glucose-6-phosphate dehydrogenase (G6PDH) and the free drug in the urine sample for a 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:

The sponsor performed precision studies in-house following the guidelines provided in CLSI EP5-A2. The study was performed using drug free urine samples spiked with amphetamine and analyzed on a Beckman Coulter / Olympus AU400e Chemistry Analyzer. Samples were measured in duplicate in two runs per day for 20 days (n = 80). The data are summarized in the following table:

Qualitative analysis (500 ng/mL cutoff)

Concentration (ng/mL)	% of cutoff	Result
0	-100	80 Neg / 0 Pos
125	-75	80 Neg / 0 Pos
250	-50	80 Neg / 0 Pos
375	-25	80 Neg / 0 Pos
500	Cutoff	48 Neg / 32 Pos
625	+25	80 Pos / 0 Neg
750	+50	80 Pos / 0 Neg
875	+75	80 Pos / 0 Neg
1000	+100	80 Pos / 0 Neg

Qualitative analysis (1000 ng/mL cutoff)

Concentration	% of cutoff	Result
0	-100	80 Neg / 0 Pos
250	-75	80 Neg / 0 Pos
500	-50	80 Neg / 0 Pos
750	-25	80 Neg / 0 Pos
1000	Cutoff	47 Neg / 33 Pos
1250	+25	80 Pos / 0 Neg
1500	+50	80 Pos / 0 Neg
1750	+75	80 Pos / 0 Neg
2000	+100	80 Pos / 0 Neg

Semi-quantitative analysis (500 ng/mL cutoff)

Concentration	% of cutoff	Result
0	-100	80 Neg / 0 Pos
125	-75	80 Neg / 0 Pos
250	-50	80 Neg / 0 Pos
375	-25	80 Neg / 0 Pos
500	Cutoff	31 Neg / 49 Pos
625	+25	80 Pos / 0 Neg
750	+50	80 Pos / 0 Neg
875	+75	80 Pos / 0 Neg
1000	+100	80 Pos / 0 Neg

Semi-quantitative analysis (1000 ng/mL cutoff)

Concentration	% of cutoff	Result
0	-100	80 Neg / 0 Pos
250	-75	80 Neg / 0 Pos
500	-50	80 Neg / 0 Pos
750	-25	80 Neg / 0 Pos
1000	Cutoff	36 Neg / 44 Pos
1250	+25	80 Pos / 0 Neg
1500	+50	80 Pos / 0 Neg
1750	+75	80 Pos / 0 Neg
2000	+100	80 Pos / 0 Neg

b. Linearity/assay reportable range:

A drug free urine pool was spiked with a high concentration of Amphetamine at a level above the highest calibrator and was used as the high value specimen. Additional pools were made by serially diluting the high value specimen with drug free urine in increments of approximately 10%. Aliquots from each pool were analyzed in duplicate in the semi-quantitative mode. The results of the study are

summarized below:

Expected concentration (ng/mL)	Measured concentration (ng/mL)	Recovery (%)
200	152.3	76.2
400	367.5	91.9
500	504.4	100.9
600	614.4	102.4
800	856.5	107.1
1000	1026.9	102.7
1200	1171.4	97.6
1400	1357.1	96.9
1600	1461.1	91.3
1800	1714.0	95.2
2000	2046.5	102.3
2200	2256.4	102.6

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

Controls and calibrators are traceable to a commercially available amphetamine standard solution. The amphetamine standard is diluted with BSA (Bovine Serum Albumin) Buffer to make the calibrator and controls to the desired concentrations. Calibrator and control concentrations are confirmed by GC/MS or LC/MS/MS.

Stability protocols and acceptance criteria for the calibrators and controls were reviewed and found to be acceptable. The sponsor claims that when stored at 2 – 8° C calibrators and controls are stable for one year. The sponsor claims that once opened, the calibrators and controls are stable for 6 months when stored at 2 – 8° C.

Values are assigned to the controls and calibrators once the GC/MS or LC/MS/MS results are within the acceptable ranges. The negative standard is prepared with BSA Buffer. The standard is compared to a reference negative standard to ensure that it is free of analyte. The value is assigned when the test is within the acceptable range.

d. *Detection limit:*

Not applicable.

e. *Analytical specificity:*

Potential interference from non-structurally related compounds and endogenous compounds was evaluated in both the qualitative and semi-quantitative modes by following CLSI EP 7-A2: Interference Testing in Clinical Chemistry. Potential interferents were spiked into drug free urine containing Amphetamine at ± 25% of the cutoff (375 ng/mL and 625 ng/mL for the 500 ng/mL cutoff or 750 ng/mL and 1,250

ng/mL for the 1,000 ng/mL cutoff). The following compounds at 100,000 ng/mL were found not to interfere with the assay at either cutoff in qualitative or semi-quantitative mode.

4-Bromo-2,5, Dimethoxyphenethylamine
6-Acetylmorphine
7-Aminoclonazepam
Alprazolam
Amitriptyline
Amobarbital
Benzylpiperazine
Bromazepam
Buprenorphine
Bupropion
Butabarbital
Caffeine
Carbamazepine
Chlorpromazine
Chrlordiazepoxide
Tramadol
Clobazam
Clomipramine
Clonazepam
Cocaine
Codeine
Cyclobenzaprine
N-Desmethyltapentadol
Delta-9-THC
Desipramine
Dextromethorphan
Diazepam
Dihydrocodeine
Doxepin
EDDP
Ethyl β-D-glucuronide
Ethylmorphine
Flunitrazepam
Fluoxetine
Flurazepam
Heroin

Hexobarbital
Hydrocodone
Hydromorphone
11-hydroxy-delta-9-THC
Ibuprofen
Imipramine
Ketamine
Levorphanol Tartrate
Lidocaine
Lorazepam
LSD
Maprotiline
Meperidine
Meprobamate
Methaquolone
Methylphenidate
Morphine
Morphine-6 -glucuronide
Nalorphine
Naloxone
Naltrexone
Nitrazepam
Norbuprenorphine
Norcodeine
Nordiazepam
Normorphine
Norpropoxyphene
Nortriptyline
Oxazepam
Oxycodone
Oxymorphone
PCP
Pentazocine
Pentobarbital
Phenobarbital
Phenytoin

Prazepam
Propranolol
Protriptyline
Ranitidine
Ritalinic Acid
Secobarbial
Sufentanil Citrate
Temazepam
11-nor-9 carboxy THC
Thioridazine
Triazolam
Trifluoromethylphenyl-piperazine
Trimipramine
Venlafaxine

In addition, Acetaminophen, Acetylsalicylic Acid, Benzoylecgonine, and Methadone were found not to interfere at a concentration of 500,000 ng/mL.

The following endogenous substances at the concentrations listed below did not interfere with the assay at either cutoff in qualitative or semi-quantitative mode:

Compound	Concentration Tested
Acetone	1.0 g/dL
Ascorbic Acid	1.5 g/dL
Bilirubin	0.002 g/dL
Creatinine	0.5 g/dL
Ethanol	1.0 g/dL
Galactose	0.01 g / dL
γ-Globulin	0.5 g/dL
Glucose	2.0 g/dL
Hemoglobin	0.150 g/dL
Human Serum Albumin	0.5 g/dL
Oxalic Acid	0.1 g/dL
Riboflavin	0.0075 g/dL
Sodium Azide	1% w/v
Sodium Chloride	6.0 g/dL
Sodium Fluoride	1% w/v
Urea	6.0 g/dL

Boric acid at a concentration of 1% w/v did not interfere with the assay at either cutoff in qualitative or semi-quantitative mode. However, the labeling conservatively recommends that Boric Acid not be used as a preservative for urine samples.

Effect of pH: The sponsor evaluated the effect of pH on the test results using both qualitative and semi-quantitative modes. Drug free urine containing Amphetamine at ± 25% of the cutoff (375 ng/mL and 625 ng/mL for the 500 ng/mL cutoff and 750 ng/mL and 1250 ng/mL for the 1000 ng/mL cutoff) were pH adjusted using hydrochloric acid or sodium hydroxide. pH values of 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0 did not interfere with the test result at either cutoff in qualitative or semi-quantitative mode.

Effect of specific gravity: The sponsor evaluated the effect of specific gravity on the test results using both qualitative and semi-quantitative modes. Drug free urine containing Amphetamine at ± 25% of the cutoff (375 ng/mL and 625 ng/mL for the 500 ng/mL cutoff and 750 ng/mL and 1250 ng/mL for the 1000 ng/mL cutoff) were adjusted using salt or albumin. Specific Gravity values of 1.000, 1.002, 1.005, 1.010, 1.015, 1.020, 1.025 and 1.030 did not interfere with the test result at either cutoff in qualitative or semi-quantitative mode.

Cross reactivity from structurally related compounds was evaluated in the qualitative and semi-quantitative modes by spiking into drug-free urine. Each potential cross-reacting compound was spiked and evaluated independently, and each spiked sample was tested in singlicate. The compounds tested and the concentration approximately equivalent to the 500 and 1000 ng/mL cutoffs are listed below:

500 ng/mL cutoff		
Compound	Concentration Tested (ng/mL)	Cross-Reactivity (%)
(+) Amphetamine	500	100.0
(-) Amphetamine	100,000	0.5
(±) Amphetamine	1,300	38.5
3,4-Methylenedioxyamphetamine (MDA)	1,500	33.3
para-Methoxyamphetamine (PMA)	2,000	25.0
Tyramine	100,000	0.5
3,4-Methylenedioxy-methamphetamine (MDMA)	500,000	0.1
Methylenedioxyethylamphetamine (MDEA)	100,000	0.5
Phenylpropanolamine	500,000	0.1
Phentermine	1,000,000	0.05
(+) Methamphetamine	1,000,000	0.05
(-) Methamphetamine	1,000,000	N.D.
(+) Ephedrine	1,000,000	N.D.
(-) Ephedrine	1,000,000	N.D.
(+) Pseudoephedrine	1,000,000	N.D.
(-) Pseudoephedrine	1,000,000	N.D.
Phenylephrine	1,000,000	N.D.
Diphenylhydramine	1,000,000	N.D.
Fenfluramine	1,000,000	N.D.

1000 ng/mL cutoff		
Compound	Concentration Tested (ng/mL)	Cross-Reactivity (%)

1000 ng/mL cutoff		
(+) Amphetamine	1000	100.0
(-) Amphetamine	200,000	0.5
(±) Amphetamine	2500	40.0
MDA	2000	50.0
PMA	4000	25.0
Tyramine	400,000	0.3
MDMA	1,000,000	N.D.
MDEA	400,000	0.3
Phenylpropanolamine	1,000,000	N.D.
Phentermine	1,000,000	N.D.
(+) Methamphetamine	1,000,000	N.D.
(-) Methamphetamine	1,000,000	N.D.
(+) Ephedrine	1,000,000	N.D.
(-) Ephedrine	1,000,000	N.D.
(+) Pseudoephedrine	1,000,000	N.D.
(-) Pseudoephedrine	1,000,000	N.D.
Phenylephrine	1,000,000	N.D.
Diphenylhydramine	1,000,000	N.D.
Fenfluramine	1,000,000	N.D.

f. Assay cut-off:

Analytical performance of the device around the claimed cutoff 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 80 samples were analyzed using the 500 ng/mL cutoff, and 81 were analyzed using the 1000 ng/mL cutoff. 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). The results of the assay performance compared to LC/MS are summarized below:

500 ng/mL cutoff (n = 80)

Candidate Device Result	Amphetamine Concentration by LC/MS (ng/mL)			
	< 250	250 – 499	500 – 750	> 750
Qualitative / POS	0	0	4	36
Qualitative / NEG	36	4	0	0
Semi-quant / POS	0	0	4	36
Semi-quant / NEG	36	4	0	0

Qualitative agreement among positives = 40 / 40 (100%)
Qualitative agreement among negatives = 40 / 40 (100%)
Semi-quant agreement among positives = 40 / 40 (100%)
Semi-quant agreement among negatives = 40 / 40 (100%)

1000 ng/mL cutoff (n = 81)

Candidate Device Result	Amphetamine Concentration by LC/MS (ng/mL)			
	< 500	500 – 999	1000 – 1500	> 1500
Qualitative / POS	0	0	4	36
Qualitative / NEG	34	6	*1	0
Semi-quant / POS	0	0	4	36
Semi-quant / NEG	34	6	*1	0

Qualitative agreement among positives = 40 / 41 (98%)
Qualitative agreement among negatives = 40 / 40 (100%)
Semi-quant agreement among positives = 40 / 41 (98%)
Semi-quant agreement among negatives = 40 / 40 (100%)

*Discordant sample – 1000 ng/mL cutoff

Candidate Device Result				LC/MS result (ng/mL)
Sample ID	Qualitative result	Semi-quantitative		
		Conc (ng/mL)	Result	
395246ZA	NEG	620.6	NEG	1173

b. Matrix comparison:

Not applicable. This device is intended to be used with urine samples only.

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.