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

A. 510(k) Number:

k181553

B. Purpose for Submission:

New Device

C. Measurand:

Ethyl Alcohol (Ethanol)

D. Type of Test:

Automated quantitative enzymatic assay

E. Applicant:

Immunalysis Corporation

F. Proprietary and Established Names:

Immunalysis Ethyl Alcohol Enzyme Assay

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
DIC	Class II	21 CFR 862.3040, Alcohol Test System	Toxicology (91)

H. Intended Use:

1. Intended use(s):

   Refer to Indications for Use below.

2. Indication(s) for use:

   The Immunalysis Ethyl Alcohol Enzyme Assay is an in vitro diagnostic device for the quantitative analysis of ethyl alcohol (ethanol) in human urine, serum or plasma with automated clinical chemistry analyzers. The measurement of ethanol is used for the

diagnosis and treatment of alcohol intoxication and poisoning. This assay is calibrated against ethyl alcohol. This device is intended for prescription use only.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   Beckman Coulter AU400e Chemistry Analyzer

I. Device Description:

The Immunalysis Ethyl Alcohol Enzyme Assay consists of ready to use reagents on automated clinical chemistry analyzers. The vials of liquid reagents are provided in multiple kit sizes by volume: 25 mL, 60 mL, 100 mL and 500 mL. Each kit box contains the following reagents:

- 1 X Reagent 1 (R1) – This contains Tris buffer with 0.1% Sodium Azide as a preservative.
- 1 X Reagent 2 (R2) – This contains alcohol dehydrogenase (ADH) and nicotinamide adenine dinucleotide (NAD) in Tris buffer with stabilizers and 0.1% Sodium Azide as a preservative.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Lin-Zhi Ethyl Alcohol Enzymatic Assay

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

   k032461

3. Comparison with predicate:

Similarities		
Item	Candidate Device: Immunalysis Ethyl Alcohol Enzyme Assay	Predicate Device: Lin-Zhi Ethyl Alcohol Enzymatic Assay (k032461)
Intended Use	For quantitative analysis of ethyl alcohol in human urine, serum, or plasma.	Same
Test Principle or Method	Enzymatic assay	Same

Similarities		
Item	Candidate Device: Immunalysis Ethyl Alcohol Enzyme Assay	Predicate Device: Lin-Zhi Ethyl Alcohol Enzymatic Assay (k032461)
Sample Type	Urine, serum, or plasma	Same
Intended Use Environment	For prescription use only	Same
Assay Measuring Range	3-550 mg/dL	Same
Reagent Storage	2-8°C until expiration date	Same
Differences		
Item	Candidate Device: Immunalysis Ethyl Alcohol Enzyme Assay	Predicate Device: Lin-Zhi Ethyl Alcohol Enzymatic Assay (k032461)
Analyzer used to collect performance data	Beckman Coulter AU400e analyzer	Hitachi 717 analyzer

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

CLSI EP5-A3: "Evaluation of Precision of Quantitative Measurement Procedures: Approved Guideline-Third Edition"

CLSI EP12-A2: "User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline-Second Edition"

CLSI EP17-A2: "Evaluation of Detection Capability for Clinical Laboratory Measurement"

L. Test Principle:

The Immunalysis Ethanol Alcohol Enzyme Assay technology is based on the oxidation of ethyl alcohol to acetaldehyde by alcohol dehydrogenase (ADH) and nicotinamide adenine dinucleotide (NAD) reduced to NADH resulting in an absorbance change measured spectrophotometrically at 340nm. The concentration of ethanol in the sample is directly proportional to the ADH activity.

M. Performance Characteristics:

1. Analytical performance:

a. Precision/Reproducibility:

The precision studies were performed on drug free urine, serum and plasma spiked with ethanol to concentrations of 25 mg/dL, 75 mg/dL, 125 mg/dL, 150 mg/dL, 175

mg/dL and 200 mg/dL, and calibrators and controls at concentrations of 50 mg/dL, 100 mg/dL and 300 mg/dL. The spiked concentrations were confirmed by gas chromatography. Each sample was run in duplicate twice a day for twenty days for a total of 80 measurements at each concentration. The data are summarized in the following tables:

Precision - Urine

Conc. (mg/dL)	n	Mean Conc. (mg/dL)	Repeatability		Between run		Within -Lab	
			SD	% CV	SD	% CV	SD	% CV
0	80	0.0	0.1	N/A	0.0	N/A	0.1	N/A
25	80	24.5	0.3	1.2	0.3	1.1	0.5	1.8
50	80	49.0	0.4	0.9	0.6	1.2	0.8	1.5
75	80	74.0	0.7	0.9	1.0	1.3	1.1	1.5
100	80	99.6	0.8	0.8	1.6	1.6	1.6	1.6
125	80	121.3	1.2	1.0	1.5	1.2	1.8	1.5
150	80	146.1	1.3	0.9	1.9	1.3	2.4	1.7
175	80	169.1	1.4	0.9	2.0	1.1	2.7	1.6
200	80	193.9	1.8	0.9	2.6	1.3	3.4	1.7
300	80	292.0	2.2	0.8	3.8	1.3	4.6	1.6

Precision - Serum

Conc. (mg/dL)	n	Mean Conc. (mg/dL)	Repeatability		Between run		Within -Lab	
			SD	%CV	SD	%CV	SD	%CV
0	80	0.4	0.2	N/A	0.1	N/A	0.2	N/A
25	80	25.8	0.2	0.9	0.5	2.0	0.8	3.2
50	80	49.3	0.3	0.7	1.0	2.0	1.0	2.0
75	80	75.7	0.4	0.5	1.4	1.8	2.2	3.0
100	80	99.6	0.5	0.5	2.0	2.1	2.1	2.1
125	80	125.9	0.8	0.6	2.4	1.9	3.9	3.1
150	80	148.4	1.0	0.6	2.6	1.7	3.8	2.6
175	80	172.3	1.0	0.6	3.1	1.8	5.1	3.0
200	80	195.2	1.3	0.7	3.4	1.8	4.9	2.5
300	80	290.4	1.4	0.5	5.5	1.9	6.0	2.1

Precision - Plasma

Conc. (mg/dL)	n	Mean Conc. (mg/dL)	Repeatability		Between run		Within -Lab	
			SD	%CV	SD	%CV	SD	%CV
0	80	0.2	0.1	N/A	0.1	N/A	0.1	N/A
25	80	24.3	0.2	0.7	0.2	1.0	1.2	4.9
50	80	49.9	0.3	0.6	0.5	0.9	0.7	1.5
75	80	69.8	0.4	0.5	0.7	1.0	1.8	2.6

100	80	100.5	0.5	0.5	1.0	1.0	1.6	1.6
125	80	118.3	0.6	0.5	1.0	0.9	2.7	2.2
150	80	155.8	0.9	0.6	1.2	0.8	4.1	2.6
175	80	170.6	0.9	0.5	1.5	0.9	3.2	1.9
200	80	197.6	1.1	0.6	1.4	0.7	3.9	2.0
300	80	292.7	2.0	0.7	2.7	0.9	5.2	1.8

b. *Linearity/assay reportable range:*

To evaluate linearity and recovery, a study was conducted on the Beckman Coulter AU400e analyzer by measuring twelve levels of human urine, serum and plasma samples in triplicate, with the mean of the three measurements used to calculate the recovery. Drug-free human urine, serum and plasma were spiked with ethanol (EtOH) to prepare the high pool at approximately 550 mg/dL, which was confirmed by gas chromatography. The remainder of the samples were prepared by intermixing the high pool with a low pool to obtain concentrations across the measuring range. The linearity results are shown below:

Linearity/recovery test – Urine

Expected conc. (mg/dL)	Mean conc. (mg/dL)	%Recovery
0	0.0	n/a
3	3.1	102.2
12	12.0	100.3
72	73.9	102.6
132	132.2	100.2
192	195.4	101.8
252	250.3	99.3
312	308.0	98.7
372	363.0	97.6
432	421.3	97.5
492	484.4	98.5
552	565.8	102.5

Slope	0.996
Intercept	-0.172
r^2	0.999

Linearity/recovery test – Serum

Expected conc. (mg/dL)	Mean conc. (mg/dL)	%Recovery
0	0.0	n/a
3	3.0	101.1
23.2	23.1	99.4
83.2	87.2	104.8
143.2	146.1	102.0

203.2	199.8	98.3
263.2	262.2	99.6
323.2	317.8	98.3
383.2	385.9	100.7
443.2	441.4	99.6
503.2	493.6	98.1
563.2	544.3	96.6

Slope	0.978
Intercept	2.869
r^2	0.999

Linearity/recovery test – Plasma

Expected conc. (mg/dL)	Mean conc. (mg/dL)	%Recovery
0	0.0	n/a
3	3.0	98.9
9.9	10.5	106.1
69.9	69.7	99.8
129.9	127.5	98.2
189.9	188.4	99.2
249.9	242.3	96.9
309.9	299.9	96.8
369.9	351.9	95.1
429.9	410.0	95.4
489.9	470.4	96.0
549.9	547.0	99.5

Slope	0.970
Intercept	0.307
r^2	0.999

The results of the linearity testing confirmed the sponsor's claimed measuring range of 3 – 550 mg/dL

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

Traceability

The controls and calibrators are traceable to the NIST ethanol standard.

d. *Detection limit:*

Limit of Blank (LoB): LoB was evaluated by performing sixty (60) blank

measurements from unaltered drug free negative human urine, serum and plasma. The Limit of blank (LoB) for each matrix was calculated using two lots, and the higher calculated LoB of the two lots was used as the claimed LoB and to calculate the Limit of Detection (LoD). The claimed Limit of Blank (LoB) of the assay is 0.481 mg/dL for urine, 0.668 mg/dL for serum and 0.652 mg/dL for plasma.

Limit of Detection (LoD): Four low level samples of each matrix containing concentrations in the range LoB to 4 x LoB were tested in replicates of five on two reagent lots over three days for a total of 60 replicates per lot. The LoD was calculated according to the formula $LoD = LoB + (Cp \times SD_L)$ where Cp is derived from the 95th percentile of the standard Gaussian distribution and SD_L is the estimated standard deviation. The sponsor claims an LoD of 1.3 mg/dL for urine, 1.7 mg/dL for serum and 1.7 mg/dL for plasma.

Limit of quantitation (LoQ): Four independent samples of each matrix spiked at low concentration of ethanol were measured in two runs in replicates of five for three days on two reagent lots for a total of 60 replicates per lot. The LoQ was calculated based on % CV ≤10% and Bias ≤10% and was determined to be 2.9 mg/dL for urine, serum and plasma.

e. *Analytical specificity:*

1. Interference studies were performed in accordance to CLSI EP07-A2. Test solutions for each compound were prepared by spiking the potential interfering compound into drug-free negative urine containing ethanol concentrations of 10 mg/dL and 100 mg/dL. The following substances at were tested at the indicated concentrations, and none of these compounds demonstrated significant interference (defined by the sponsor as ≥10% bias).

Non-Interfering Structurally Unrelated Compounds in Urine:

Compound	Compound Conc. (mg/dL)
Acetaminophen	500,000
6-Acetylcodeine	100,000
6-Acetylmorphine	100,000
Acetylsalicylic Acid	500,000
Alphenal	100,000
Alprazolam	100,000
7-Aminoclonazepam	40,000
7-Aminoflurnitrazepam	100,000
7-Aminonitrazepam	100,000
Amitriptyline	100,000
Amobarbital	100,000
S-(+)-Amphetamine	100,000
Aprobarbital	100,000
Barbital	100,000

Compound	Compound Conc. (mg/dL)
Benzoylecgonine	100,000
Benzylpiperzine	100,000
Bromazepam	100,000
4-bromo 2-5, dimethoxyphenethylamine	100,000
Buprenorphine	50,000
Bupropion	100,000
Butabarbital	100,000
Butalbital	100,000
Caffeine	500,000
Cannabidiol	100,000
Cannabinol	100,000
Carbamazpine	100,000
Carisoprodol	100,000
Chlordiazepoxide	100,000
Chlorpromazine	100,000
Clobazam	100,000
Clomipramine	100,000
Clonazepam	100,000
Clozapine	100,000
Cocaine	100,000
Codeine	100,000
Cotinine	100,000
Cyclobenzaprine	100,000
Cyclopentobarbital	100,000
Demoxepam	100,000
Deslkylflurazepam	100,000
Desipramine	100,000
Dextromethorphan	100,000
Diazepam	100,000
Digoxin	100,000
Dihydrocodeine	100,000
Diphenhydramine	500,000
Dehydronorketamine	40,000
Delta 9 THC	100,000
Doxepin	100,000
Doxylamine	100,000
ecgonine	100,000
ecgonine methyl ester	100,000
EDDP	100,000
EMDP	100,000
1R,2S Ephedrine	100,000
1S,2R Ephendrine	100,000
Ethyl-β-D-Glucuronide	50,000

Compound	Compound Conc. (mg/dL)
Ethylmorphine	100,000
Fenfluramine	100,000
Fentanyl	100,000
Flunitrazepam	100,000
Fluoxetine	100,000
Flurazepam	100,000
Haloperidol	100,000
Heroin	100,000
Hexobarbital	100,000
hydrocodone	100,000
hydromorphone	100,000
11-hydroxy-delta9 THC	100,000
Ibuprofen	100,000
Imipramine	100,000
Ketamine	100,000
Lamotrignine	100,000
Levorphanol Tartrate	100,000
Lidocaine	100,000
Lorazepam	100,000
Lorazepam Glucuronide	50,000
Lormetrazepam	100,000
LSD	100,000
Maprotinline	100,000
MDA	100,000
MDEA	100,000
MDMA	100,000
Meperidine	100,000
Meprobamate	100,000
Methamphetamine	100,000
Methadone	500,000
Methaquolone	100,000
Methoxetamine	100,000
Methylone	100,000
Methylphenidate	100,000
Midazolam	100,000
Morphine	100,000
Morphine-3-glucuronide	100,000
Morphine-6-glucuronide	100,000
N-Desmethyltapentadol	100,000
Nalorphine	100,000
Naloxone	100,000
Naltrexone	100,000
Naproxen	100,000
Nitrazepam	100,000

Compound	Compound Conc. (mg/dL)
11-nor-9-carboxy-delta9-THC	100,000
Norbuprenorphine	50,000
Norcodeine	100,000
Nordiazepam	100,000
Norketamine	100,000
Normorphine	100,000
Norproxyphene	100,000
Norpseudoephedrine	50,000
Nortripyline	100,000
N-desmethyltramadol	100,000
N-desmethylvenlafaxine	100,000
O-desmethyltramadol	100,000
O-desmethylvenlafaxine	100,000
Olanzapine	100,000
Oxycodone	100,000
Oxymorphone	100,000
Oxazepam	100,000
PCP	100,000
Pentazocine	100,000
Pentobarbital	100,000
Phenazepam	100,000
Phenobarbital	100,000
Phentermine	100,000
Phenylephedrine	100,000
Phenylpropanolamine	100,000
Phenytoin	100,000
PMA	100,000
Prazepam	100,000
Propranolol	100,000
Propoxyphene	100,000
Protripyline	100,000
R,R Psuedoephedrine	100,000
S,S Psuedoephedrine	100,000
Ritalinic Acid	100,000
Salicylic Acid	100,000
Sertaraline	100,000
Sufentanil Citrate	50,000
Talbutal	50,000
Tapentadol	100,000
Temazepam	100,000
Theophylline	100,000
Thiopental	100,000
Thiordazine	100,000
Tramadol	100,000

Compound	Compound Conc. (mg/dL)
Trazadone	100,000
Triazolam	100,000
Trifluoromethylphenyl-piperazine	100,000
Trimipramine	100,000
Venlafaxine	100,000
Verapamil	100,000
Zolpidem Tartrate	100,000
O-desmethylvenlafaxine	100,000
Olanzapine	100,000
Oxycodone	100,000
Oxymorphone	100,000
Oxazepam	100,000
PCP	100,000
Pentazocine	100,000
Pentobarbital	100,000
Phenazepam	100,000
Phenobarbital	100,000
Phentermine	100,000
Phenylephedrine	100,000
Phenylpropanolamine	100,000
Phenytoin	100,000
PMA	100,000
Prazepam	100,000
Propranolol	100,000
Propoxyphene	100,000
Protripyline	100,000
R,R Psuedoephedrine	100,000
S,S Psuedoephedrine	100,000
Ritalinic Acid	100,000
Salicylic Acid	100,000
Sertaraline	100,000
Sufentanil Citrate	50,000
Talbutal	50,000
Tapentadol	100,000
Temazepam	100,000
Theophylline	100,000
Thiopental	100,000
Thiordazine	100,000
Tramadol	100,000
Trazadone	100,000
Triazolam	100,000
Trifluoromethylphenyl-piperazine	100,000
Trimipramine	100,000
Venlafaxine	100,000

Compound	Compound Conc. (mg/dL)
Verapamil	100,000
Zolpidem Tartrate	100,000

Non-Interfering Endogenous Compounds and Urine Preservatives

Compound	10 mg/dL and 100 mg/dL
	Compound Conc.
Ascorbic Acid	1.5 g/dL
Bilirubin	0.02 g/dL
Creatinine	0.5 g/dL
Galactose	0.01 g/dL
γ-Globulin	0.5 g/dL
Glucose	2.0 g/dL
Hemoglobin	0.3 g/dL
Human Serum Albumin	0.5 g/dL
Oxalic Acid	0.1 g/dL
Riboflavin	0.0075 g/dL
Sodium Chloride	6.0 g/dL
Urea	6.0 g/dL
Boric Acid	1% w/v
Sodium Azide	1% w/v
Sodium Fluoride	1% w/v

Non-interfering Substances – Serum and Plasma

Compound	10 mg/dL	100 mg/dL
	Compound Conc.	Compound Conc.
Acetaminophen	20 mg/dL	20 mg/dL
Amikacin	8 mg/dL	8 mg/dL
Ampicillin	5.3 mg/dL	5.3 mg/dL
Ascorbic Acid	6 mg/dL	6 mg/dL
Bilirubin total	60 mg/dL	80 mg/dL
Bilirubin direct	60 mg/dL	80 mg/dL
Caffeine	6 mg/dL	6 mg/dL
Carbamazepine	3 mg/dL	3 mg/dL
Chloramphenicol	5 mg/dL	5 mg/dL
Chlordiazepoxide	1 mg/dL	1 mg/dL
Chlorpromazine	0.2 mg/dL	0.2 mg/dL
Cholesterol	30 mg/dL	503 mg/dL
Cimetidine	2 mg/dL	2 mg/dL
Creatinine	30 mg/dL	30 mg/dL
Dextran	6000 mg/dL	6000 mg/dL
Diazepam	0.51 mg/dL	0.51 mg/dL

Compound	10 mg/dL	100 mg/dL
	Compound Conc.	Compound Conc.
Digoxin	6.1 mg/dL	6.1 mg/dL
Erythromycin	6 mg/dL	6 mg/dL
Ethosuximide	25 mg/dL	25 mg/dL
Furosemide	6 mg/dL	6 mg/dL
Gentamicin	1 mg/dL	1 mg/dL
Hemoglobin	62.5 mg/dL	1,000 mg/dL
Heparin	3 U/mL	3 U/mL
Ibuprofen	50 mg/dL	50 mg/dL
IgG	5,000 mg/dL	5,000 mg/dL
Intralipid	187.5 mg/dL	750 mg/dL
Lactate Dehydrogenase	1,000 U/L	237,500 U/L
Lactate	150 mg/dL	901 mg/dL
Lidocaine	1.2 mg/dL	1.2 mg/dL
Lithium	2.2 mg/dL	2.2 mg/dL
Mannitol	500 mg/dL	500 mg/dL
Penicillin	25 U/mL	25 U/mL
Pentobarbital	8 mg/dL	8 mg/dL
Phenobarbital	10 mg/dL	10 mg/dL
Phenytoin	5 mg/dL	5 mg/dL
Primidone	4 mg/dL	4 mg/dL
Propoxyphene	0.16 mg/dL	0.16 mg/dL
HSA (albumin)	37.5 mg/dL	1,250 mg/dL
Protein (total)	50 mg/dL	1,500 mg/dL
Salicylic Acid	60 mg/dL	60 mg/dL
Theophylline	4 mg/dL	4 mg/dL
Triglycerides	500 mg/dL	500 mg/dL
Urea	500 mg/dL	500 mg/dL
Uric Acid	20 mg/dL	20 mg/dL
Valproic Acid	50 mg/dL	50 mg/dL

Non-interfering Anticoagulants –Plasma

Compound	10 mg/dL	100 mg/dL
	Compound Conc.	Compound Conc.
Heparin	3 U/mL	3 U/mL
EDTA	2 mg/mL	2 mg/mL
Oxalic Acid	2 mg/mL	2 mg/mL
Sodium Citrate	3.8 g/dL	3.8 g/dL
Sodium Fluoride	2 mg/mL	2 mg/mL

2. To evaluate cross-reactivity with structurally similar compounds, the potential cross-reactants were spiked into alcohol free urine, serum, and plasma and measured for ethanol using the candidate assay. Results were as follows:

Compound	Matrix	% cross-reactivity
Acetaldehyde	Urine	<3.3
	Serum	<3.3
	Plasma	<3.3
Acetone	Urine	<3.3
	Serum	<3.3
	Plasma	<3.3
n-Butanol	Urine	<3.3
	Serum	<3.3
	Plasma	<3.3
Ethylene Glycol	Urine	<3.3
	Serum	<3.3
	Plasma	<3.3
Isopropanol	Urine	<3.3
	Serum	<3.3
	Plasma	<3.3
Methanol	Urine	<3.3
	Serum	<3.3
	Plasma	<3.3
n-Propanol	Urine	16.7
	Serum	11.1
	Plasma	11.1
Propylene Glycol	Urine	<3.3
	Serum	<3.3
	Plasma	<3.3

3. Interference – pH
   To evaluate potential interference from the effect of urine pH, device performance in the quantitative mode was tested using a range of urine pH values. All test samples were prepared in drug-free urine containing EtOH at concentration of 10 mg/dL and 100 mg/dL. No interference was observed at urine pH values ranging from 3.0 to 11.0.

4. Interference - Specific Gravity
   To evaluate potential interference from the specific gravity of urine, device performance in the quantitative mode was tested using a range of physiologically relevant urine specific gravity values. All test samples were prepared in drug free urine containing EtOH at concentration of 10 mg/dL and 100 mg/dL. No interference was observed at urine specific gravity values ranging from 1.000 to 1.030.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed using unaltered clinical urine, serum and plasma samples. 80 urine samples, 76 serum samples and 64 plasma samples with concentrations covering the measuring range of candidate device were evaluated. Results were compared to the predicate device, the Lin-Zhi Ethyl Alcohol Enzymatic Assay, using linear regression and the study results are summarized in the table below.

Matrix	n	Slope	Intercept	r^2
Urine	80	1.0284	-2.7848	0.9964
Serum	76	1.0067	-5.8802	0.9948
Plasma	64	1.0595	-7.3902	0.9932

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.