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

## A. 510(k) Number:

k181159

## B. Purpose for Submission:

New device

## C. Measurand:

Norfentanyl

## D. Type of Test:

Qualitative

## E. Applicant:

Lin-Zhi International, Inc.

## F. Proprietary and Established Names:

LZI Fentanyl Enzyme Immunoassay

## G. Regulatory Information:

| Product Code | Classification | Regulation Section | Panel |
|---|---|---|---|
| DJG | Class II | 21 CFR 862.3650, Opiate test system | Toxicology (91) |

## H. Intended Use:

1. Intended use(s):

   See Indication(s) for use below.

2. Indication(s) for use:

   The LZI Fentanyl Enzyme Immunoassay is intended for the qualitative determination of norfentanyl in human urine at the cutoff value of 5 ng/mL when calibrated against norfentanyl. The assay is designed for prescription use with a number of automated clinical chemistry analyzers.

The assay provides only a preliminary analytical result. A more specific alternative chemical method (e.g., gas or liquid chromatography and mass spectrometry) must be used in order to obtain a confirmed analytical result. Clinical consideration and professional judgment should be exercised with any drug of abuse test result, particularly when the preliminary test result is positive.

3. Special conditions for use statement(s):

   For prescription use only.

   For in vitro diagnostic use only.

4. Special instrument requirements:

   Beckman Coulter AU680 Clinical Chemistry Analyzer

## I. Device Description:

The LZI Fentanyl Enzyme Immunoassay is a kit comprised of two reagents, R1 and R2, which are bottled separately but sold together within the kit.

The R1 solution contains mouse monoclonal anti-norfentanyl antibody, glucose-6-phosphate (G6P) nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09 %) as a preservative. The R2 solution contains glucose-6-phosphate dehydrogenase (G6PDH) labeled with norfentanyl in buffer with sodium azide (0.09 %) as a preservative.

## J. Substantial Equivalence Information:

1. Predicate device name(s):

   Immunalysis SEFRIA Fentanyl Urine Enzyme Immunoassay

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

   k161216

3. Comparison with predicate:

| **Similarities and Differences** | | |
|---|---|---|
| Item | Candidate Device<br>LZI Fentanyl Enzyme Immunoassay | Predicate<br>Immunalysis SEFRIA Fentanyl Urine Enzyme Immunoassay<br>k161216 |
| Intended Use | In vitro diagnostic device intended for the qualitative | Same |

| Similarities and Differences | | |
|---|---|---|
| Item | Candidate Device<br>LZI Fentanyl Enzyme Immunoassay | Predicate<br>Immunalysis SEFRIA Fentanyl Urine Enzyme Immunoassay<br>k161216 |
| | determination of the presence of drugs of abuse in human urine | |
| Analyte | Norfentanyl | Fentanyl |
| Cutoff | 5 ng/mL | 1 ng/mL |
| Matrix | Urine | Same |
| Calibrator Levels | 0, 2.5, 5, 10, 20 ng/mL | 0, 1, 2, 4 ng/mL |
| Assay methodology | Absorbance change measured spectrophotometrically at 340 nm | Absorbance change measured spectrophotometrically at 570 nm |

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

Not applicable.

**L. Test Principle:**

The LZI Fentanyl Enzyme Immunoassay is a homogeneous enzyme immunoassay with ready-to-use liquid reagents. It detects and is calibrated against norfentanyl, the major metabolite of fentanyl in human urine. The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody in the reagent. In the absence of drug in the sample, norfentanyl-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. When free norfentanyl is present in the sample, it competes with norfentanyl-labeled G6PDH for antibody binding, allowing for maximal enzyme activity. Thus G6PDH activity is proportional to the amount of free fentanyl in the sample. Active G6PDH converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that can be measured spectrophotometrically at 340 nm.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

*a. Precision/Reproducibility:*

The sponsor conducted a qualitative precision study on one Beckman Coulter AU680 analyzer using samples containing norfentanyl. Samples were prepared by spiking pooled negative human urine with norfentanyl to concentrations of ±25%, ±50%, ±75%, and ±100% of the cutoff. All concentrations were confirmed by GC/MS

testing. Samples were tested in two replicates per run, two runs per day for 22 days, total n = 88. Results are presented below. For within-run precision, the mean test results of each day's four data points were calculated and averaged for all 22 days.

| 5 ng/mL Cutoff Result | | Within Run (N=22) | | Total Precision (N=88) | |
|---|---|---|---|---|---|
| **Norfentanyl Concentration** | **% of Cutoff** | **N** | **Assay Result** | **N** | **Assay Result** |
| 0 ng/mL | 0 % | 22 | 22 Negative | 88 | 88 Negative |
| 1.25 ng/mL | 25.0 % | 22 | 22 Negative | 88 | 88 Negative |
| 2.5 ng/mL | 50.0 % | 22 | 22 Negative | 88 | 88 Negative |
| 3.75 ng/mL | 75.0 % | 22 | 22 Negative | 88 | 88 Negative |
| 5 ng/mL | 100.0 % | 22 | 2 Positive/20 Negative | 88 | 26 Positive/62 Negative |
| 6.25 ng/mL | 125.0 % | 22 | 22 Positive | 88 | 88 Positive |
| 7.5 ng/mL | 150.0 % | 22 | 22 Positive | 88 | 88 Positive |
| 8.75 ng/mL | 175.0 % | 22 | 22 Positive | 88 | 88 Positive |
| 10 ng/mL | 200.0 % | 22 | 22 Positive | 88 | 88 Positive |

b. *Linearity/assay reportable range:*

Not applicable, this device is intended for qualitative use only.

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

The LZI Fentanyl Enzyme Immunoassay is traceable to a commercially available norfentanyl standard.

d. *Detection limit:*

Not applicable.

e. *Analytical specificity:*

Cross reactivity from structurally related compounds was evaluated by testing pooled negative urine samples spiked with these compounds. Samples were tested in duplicate. The compounds and concentrations tested and the calculated cross-reactivity are provided in the table below. Percent cross-reactivity was calculated as the lowest concentration of the compound where the assay response was positive/the cutoff concentration x 100.

| Compound | Concentration Tested (ng/mL) | % Cross-Reactivity | Test Result |
|---|---|---|---|
| Fentanyl | 2.50 | 200.0 % | Positive |
| Norfentanyl | 5.00 | 100.0 % | Positive |
| 4-Fluoro-isobutyryl Fentanyl | 35 | 14.29% | Negative |
| 9-HydroxyRisperidone | 100,000 | 0.01% | Negative |
| Acetyl Fentanyl | 7 | 71.43% | Positive |

| Compound | Concentration Tested (ng/mL) | % Cross-Reactivity | Test Result |
|---|---|---|---|
| Acetyl Norfentanyl | 100 | 5.00% | Negative |
| Acryl Fentanyl | 3.5 | 142.86% | Positive |
| Alfentanil | 100,000 | 0.01% | Negative |
| Butyryl Fentanyl | 3.5 | 142.86% | Positive |
| Carfentanil Oxalate | 100,000 | 0.01% | Negative |
| Cis-d, I 3-Methylfentanyl | 8.5 | 58.82% | Negative |
| Despropionylfentanyl(4-ANPP) | 100,000 | 0.01% | Negative |
| Furanyl Fentanyl | 6 | 81.97% | Positive |
| Isobutyryl Fentanyl | 20 | 25.00% | Negative |
| Labetalol Hydrochloride | 100,000 | 0.01% | Negative |
| MT-45 | 100,000 | 0.01% | Negative |
| Norcarfentail Oxalate | 100,000 | 0.01% | Negative |
| Ocfentanil | 3.5 | 142.86% | Positive |
| Para-fluoro butyrl Fentanyl (P-FBF) | 5.5 | 90.91% | Positive |
| para-Fluorofentanyl | 3.05 | 163.93% | Positive |
| Remifentanil | 100,000 | 0.01% | Negative |
| Risperidone | 100,000 | 0.01% | Negative |
| Sufentanil | 100,000 | 0.01% | Negative |
| Thienyl Fentnayl | 3.5 | 142.86% | Negative |
| Thiofentanyl | 3.2 | 156.25% | Positive |
| Trans-d, I 3-Methylfentanyl | 6 | 83.33% | Positive |
| Trazodone | 100,000 | 0.01% | Negative |
| U-47700 | 100,000 | 0.01% | Negative |
| Valeryl Fentanyl | 95 | 5.26% | Negative |
| ω-1-Hydroxy Fentanyl | 320.0 | 1.56% | Negative |

Potential interference from endogenous substances was evaluated by spiking these substances into pooled negative human urine containing norfentanyl at +25% and -25% of the 5 ng/mL cutoff (3.75 ng/mL and 6.25 ng/mL). Samples were tested in duplicate.

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

| Compound | Concentration Tested (mg/dL) |
|---|---|
| Acetone | 1000 |
| Ascorbic Acid | 1500 |
| Bilirubin | 2 |
| Creatinine | 500 |
| Ethanol | 1000 |
| Galactose | 10 |
| Gamma globulin | 500 |
| Glucose | 3000 |

| Compound | Concentration Tested (mg/dL) |
|---|---|
| Hemoglobin | 300 |
| Beta-Hydroxybutyric Acid | 100 |
| Human Serum Albumin | 500 |
| Oxalic Acid | 100 |
| Riboflavin | 7.5 |
| Urea | 6000 |
| Uric Acid | 10 |
| Sodium Azide | 1000 |
| Sodium Chloride | 6000 |

Citric Acid (800 mg/dL) and Potassium Chloride (6000 mg/dL) were evaluated by spiking these compounds into processed negative urine containing norfentanyl at + 50% and -50% of the 5 ng/mL cutoff (2.5 ng/mL and 7.5 ng/mL). The sponsor stated that no significant interference was observed.

Boric acid at a concentration of 1% w/v was evaluated by spiking the potential interferent into processed negative urine containing norfentanyl at +25% and -25% of the 5 ng/mL cutoff (3.75 ng/mL and 6.25 ng/mL). The labeling contains the following limitation:

*Boric acid at 1% w/v may cause false negative results. Boric acid is not recommended as a preservative for urine.*

Effect of pH: The sponsor evaluated the effect of pH using pooled negative human urine containing norfentanyl at +25% and -25% of the 5 ng/mL cutoff (3.75 ng/mL and 6.25 ng/mL). pH values of 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0 did not significantly interfere with the test.

Effect of specific gravity: The sponsor evaluated the effect of specific gravity on the test results using drug free urine samples containing norfentanyl at +25% and -25% of the 5 ng/mL cutoff (3.75 ng/mL and 6.25 ng/mL). Specific Gravity values of 1.003, 1.004, 1.007, 1.011, 1.012, 1.017, 1.018, 1.021, 1.024, 1.028 did not interfere with the test.

Potential interference from structurally unrelated compounds was evaluated by spiking these compounds into pooled negative human urine containing norfentanyl at +25% and -25% of the 5 ng/mL cutoff (3.75 ng/mL and 6.25ng/mL). Samples were tested in duplicate. The following non-structurally related substances at the concentrations listed below did not significantly interfere with the assay.

| Compound | Concentration Tested (ng/mL) |
|---|---|
| Acetaminophen | 100,000 |
| 6-Acetylmorphine | 10,000 |

| Compound | Concentration Tested (ng/mL) |
|---|---|
| Acetylsalicylic Acid | 100,000 |
| Amitriptyline | 100,000 |
| Amlodipine Besylate | 100,000 |
| Amoxicillin | 100,000 |
| d-Amphetamine | 100,000 |
| Atorvastatin | 20,000 |
| Buprenorphine | 100,000 |
| Bupropion | 100,000 |
| Caffeine | 100,000 |
| Carbamazepine | 100,000 |
| Cetirizine | 100,000 |
| Chlorpheniramine | 100,000 |
| Chlorpromazine | 100,000 |
| Clomipramine | 100,000 |
| Codeine | 100,000 |
| Desipramine | 100,000 |
| Diphenhydramine | 100,000 |
| Duloxetine | 100,000 |
| Fluoxetine | 100,000 |
| Fluphenazine | 100,000 |
| Gabapentin | 100,000 |
| Hydrocodone | 100,000 |
| Hydromorphone | 100,000 |
| Ibuprofen | 100,000 |
| Imipramine | 100,000 |
| Lisinopril | 100,000 |
| Losartan | 10,000 |
| Loratidine | 100,000 |
| MDA (3,4-methylene dioxyamphetamine) | 100,000 |
| MDEA | 100,000 |
| MDMA (3,4-methylenedioxymethamphetamine) | 100,000 |
| Meperidine | 100,000 |
| Metformin | 100,000 |
| Metoprolol | 100,000 |
| Methadone | 100,000 |
| Morphine | 100,000 |
| Nicotine | 100,000 |
| Nortriptyline | 100,000 |
| Omeprazole | 100,000 |
| Oxazepam | 100,000 |
| Oxycodone | 100,000 |

| Compound | Concentration Tested (ng/mL) |
|---|---|
| Oxymorphone | 100,000 |
| Phencyclidine (PCP) | 100,000 |
| Phenobarbital | 100,000 |
| (1S,2S)-(+)Pseudoephedrine | 100,000 |
| Quetiapine | 100,000 |
| Ranitidine | 100,000 |
| Salbutamol (Albuterol) | 100,000 |
| Sertraline | 100,000 |
| THC-COOH (11-Nor-Delta-9-THC-9-carboxylic acid) | 100,000 |
| L-Thyroxine | 10,000 |
| Tramadol | 100,000 |
| Zolpidem | 10,000 |

Dextromethorphan was found to interfere at 40,000 ng/mL:

| Compound | Concentration tested (ng/mL) | Test Result | | |
|---|---|---|---|---|
| | | Negative | -25% Cutoff | + 25% Cutoff |
| Dextromethorphan | 40,000 | Positive | Positive | Positive |

The labeling includes the following limitations:

*Dextromorphan may cause false positive results at concentrations greater than 5 µg/mL.*

*f. Assay cut-off:*

Analytical performance of the device around the claimed cutoff (5 ng/mL) is described in the precision section M.1.a above and accuracy section M.2.a below.

2. Comparison studies:

*a. Method comparison with predicate device:*

A total of 101 unaltered clinical samples were tested with the LZI Fentanyl Enzyme Immunoassay on the AU680 automated clinical analyzer. All samples were confirmed with LC/MS for norfentanyl concentrations. Results are shown below:

| **Candidate Device Results** | **Negative** by LC/MS analysis | **< 50 % of the cutoff concentration** by LC/MS analysis | **Near Cutoff Negative** (Between 50 % below the cutoff and the cutoff concentration by LC/MS analysis) | **Near Cutoff Positive** (Between the cutoff and 50 % above the cutoff concentration by LC/MS analysis) | **High Positive** (Greater than 50 % above the cutoff concentration by LC/MS analysis) |
|---|---|---|---|---|---|
| **Positive** | 0 | 1 | 6 | 8 | 41 |
| **Negative** | 21 | 19 | 5 | 0 | 0 |

Discordant samples:

| **Sample #** | **LC/MS Norfentanyl (ng/mL)** | **Candidate Device Result** |
|---|---|---|
| 38* | 1.5 | Positive |
| 44 | 3.0 | Positive |
| 46 | 3.3 | Positive |
| 47 | 3.5 | Positive |
| 48 | 3.8 | Positive |
| 50 | 4.16 | Positive |
| 52 | 4.6 | Positive |

* This sample contained levels of fentanyl that contributed to the false positive result.

The sponsor provided additional information for FDA review that supports the clinical validity of a 5 ng/mL norfentanyl cutoff. Over 7,000 de-identified urine samples, originating from patients presenting at various hospital clinics, substance abuse clinics, and emergency departments were evaluated for the presence of fentanyl and norfentanyl using an LC/MS method. Approximately 600 samples contained amounts of either fentanyl or norfentanyl above the LC/MS method detection limit. Assuming a 1 ng/mL fentanyl cutoff (previously cleared in k161216), 89.1% of these samples would have been determined to be positive for fentanyl. Assuming a 5 ng/mL norfentanyl cutoff, 93.6% of these samples would have been determined to be positive for norfentanyl. 9.0% of these samples were not above the 1 ng/mL fentanyl cutoff, but were above the 5 ng/mL norfentanyl cutoff. 4.5% of these samples were above the 1 ng/mL fentanyl cutoff, but were not above the 5 ng/mL norfentanyl cutoff. Therefore, the sponsor provided sufficient information to support that a 5 ng/mL norfentanyl cutoff demonstrates similar positivity and negativity rates to the 1 ng/mL fentanyl cutoff.

*b. Matrix comparison:*

Not applicable. This assay 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 Parts 801 and 809, as applicable.

**O. Conclusion:**

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.