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

A. 510(k) Number:

k161216

B. Purpose for Submission:

New device

C. Measurand:

Fentanyl

D. Type of Test:

Qualitative

E. Applicant:

Immunalysis Corporation

F. Proprietary and Established Names:

Immunalysis SEFRIA Fentanyl Urine Enzyme Immunoassay
Immunalysis SEFRIA Fentanyl Urine Calibrators

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
DJG	Class II	21 CFR 862.3650, Opiate test system	Toxicology (91)
DLJ	Class II	21 CFR 862.3200, Clinical toxicology calibrator	Toxicology (91)

H. Intended Use:

1. Intended use(s):

Refer to Indications for Use below.

2. Indication(s) for use:

The Immunalysis SEFRIA Fentanyl Urine Enzyme Immunoassay is an enzyme immunoassay with a cutoff of 1.0 ng/mL. The assay is intended for use in laboratories for the qualitative analysis of Fentanyl in human urine with automated clinical chemistry analyzers. This assay is calibrated against Fentanyl. This in-vitro diagnostic device is for prescription use only.

The Immunalysis SEFRIA Fentanyl 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. 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.

The Immunalysis Fentanyl Urine Calibrators are used as calibrators in the Immunalysis SEFRIA Fentanyl Urine Enzyme Immunoassay for the qualitative determination of Fentanyl 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 accurately, and measuring enzyme rates precisely at 570nm.

I. Device Description:

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

- Antibody/Enzyme Donor (EA) – This reagent contains rabbit antibodies to fentanyl, and a partial sequence to galactosidase in PIPES buffer with Sodium Azide as a preservative.
- Substrate/Enzyme Acceptor (ED) – This reagent contains contain the complementary sequence of β-galactosidase labeled with fentanyl in malic acid buffer with Sodium Azide as a preservative.

All of the Immunalysis Fentanyl Urine Calibrators are sold as individual bottles and are liquid and ready to use. The negative calibrator is a processed, drug-free synthetic urine matrix with sodium azide as a preservative. Each calibrator (1, 2, and 4 ng/mL) contains a

known concentration of Fentanyl spiked into the negative calibrator matrix.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Siemens Healthcare Diagnostics, Inc. Emit II Plus Buprenorphine Assay

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

   k150606

3. Comparison with predicate:

Similarities - Reagent		
Item	Predicate Device Siemens Healthcare Diagnostics. Emit II Plus Buprenorphine Assay k150606	Candidate Device Immunalysis SEFRIA Fentanyl Urine Enzyme Immunoassay
Test System	Homogenous enzyme immunoassay	Same
User Environment	Clinical laboratories	Same
Sample Matrix	Urine	Same
Mass Spectrometry Confirmation	Required to confirm preliminary positive analytical results	Same
Storage	2 – 8°C until expiration date	Same
Materials	Antibody coated tube and reagents	Antibody/substrate reagents and enzyme labeled conjugate

Differences - Reagent		
Item	Predicate Device Siemens Healthcare Diagnostics. Emit II Plus Buprenorphine Assay k150606	Candidate Device Immunalysis SEFRIA™ Fentanyl Urine Enzyme Immunoassay
Intended Use	For the qualitative and semi-quantitative determination of Buprenorphine in human urine at a cutoff of 5 ng/mL	For the qualitative determination of Fentanyl in human urine at a cutoff of 1 ng/mL
Detection	Absorbance change measured spectrophotometrically at	Absorbance change measured spectrophotometrically at

	340 nm	570 nm
Measurand Analytes	Buprenorphine	Fentanyl
Cutoff Levels	5 ng/mL	1 ng/mL
Antibody	Mouse monoclonal antibody to Buprenorphine	Enzyme Acceptor protein and rabbit antibodies to Fentanyl
Reagents Form	R1 and R2-Liquid-Ready to Use	EA and ED: Liquid-Ready to Use

Calibrators		
Item	Predicate Device Siemens Healthcare Diagnostics. Emit II Plus Specialty Drug Calibrator/Control Levels 1 - 4 k150606	Candidate Device Immunalysis Immunalysis Fentanyl Urine Calibrators
Analytes	Buprenorphine	Fentanyl
Calibrator Levels	One negative and four levels (2.5, 5, 15, 25 ng/mL)	One negative and three levels (1, 2, and 4 ng/mL)
Calibrator Form	Liquid	Same
Storage	2 – 8°C until expiration date	Same

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

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

CLSI EP07-A2: "Interference Testing in Clinical Chemistry: Approved Guideline - Second Edition"

ISO 14971 Second edition 2007-03-01, "Medical devices – application of risk management to medical devices"

L. Test Principle:

The SEFRIA technology is based on artificial fragments of the *E. coli* enzyme β-galactosidase. A mutant enzyme, termed Enzyme Acceptor (EA), is created by deletion of 28 amino acids in the amino-terminal region of the sequence for β-galactosidase. EA is inactive, but can combine Enzyme Donors (ED's), containing the deleted sequence, to form active β-galactosidase. This process is termed complementation, and the active enzyme formed as a result can be measured by hydrolysis of a chromogenic substrate such as chlorophenolred β-D-galactopyranoside (CPRG). The ED peptides are attached to a derivative of fentanyl that does not interfere with the

formation of active β-galactosidase. However, when fentanyl antibodies bind to the ED-fentanyl conjugate complementation is blocked, thus preventing the formation of active β-galactosidase.

The assay is based on the competition of fentanyl in a urine sample with the ED-fentanyl conjugate for the fixed amount of antibody binding sites. In the absence of the free drug in the sample, the antibody binds the ED-fentanyl conjugate, resulting in inhibition of enzyme formation. As the fentanyl concentration in the sample increases, ED-fentanyl becomes available for complementation, creating a dose response relationship between fentanyl concentration in the urine and enzyme formation. The β- galactosidase activity is determined spectrophotometrically at 570 nm by the conversion of CPRG (orange) to chlorophenol red (red) and galactose.

M. Performance Characteristics:

1. Analytical performance:

   a. *Precision/Reproducibility:*

   The sponsor performed precision studies for 20 days, 2 runs per day in duplicate (N=80) on drug-free negative urine samples spiked with Fentanyl to concentrations of ±25%, ±50%, ±75%, and ±100% of the cutoff. Fentanyl concentrations in spiked samples were confirmed by mass spectrometry. Results were analyzed on a Beckman Coulter / Olympus AU400e Chemistry Analyzer. The data are summarized in the following tables:

   Qualitative analysis (1.0 ng/mL cutoff)

Concentration (ng/mL)	% of cutoff	Result
0	-100	80 Neg / 0 Pos
0.25	-75	80 Neg / 0 Pos
0.5	-50	80 Neg / 0 Pos
0.75	-25	80 Neg / 0 Pos
1.0	Cutoff	32 Neg / 48 Pos
1.25	+25	80 Pos / 0 Neg
1.5	+50	80 Pos / 0 Neg
1.75	+75	80 Pos / 0 Neg
2.0	+100	80 Pos / 0 Neg

   b. *Linearity/assay reportable range:*

   Not applicable, this device is intended for qualitative use only

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

   Traceability

   The analytes in the calibrators are traceable to a commercially available standard solution. The standard is certified material with the concentration verified by GC-MS or LC/MS-MS.

Value Assignment/Expected Values

The calibrators are prepared by spiking known concentrations of Fentanyl into a negative calibrator matrix. Concentrations are confirmed by GC/MS or LC/MS/MS.

Stability

Real time, closed vial and on-board stability studies for calibrators were conducted. Stability protocols and acceptance criteria were reviewed and found to be acceptable, and support that, when stored at 2 – 8 °C, calibrators are stable for one year. The results support that, once opened, calibrators are stable for 60 days when stored at 2 – 8 °C.

d. Detection limit:

Not applicable.

e. Analytical specificity:

Cross reactivity from structurally related compounds was evaluated by testing drug-free urine samples spiked with these compounds. Each potential cross-reacting compound was spiked and evaluated independently, and each spiked sample was tested in singlicate. Compounds were tested at a high concentration, and if cross-reactivity was observed, were diluted to identify the lowest concentration that produced a positive result. These concentrations were used to determine the percent cross-reactivity. The compounds and concentrations tested, and the approximate cross-reactivity are provided in the table below.

Compound	Concentration Tested (ng/mL)	Result	Cross-Reactivity (%)
Fentanyl	1	Positive	100
Butryl Fentanyl	0.8	Positive	125
Acetyl Fentanyl	1	Positive	100
Despropionyl Fentanyl	40	Positive	2.5
Sufentanil	175	Negative	<0.5714
Norfentanyl	20,000	Positive	0.005
6-Acetyl Morphine	100,000	Negative	<0.0010
Codeine	100,000	Negative	<0.0010
Methadone	100,000	Negative	<0.0010
Hydrocodone	100,000	Negative	<0.0010
Hydromorphone	100,000	Negative	<0.0010
Oxycodone	100,000	Negative	<0.0010
Morphine	100,000	Negative	<0.0010
Morphine-3-glucuronide	100,000	Negative	<0.0010

Compound	Concentration Tested (ng/mL)	Result	Cross-Reactivity (%)
Ethylmorphine			
EDDP	100,000	Negative	<0.0010
EMDP	100,000	Negative	<0.0010
Levorphanol	100,000	Negative	<0.0010
Oxymorphone	100,000	Negative	<0.0010
Tramadol	100,000	Negative	<0.0010
Nalorphine	100,000	Negative	<0.0010
Naloxone	100,000	Negative	<0.0010
Naltrexone	100,000	Negative	<0.0010
Normorphine	100,000	Negative	<0.0010
6-Acetyl Codeine	100,000	Negative	<0.0010
Dihydrocodeine	100,000	Negative	<0.0010
Diacetyl Morphine	100,000	Negative	<0.0010
Pentazocine	75,000	Positive	0.0013
Meperidine	100,000	Negative	<0.0010
Morphine-6-glucuronide	100,000	Negative	<0.0010
Buprenorphine	100,000	Negative	<0.0010
Norcodeine	100,000	Negative	<0.0010
Propoxyphene	100,000	Negative	<0.0010
Labetalol	15,000	Positive	0.0067
Nordiazepam	100,000	Negative	<0.0010
Trimethoprim	100,000	Negative	<0.0010
Fluoxetine	60,000	Positive	0.0017
Amitryptyline	75,000	Positive	0.0013
Doxepin	100,000	Positive	0.001
Nortriptyline	100,000	Positive	0.001
Protryptyline	100,000	Negative	<0.0010
Trimipramine	100,000	Negative	<0.0010
Buproprion	100,000	Negative	<0.0010
Trazodone	10,000	Positive	0.01
Clomipramine	45,000	Positive	0.0022
Desipramine	100,000	Positive	0.001
Imipramine	100,000	Positive	0.001
Haloperidol	1,250	Positive	0.08
Pipamperone	1,500	Positive	0.0667
Risperidone	2,500	Positive	0.04
Chlorpromazine	75,000	Positive	0.0013
Meta-chlorphenyl piperazine	100,000	Positive	0.001
Venlafaxine	100,000	Negative	<0.0010
PCP	100,000	Positive	0.001
Diphenhydramine	100,000	Positive	0.001

Compound	Concentration Tested (ng/mL)	Result	Cross-Reactivity (%)
Methamphetamine	70,000	Positive	0.0014
Benzylpiperazine	50,000	Positive	0.002
Fenfluramine	60,000	Positive	0.0017
Cyclobenzaprine	100,000	Positive	0.001

Potential interference from endogenous substances was evaluated by spiking these substances into drug free urine containing fentanyl at ±50% of the 1.0 cutoff (0.5 ng/mL and 1.5 ng/mL).

The following endogenous substances, at the concentrations listed below, did not interfere with the assay:

Compound	Concentration Tested
Acetone	1.0 g/dL
Ascorbic Acid	0.56 g/dL
Bilirubin	2.0 mg/dL
Creatinine	0.5 g/dL
Ethanol	1.0 g/dL
Galactose	10 mg /dL
γ-Globulin	0.5 g/dL
Glucose	2.0 g/dL
Hemoglobin	0.5 g/dL
Human Serum Albumin	0.5 g/dL
Oxalic Acid	0.1 g/dL
Riboflavin	7.5 mg/dL
Sodium Azide	1% w/v
Sodium Chloride	6.0 g/dL
Sodium Fluoride	1% w/v
Urea	2.0 g/dL

Boric acid at a concentration of 1% w/v was evaluated by spiking the potential interferent into drug free urine containing Fentanyl at ± 50% of the cutoff (0.5 ng/mL and 1.5 ng/mL).

Effect of pH: The sponsor evaluated the effect of pH using drug free urine containing Fentanyl at ± 50% of the cutoff (0.5 ng/mL and 1.5 ng/mL). The pH was 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.

Effect of specific gravity: The sponsor evaluated the effect of specific gravity on the test results using drug free urine containing Fentanyl at ± 50% of the cutoff (0.5 ng/mL and 1.5 ng/mL) that 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.

Potential interference from non-structurally related compounds were evaluated by spiking these compounds into drug free urine containing fentanyl at ±50% of the 1.0 cutoff (0.5 ng/mL and 1.5ng/mL). The following non-structurally related substances at the concentrations listed below did not interfere with the assay:

Compound	Concentration Tested (ng/mL)
11-hydroxy-delta-9-THC	75,000
11-nor-9 carboxy THC	100,000
1S, 2R(+)-Ephedrine	100,000
7-Aminoclonazepam	100,000
Aminoflunitrazepam	100,000
7-Aminonitrazepam	100,000
Acetaminophen	500,000
Amobarbital	100,000
Barbital	100,000
Benzoylecgonine	100,000
Bromazepam	100,000
Butabarbital	100,000
Caffeine	100,000
Cannabidiol	100,000
Cannabinol	75,000
Carbamazepine	100,000
Carisoprodol	100,000
Chlordiazepoxide	100,000
cis-Tramadol	100,000
Clobazam	100,000
Clonazepam	100,000
Cotenine	100,000
Delta-9-THC	100,000
Demoxepam	100,000
Ecgonine	100,000
Ecgonine methyl ester	100,000
Ethylbeta-D glucuronide	100,000
Flunitrazepam	100,000
Heroin	100,000
Hexobarbital	100,000
Ibuprofen	100,000
Ketamine	100,000
Lamotrignine	100,000
Lidocaine	100,000
Lorazepam Glucuronide	50,000

Compound	Concentration Tested (ng/mL)
LSD	100,000
Mephobarbital	100,000
Methaquolone	100,000
Naproxen	100,000
Nitrazepam	100,000
Normorphine	100,000
Norpseudoephedrine	100,000
Oxazepam	100,000
Pentobarbital	100,000
Phenobarbital	100,000
Phenylephedrine	100,000
Salicylic Acid	100,000
Secobarbital	100,000
Temazepam	100,000
Phenytoin	100,000
PMA	100,000
Propranolol	100,000
(+)-MDA	75,000
4-Bromo-2,5,Dimethoxyphenethylamine	75,000
Desalkyflurazepam	75,000
Dextromethorphan	75,000
Diazepam	75,000
Flurazepam	75,000
Lorazepam	75,000
Lormetazepam	75,000
Maprotiline	75,000
Medezapam	75,000
Meprobamate	75,000
Methyphenidate	75,000
Midazolam	75,000
N Desmethyltapentadol	75,000
Oxazepam glucuronide	75,000
Phentermine	75,000
Phenylpropanolamine	75,000
R,R(-)-Pseudoephedrine-	75,000
Ranitidine	75,000
Ritalinic Acid	75,000
Sertraline	75,000

Compound	Concentration Tested (ng/mL)
Theophylline	75,000
Thioridazine	75,000
Zolpidem Tartrate	75,000
Cocaine	40,000
MDEA	50,000
MDMA	50,000
S-(+) Amphetamine	50,000
Triazolam	50,000
Trifluoromethylphenyl-piperazine	40,000

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:

A method comparison study was performed using unaltered, clinical urine samples obtained from clinical testing laboratories. A total of 80 samples were analyzed 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:

Candidate Device Result	Fentanyl Concentration by LC/MS (ng/mL)			
	< 0.5 <50%	0.5-0.9 ≥50% <100%	1.0-1.5 ≥100% ≤150%	> 1.5 >150%
POS	0	1*	9	31
NEG	30	9	0	0

*Discordant sample: One sample was determined as positive by the candidate device, whereas this sample was determined to be negative by the confirmatory LC/MS method (0.9 ng/mL).

The sponsor conducted a supplemental method comparison study, which included twenty (20) subjects who were prescribed fentanyl and twenty (20) subjects who were suspected fentanyl abuse users. The urine samples from all study subjects were determined to be positive for fentanyl using the candidate device. Subject urine concentrations, as determined by LC/MS, ranged from 1.0 to 367 ng/mL for the

prescription fentanyl users and from 0.12 to 385 ng/mL for the suspected fentanyl abuse users. Two (2) of the twenty (20) subjects in the suspected fentanyl abuser sub-population were determined as false positive*.

*Discordant samples:

The first sample was confirmed negative for fentanyl by LC/MS (fentanyl concentration: 0.12 ng/mL). Further analysis identified despropionyl fentanyl at a concentration consistent with a positive result for the candidate device, as determined by their cross-reactivity study (despropionyl fentanyl concentration: 46.6 ng/mL)

The second sample was confirmed negative for fentanyl by LC/MS (fentanyl concentration: 0.17 ng/mL). Further analysis identified acetyl fentanyl at a concentration consistent with a positive result for the candidate device, as determined by their cross-reactivity study (acetyl fentanyl concentration: 1.03 ng/mL)

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.