DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

June 14, 2017

IMMUNALYSIS CORPORATION
MICHELLE BODIEN, ASSOCIATE DIRECTOR, REGULATORY AFFAIRS
829 TOWNE CENTER DRIVE
POMONA, CA 91767

Re: k161216
Trade/Device Name: Immunalysis SEFRIA Fentanyl Urine Enzyme Immunoassay and Immunalysis Fentanyl Urine Calibrators
Regulation Number: 21 CFR 862.3650
Regulation Name: Opiate test system
Regulatory Class: II
Product Code: DJG, DLJ
Dated: May 30, 2017
Received: June 1, 2017

Dear Ms. Bodien:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Kellie B. Kelm -S

for Courtney H. Lias, Ph.D.
Director
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

# Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
*See PRA Statement below.*

510(k) Number *(if known)*
k161216

Device Name
Immunalysis SEFRIA Fentanyl Urine Enzyme Immunoassay,
Immunalysis Fentanyl Urine Calibrators

Indications for Use *(Describe)*
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.

Immunalysis Fentanyl Urine Calibrators:
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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

**CONTINUE ON A SEPARATE PAGE IF NEEDED.**

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# 510(k) SUMMARY

A. General Information

1. Applicant Name: Immunalysis Corporation
   829 Towne Center Drive
   Pomona, CA 91767
2. Company Contact: Michelle Bodien
   Associate Director, Regulatory Affairs
   Phone: (858) 805-2283
   Email: michelle.bodien@alere.com
3. Date prepared: June 13, 2017

B. Device Identification

1. Trade Name: Immunalysis SEFRIA™ Fentanyl Urine Enzyme Immunoassay
   Immunalysis Fentanyl Urine Calibrators
2. Common Name: Fentanyl Urine Enzyme Immunoassay
   Fentanyl Urine Calibrators

C. Regulatory Information

1. Device Classification: II
2. Regulation Number: 21 CFR 862.3650 Enzyme Immunoassay,Opiates
   21 CFR 862.3200 Calibrators, Drug Specific
3. Panel: Toxicology (91)
4. Product Code: DJG
   DLJ
5. Predicate Device: Emit® II Plus Buprenorphine Assay
6. Predicate Company: Siemens Healthcare Diagnostics Inc.
7. Predicate K Number: K150606

D. Device Description

The assay consists of antibody/ substrate reagent and enzyme conjugate reagent. The antibody/ substrate reagent includes EA protein and rabbit antibodies to Fentanyl in PIPES buffer with sodium azide as a preservative. The enzyme conjugate reagent includes ED peptide labeled with Fentanyl and CPRG substrate in malic acid buffer with sodium azide as a preservative. Calibrators and controls are included as part of the test system and provided separately. The Fentanyl calibrators consist of a Level 1 calibrator at 1 ng/mL, a Level 2 calibrator at 2 ng/mL, and a Level 3 calibrator at 4 ng/mL. The control set contains a LOW control at 0.5 ng/mL and a HIGH control at 1.5 ng/mL.

Automated clinical chemistry analyzers capable of maintaining a constant temperature, pipetting samples and reagents, mixing reagents, timing the reaction accurately and measuring enzymatic rates at 570nm can be used to perform the assay.

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. EA is inactive, but can combine with peptides, termed 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 can be modified by attachment of a derivative of fentanyl, which does not interfere with the formation of active β-galactosidase. However antibodies to fentanyl bind to the ED-fentanyl conjugate, and block complementation. 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.

E. Intended Use

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

   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.

2. Immunalysis Fentanyl Urine Calibrators
   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.

F. Comparison With Predicate

| Attribute | **Predicate Device K150606 Emit® II Plus Buprenorphine Assay** | **Candidate Device Immunalysis Fentanyl Urine EIA** |
|---|---|---|
| **Similarities** | | |
| **Test System** | Homogeneous enzyme immunoassay | Same |
| **Sample Matrix** | Urine | Same |
| **User Environment** | For use in laboratories | Same |
| **Mass Spectrometry Confirmation** | Required for preliminary positive analytical results | Same |
| **Storage** | 2 – 8° C | Same |
| **Materials** | Antibody/substrate reagents and enzyme labeled conjugate | Same |
| **Calibrator Form** | Liquid | Same |
| **Differences** | | |
| **Intended Use** | For the qualitative and semi-quantitative determination of the presence of Buprenorphine in human urine at a cutoff of 5 ng/mL | For the qualitative determination of the presence of Fentanyl in human urine at a cutoff of 1 ng/mL |
| **Detection** | Absorbance change measured spectrophotometrically at 340 nm | Absorbance change measured spectrophotometrically at 570 nm |
| **Measured Analytes** | Buprenorphine | Fentanyl |
| **Cutoff Levels** | 5 ng/mL of Buprenorphine | 1 ng/mL of Fentanyl |
| **Antibody** | Mouse monoclonal antibody to buprenorphine | Rabbit antibodies to Fentanyl |
| **Reagents Form** | R1 and R2: Liquid – Ready to use | EA and ED: Liquid – Ready to use |
| **Calibrator Levels** | One negative and four levels (0, 2.5, 5, 15 and 25 ng/mL) | One negative and three levels (0, 1, 2 and 4 ng/mL |
| | | |

G. Performance Characteristics:

The following laboratory performance studies were performed to determine substantial equivalence of the Immunalysis SEFRIA™ Fentanyl Urine Enzyme Immunoassay to the predicate. All studies utilized the Beckman Coulter AU 400e instrument.

1. Precision/ Cutoff Characterization/ Reproducibility – Study was performed for 10 days, 2 runs per day in replicates of 4 on drug free urine (N=80) spiked with fentanyl to concentrations of ±25%, ±50%, ±75%, and ±100% of the cutoff (1.0 ng/mL). The spiked concentrations were confirmed by mass spectrometry (MS). The study verified that the cutoff serves as a boundary between a negative and positive interpretation of a qualitative result.

   The following is a summary table precision results for the 1.0 ng/mL cutoff test data results.

**Table 1 - Precision Results**

| Concentration (ng/mL) | % of cutoff | # of determinations | Total Result |
|---|---|---|---|
| 0 | -100% | 80 | 80 Negative |
| 0.25 | -75% | 80 | 80 Negative |
| 0.5 | -50% | 80 | 80 Negative |
| 0.75 | -25% | 80 | 80 Negative |
| 1.0 | Cutoff | 80 | 32 Negative/48 Positive |
| 1.25 | +25% | 80 | 80 Positive |
| 1.5 | +50% | 80 | 80 Positive |
| 1.75 | +75% | 80 | 80 Positive |
| 2.0 | +100% | 80 | 80 Positive |

2. Specificity and Cross-Reactivity – Structurally similar compounds were spiked into drug free urine at levels that will yield a result that is equivalent to the cutoff. The study verified assay performance relative to the ability of the device to exclusively determine certain drugs.

The following is a summary table of results:

| Table 3 - Structurally Related Compounds | | | |
|---|---|---|---|
| Compound | Concentration Tested (ng/mL) | Result | Cross-Reactivity (%) |
| Fentanyl | 1 | Positive | 100.0000 |
| Butyryl Fentanyl | 0.8 | Positive | 125.0000 |
| Acetyl Fentanyl | 1 | Positive | 100.0000 |
| Despropionyl Fentanyl | 40 | Positive | 2.5000 |
| Sufentanil | 175 | Negative | <0.5714 |
| Haloperidol | 1,250 | Positive | 0.0800 |
| Pipamperone | 1,500 | Positive | 0.0667 |
| Risperidone | 2,500 | Positive | 0.0400 |
| Norfentanyl | 20,000 | Positive | 0.0050 |
| Trazodone | 10,000 | Positive | 0.0100 |
| Labetalol | 15,000 | Positive | 0.0067 |
| Clomipramine | 45,000 | Positive | 0.0022 |
| Benzylpiperazine | 50,000 | Positive | 0.0020 |
| Fluoxetine | 60,000 | Positive | 0.0017 |
| Fenfluramine | 60,000 | Positive | 0.0017 |
| Methamphetamine | 70,000 | Positive | 0.0014 |
| Amitryptyline | 75,000 | Positive | 0.0013 |
| Chlorpromazine | 75,000 | Positive | 0.0013 |
| PCP | 100,000 | Positive | 0.0010 |
| Pentazocine | 75,000 | Positive | 0.0013 |
| 6-Acetyl Codeine | 100,000 | Negative | <0.0010 |
| 6-Acetyl Morphine | 100,000 | Negative | <0.0010 |
| Buprenorphine | 100,000 | Negative | <0.0010 |
| Buproprion | 100,000 | Negative | <0.0010 |
| Codeine | 100,000 | Negative | <0.0010 |
| Cyclobenzaprine | 100,000 | Positive | 0.0010 |
| Desipramine | 100,000 | Positive | 0.0010 |
| Diacetyl Morphine | 100,000 | Negative | <0.0010 |
| Dihydrocodeine | 100,000 | Negative | <0.0010 |
| Diphenhydramine | 100,000 | Positive | 0.0010 |
| Doxepin | 100,000 | Positive | 0.0010 |
| EDDP | 100,000 | Negative | <0.0010 |
| EMDP | 100,000 | Negative | <0.0010 |
| Hydrocodone | 100,000 | Negative | <0.0010 |
| Hydromorphone | 100,000 | Negative | <0.0010 |
| Imipramine | 100,000 | Positive | 0.0010 |
| Levorphanol | 100,000 | Negative | <0.0010 |
| Meta-chlorphenyl piperazine | 100,000 | Positive | 0.0010 |
| Meperidine | 100,000 | Negative | <0.0010 |

| Table 3 - Structurally Related Compounds | | | |
|---|---|---|---|
| Compound | Concentration Tested (ng/mL) | Result | Cross-Reactivity (%) |
| Methadone | 100,000 | Negative | <0.0010 |
| Morphine | 100,000 | Negative | <0.0010 |
| Morphine-3-glucuronide | 100,000 | Negative | <0.0010 |
| Morphine-6-glucuronide | 100,000 | Negative | <0.0010 |
| Nalorphine | 100,000 | Negative | <0.0010 |
| Naloxone | 100,000 | Negative | <0.0010 |
| Naltrexone | 100,000 | Negative | <0.0010 |
| Norcodeine | 100,000 | Negative | <0.0010 |
| Nordiazepam | 100,000 | Negative | <0.0010 |
| Normorphine | 100,000 | Negative | <0.0010 |
| Nortriptyline | 100,000 | Positive | 0.0010 |
| Oxycodone | 100,000 | Negative | <0.0010 |
| Oxymorphone | 100,000 | Negative | <0.0010 |
| Propoxyphene | 100,000 | Negative | <0.0010 |
| Protryptyline | 100,000 | Negative | <0.0010 |
| Tramadol | 100,000 | Negative | <0.0010 |
| Trimethoprim | 100,000 | Negative | <0.0010 |
| Trimipramine | 100,000 | Negative | <0.0010 |
| Venlafaxine | 100,000 | Negative | <0.0010 |

3. Interference – Structurally unrelated compounds were evaluated by spiking the potential interferent into drug free urine containing fentanyl at ±50% of the cutoff. All potential interferents analyzed verified that assay performance is unaffected by externally ingested compounds. The results of this study are indicated in Table 5 below.

| Table 5 - Structurally Unrelated Compounds | | | |
|---|---|---|---|
| Compound | Concentration Tested (ng/mL) | -50% Cutoff (0.5 ng/mL) | +50% Cutoff (1.5 ng/mL) |
| 11-hydroxy-delta-9-THC | 75,000 | Negative | Positive |
| 11-nor-9 carboxy THC | 100,000 | Negative | Positive |
| 1S, 2R(+)-Ephedrine | 100,000 | Negative | Positive |
| 7-Aminoclonazepam | 100,000 | Negative | Positive |
| 7-Aminoflunitrazepam | 100,000 | Negative | Positive |
| 7-Aminonitrazepam | 100,000 | Negative | Positive |
| Acetaminophen | 500,000 | Negative | Positive |
| Amobarbital | 100,000 | Negative | Positive |
| Barbital | 100,000 | Negative | Positive |
| Benzoylecgonine | 100,000 | Negative | Positive |
| Bromazepam | 100,000 | Negative | Positive |
| Butabarbital | 100,000 | Negative | Positive |
| Caffeine | 100,000 | Negative | Positive |
| Cannabidiol | 100,000 | Negative | Positive |
| Cannabinol | 75,000 | Negative | Positive |
| Carbamazepine | 100,000 | Negative | Positive |

| Table 5 - Structurally Unrelated Compounds | | | |
|---|---|---|---|
| Compound | Concentration Tested (ng/mL) | -50% Cutoff (0.5 ng/mL) | +50% Cutoff (1.5 ng/mL) |
| Carisoprodol | 100,000 | Negative | Positive |
| Chlordiazepoxide | 100,000 | Negative | Positive |
| cis-Tramadol | 100,000 | Negative | Positive |
| Clobazam | 100,000 | Negative | Positive |
| Clonazepam | 100,000 | Negative | Positive |
| Cotenine | 100,000 | Negative | Positive |
| Delta-9-THC | 100,000 | Negative | Positive |
| Demoxepam | 100,000 | Negative | Positive |
| Ecgonine | 100,000 | Negative | Positive |
| Ecgonine methyl ester | 100,000 | Negative | Positive |
| Ethyl beta-D-glucuronide | 100,000 | Negative | Positive |
| Flunitrazepam | 100,000 | Negative | Positive |
| Heroin | 100,000 | Negative | Positive |
| Hexobarbital | 100,000 | Negative | Positive |
| Ibuprofen | 100,000 | Negative | Positive |
| Ketamine | 100,000 | Negative | Positive |
| Lamotrignine | 100,000 | Negative | Positive |
| Lidocaine | 100,000 | Negative | Positive |
| Lorazepam Glucuronide | 50,000 | Negative | Positive |
| LSD | 100,000 | Negative | Positive |
| Mephobarbital | 100,000 | Negative | Positive |
| Methaquolone | 100,000 | Negative | Positive |
| Naproxen | 100,000 | Negative | Positive |
| Nitrazepam | 100,000 | Negative | Positive |
| Normorphine | 100,000 | Negative | Positive |
| Norpseudoephedrine | 100,000 | Negative | Positive |
| Oxazepam | 100,000 | Negative | Positive |
| Pentobarbital | 100,000 | Negative | Positive |
| Phenobarbital | 100,000 | Negative | Positive |
| Phenylephedrine | 100,000 | Negative | Positive |
| Salicylic Acid | 100,000 | Negative | Positive |
| Secobarbital | 100,000 | Negative | Positive |
| Temazepam | 100,000 | Negative | Positive |
| Phenytoin | 100,000 | Negative | Positive |
| PMA | 100,000 | Negative | Positive |
| Propranolol | 100,000 | Negative | Positive |
| (+)-MDA | 75,000 | Negative | Positive |
| 4-Bromo-2, 5,Dimethoxyphenethylamine | 75,000 | Negative | Positive |
| Desalkyflurazepam | 75,000 | Negative | Positive |
| Dextromethorphan | 75,000 | Negative | Positive |
| Diazepam | 75,000 | Negative | Positive |
| Flurazepam | 75,000 | Negative | Positive |
| Lorazepam | 75,000 | Negative | Positive |
| Lormetazepam | 75,000 | Negative | Positive |
| Maprotiline | 75,000 | Negative | Positive |
| Medezapam | 75,000 | Negative | Positive |

| Table 5 - Structurally Unrelated Compounds | | | |
|---|---|---|---|
| Compound | Concentration Tested (ng/mL) | -50% Cutoff (0.5 ng/mL) | +50% Cutoff (1.5 ng/mL) |
| Meprobamate | 75,000 | Negative | Positive |
| Methyphenidate | 75,000 | Negative | Positive |
| Midazolam | 75,000 | Negative | Positive |
| N-Desmethyltapentadol | 75,000 | Negative | Positive |
| Oxazepam glucuronide | 75,000 | Negative | Positive |
| Phentermine | 75,000 | Negative | Positive |
| Phenylpropanolamine | 75,000 | Negative | Positive |
| R,R(-)-Pseudoephedrine | 75,000 | Negative | Positive |
| Ranitidine | 75,000 | Negative | Positive |
| Ritalinic Acid | 75,000 | Negative | Positive |
| Sertraline | 75,000 | Negative | Positive |
| Theophylline | 75,000 | Negative | Positive |
| Thioridazine | 75,000 | Negative | Positive |
| Zolpidem Tartrate | 75,000 | Negative | Positive |
| Cocaine | 40,000 | Negative | Positive |
| MDEA | 50,000 | Negative | Positive |
| MDMA | 50,000 | Negative | Positive |
| S-(+) Amphetamine | 50,000 | Negative | Positive |
| Triazolam | 50,000 | Negative | Positive |
| Trifluoromethylphenyl-piperazine | 40,000 | Negative | Positive |

4. Interference – Endogenous compounds were evaluated by spiking the potential interferent into drug free urine containing fentanyl at ±50% of the cutoff. All potential interferents analyzed verified that assay performance is unaffected by internally existing physiological conditions. The results of this study are indicated in Table 6 below:

| Table 6 - Endogenous Compounds | | | |
|---|---|---|---|
| Compound | Concentration Tested (ng/mL) | -50% Cutoff (0.5 ng/mL) | +50% Cutoff (1.5 ng/mL) |
| Acetone | 1.0 g/dL | Negative | Positive |
| Ascorbic Acid | 0.56 g/dL | Negative | Positive |
| Bilirubin | 2.0 mg/dL | Negative | Positive |
| Creatinine | 0.5 g/dL | Negative | Positive |
| Ethanol | 1.0 g/dL | Negative | Positive |
| Galactose | 10 mg/dL | Negative | Positive |
| γ-Globulin | 0.5 g/dL | Negative | Positive |
| Glucose | 2.0 g/dL | Negative | Positive |
| Hemoglobin | 0.5 g/dL | Negative | Positive |
| Human Serum Albumin | 0.5 g/dL | Negative | Positive |
| Oxalic Acid | 0.1 g/dL | Negative | Positive |
| Riboflavin | 7.5 mg/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 | 2.0 g/dL | Negative | Positive |

5. Boric Acid Interference – 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. The results of this study are indicated in Table 7 below.

| **Table 7 – Boric Acid** | | | |
|---|---|---|---|
| Compound | Concentration Tested (ng/mL) | -50% Cutoff (0.5 ng/mL) | +50% Cutoff (1.5 ng/mL) |
| Boric Acid | 1% w/v | Negative | Positive |

6. pH Interference – To evaluate potential interference from the effect of urine pH, device performance 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 fentanyl at ±50% of the cutoff. No positive or negative interference was observed at urine pH values ranging from 3.0 to 11.0 for each test mode. The results of this study are indicated in Table 8 below.

| **Table 8 - Effect of pH** | | |
|---|---|---|
| pH Value | -50% Cutoff (0.5 ng/mL) | +50% Cutoff (1.5 ng/mL) |
| 3.0 | Negative | Positive |
| 4.0 | Negative | Positive |
| 5.0 | Negative | Positive |
| 6.0 | Negative | Positive |
| 7.0 | Negative | Positive |
| 8.0 | Negative | Positive |
| 9.0 | Negative | Positive |
| 10.0 | Negative | Positive |
| 11.0 | Negative | Positive |

7. Specific Gravity Interference - To evaluate potential interference from the specific gravity of urine, device performance was tested using a range of physiologically relevant 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 fentanyl at ±50% of the cutoff. No positive or negative interference was observed at urine specific gravity values ranging from 1.000 to 1.030 for each test mode. The results of this study are indicated in Table 9 below.

| **Table 9 - Effect of Specific Gravity** | | |
|---|---|---|
| Specific Gravity Value | -50% Cutoff (0.5 ng/mL) | +50% Cutoff (1.5 ng/mL) |
| 1.000 | Negative | Positive |
| 1.002 | Negative | Positive |
| 1.005 | Negative | Positive |
| 1.010 | Negative | Positive |
| 1.015 | Negative | Positive |
| 1.020 | Negative | Positive |
| 1.025 | Negative | Positive |
| 1.030 | Negative | Positive |

8. Linearity/ Recovery - Not applicable, this device is intended for qualitative use only

9. Method Comparison – Eighty deidentified, unaltered leftover clinical urine samples obtained from clinical testing laboratories were analyzed for fentanyl at an assay cutoff of 1.0 ng/mL with the Immunalysis SEFRIA™ Fentanyl Urine Enzyme Immunoassay compared to results by mass spectrometry (LC-MS/MS). The instruments used were a Beckman Coulter AU 400e and an Agilent 6430 Liquid Chromatography Tandem Mass Spectrometer.

| **Table 12 –Performance Verified by LC/MS** | | | | | |
|---|---|---|---|---|---|
| SEFRIA™ Fentanyl Urine EIA Result | Fentanyl Concentration by LC/MS | | | | Agreement (%) |
| | < 0.5 ng/mL | 0.5 ~ 0.9 ng/mL | 1.0 ~ 1.5 ng/mL | > 1.5 ng/mL | |
| Positive | 0 | 1 | 9 | 31 | 100% (40/40) |
| Negative | 30 | 9 | 0 | 0 | 98% (39/40) |

The following is a summary table of qualitative discordant results for the 1.0 ng/mL cutoff:

| **Table 13 - Discordant Result Summary** | | | |
|---|---|---|---|
| Sample ID | In-House ID | 1ng Cutoff Test Device | LC/MS Confirmation |
| **Negative 36** | **21378** | Positive | 0.9 ng/mL |

10. Immunalysis Fentanyl Urine Calibrators

    a. Traceability – all components of the calibrators have been traced to a commercially available fentanyl solution.
    b. Value Assignment – 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 fentanyl in the negative calibrator matrix. If any of the analytes are not within the acceptable range, then the calibrator is adjusted and re-tested. Values are assigned to the calibrators once the mass spectrometry results are within the acceptable ranges.

H. Conclusion

The information provided in this pre-market notification demonstrates that the Immunalysis SEFRIA™ Fentanyl Urine Enzyme Immunoassay is substantially equivalent to the legally marketed predicate device for its intended use.