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

**A. 510(k) Number:**

k180427

**B. Purpose for Submission:**

New device

**C. Measurand:**

Fentanyl

**D. Type of Test:**

Qualitative, homogeneous enzyme immunoassay

**E. Applicant:**

ARK Diagnostics, Inc.

**F. Proprietary and Established Names:**

ARK Fentanyl Assay

**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):

   Refer to Indications for Use.

2. Indication(s) for use:

   The ARK Fentanyl Assay is an immunoassay intended for the qualitative determination of fentanyl in human urine at a cutoff concentration of 1.0

ng/mL. The assay is intended for use in laboratories with automated clinical chemistry analyzers. This in vitro diagnostic device is for prescription use only.

The ARK Fentanyl Assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/tandem Mass Spectrometry (LC-MS/MS) is the preferred confirmatory method. Clinical consideration and professional judgment should be exercised with any drug test result, particularly when the preliminary test result is positive.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   Performance data was collected on the Beckman Coulter AU680 analyzer.

## I. Device Description:

The ARK Fentanyl Assay is a homogeneous enzyme immunoassay used for the detection of fentanyl in human urine. The ARK Fentanyl Assay consists of reagents R1 rabbit anti-fentanyl polyclonal antibody with substrate and R2 fentanyl derivative labeled with bacterial recombinant G6PDH enzyme. The ARK Fentanyl Assay test system includes separately provided kits for the ARK Fentanyl Assay, ARK Fentanyl Calibrator and ARK Fentanyl Control.

## 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 | | |
|---|---|---|
| Item | Candidate Device | Predicate device – k161216 |
| Intended Use | Same | For the determination of fentanyl in human urine at a cutoff concentration of 1.0 ng/mL. |
| Methodology | Same | Homogenous enzyme immunoassay (EIA) |
| Sample Matrix | Same | Urine |
| User Environment | Same | Clinical laboratories; Prescription use only |
| Platform Required | Same | Automated clinical chemistry analyzer |
| Reagents Form | Same | Liquid – Ready to use |
| Reagent Materials | Same | Two (2) reagent system: Antibody/substrate reagent and enzyme labeled conjugate Sodium azide preservative |
| Stability | Same | 2-8˚C until expiration date |
| Antibody | Same | Rabbit antibodies to fentanyl |
| Cutoff | Same | 1.0 ng/mL |

| Differences | | |
|---|---|---|
| Item | Candidate Device | Predicate device – k161216 |
| Detection Wavelength | Absorbance change measured spectrophotometrically at 340 nm. | Absorbance change measured spectrophotometrically at 570 nm. |

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

- CLSI EP07-A2: Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition
- CLSI EP12-A2: User Protocol For Evaluation Of Qualitative Test Performance; Approved Guideline - Second Edition

**L. Test Principle:**

The ARK Fentanyl Assay is a homogeneous enzyme immunoassay technique used for the analysis of fentanyl in human urine. The assay is based on competition between fentanyl in the patient specimen and fentanyl labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH) for antibody binding sites. As the latter binds antibody, enzyme activity decreases. In the presence of fentany from

the patient specimen, enzyme activity increases and is proportional to the fentanyl concentration. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH in the presence of glucose-6-phosphate (G6P), resulting in an absorbance change that is measured spectrophotometrically.

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

1. Analytical performance:

*a. Precision/Reproducibility:*

A precision study data was conducted over 20 non-consecutive days, with two runs per day in replicates of four, on a single Beckman Coulter AU680 automated clinical chemistry analyzer. Samples were prepared in a human urine pool negative for fentanyl.

Results are summarized below:

| Human Urine (ng/mL) | Relative % Cutoff | # of Results | Results |
|---|---|---|---|
| 0.00 | -100 | 160 | 160 Negative |
| 0.25 | -75 | 160 | 160 Negative |
| 0.50 | -50 | 160 | 160 Negative |
| 0.75 | -25 | 160 | 160 Negative |
| 1.00 | Cutoff | 160 | 97 Negative; 63 Positive |
| 1.25 | 25 | 160 | 160 Positive |
| 1.50 | 50 | 160 | 160 Positive |
| 1.75 | 75 | 160 | 160 Positive |
| 2.00 | 100 | 160 | 160 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):*

ARK Fentanyl Calibrators are are traceable to a commercially available certified standard solution, and are prepared by volumetric dilution, with concentrations verified by high-performance liquid chromatography.

*d. Detection limit:*

Not applicable.

*e. Analytical specificity:*

The sponsor evaluated the cross-reactivity of the following metabolites and structural analogs of Fentanyl by spiking them into drug-free, negative human urine to determine the minimum concentration that would give a positive result approximately equivalent to the 1.0 ng/mL fentanyl cutoff. These concentrations were used to determine the percent cross-reactivity according to the formula:

Percent cross-reactivity = (Cutoff concentration / lowest concentration of cross-reactant causing a positive result) x 100

Cross-reactivity:

Norfentanyl (major metabolite)

| **Compound** | **Concentration tested (ng/mL)** | **Percent Cross reactivity** |
|---|---|---|
| Norfentanyl (major metabolite)* | 2.5 | 10 |
| | 300 | 0.33 |

*Cross-reactivity of norfentanyl was observed to be non-linear

Other Metabolites and Structural Analogs of Fentanyl

| **Compound** | **Lowest concentration that produces a response approximately equivalent to the cutoff (ng/mL)** | **Percent Cross reactivity** |
|---|---|---|
| Acetyl fentanyl | 1.20 | 83.33 |
| Acrylfentanyl | 1.20 | 83.33 |
| ω-1-Hydroxyfentanyl | 1.20 | 83.33 |
| Isobutyryl fentanyl | 1.50 | 66.67 |
| Ocfentanil | 1.50 | 66.67 |
| Butyryl fentanyl | 1.60 | 62.50 |
| Furanyl fentanyl | 1.75 | 57.14 |
| Valeryl fentanyl | 2.50 | 40.00 |
| (±) β-hydroxythiofentanyl | 2.80 | 35.71 |

| Compound | Lowest concentration that produces a response approximately equivalent to the cutoff (ng/mL) | Percent Cross reactivity |
|---|---|---|
| 4-Fluoro-isobutyryl fentanyl | 3.00 | 33.33 |
| Para-fluorobutyryl fentanyl (p-FBF) | 3.00 | 33.33 |
| Para-fluoro fentanyl | 3.00 | 33.33 |
| (±)-3-cis-methyl fentanyl | 5.00 | 20.00 |
| Despropionyl fentanyl (4- ANPP) | 75.00 | 1.33 |
| Carfentanil | 500 | 0.20 |
| Sufentanil | 625 | 0.16 |
| Norcarfentanil | 5,000 | <0.02 |
| Acetyl norfentanyl | 10,000 | 0.01 |
| Remifentanil | 10,000 | <0.01 |
| Alfentanil | 100,000 | <0.001 |

The sponsor also provided results from a study evaluating whether other structurally similar compounds cross-reacted with the candidate device. None of the compounds below caused a positive result at the concentrations indicated.

| Compound | Concentration Tested (ng/mL) |
|---|---|
| 6-Acetyl morphine | 10,000 |
| Amphetamine | 100,000 |
| Buprenorphine | 100,000 |
| Buprenorphine glucuronide | 50,000 |
| Codeine | 100,000 |
| Dextromethorphan | 100,000 |
| Dihydrocodeine | 100,000 |
| EDDP | 100,000 |
| EMDP | 50,000 |
| Fluoxetine | 50,000 |
| Heroin | 30,000 |

| Compound | Concentration Tested (ng/mL) |
|---|---|
| Hydrocodone | 100,000 |
| Hydromorphone | 100,000 |
| Ketamine | 100,000 |
| Levorphanol | 50,000 |
| Meperidine | 100,000 |
| Methadone | 100,000 |
| Morphine | 100,000 |
| Morphine-3-glucuronide | 50,000 |
| Naloxone | 50,000 |
| Naltrexone | 50,000 |
| Norbuprenorphine | 50,000 |
| Norcodeine | 50,000 |
| Norketamine | 100,000 |
| Normeperidine | 100,000 |
| Normorphine | 50,000 |
| Noroxycodone | 100,000 |
| Oxycodone | 100,000 |
| Oxymorphone | 50,000 |
| Pentazocine (Talwin) | 10,000 |
| Pipamperone | 100,000 |
| Risperidone | 2,000 |
| Tapentadol | 50,000 |
| Thioridazine | 50,000 |
| Tilidine | 50,000 |
| Tramadol | 100,000 |
| Tramadol-O-Desmethyl | 100,000 |
| Tramadol-N-Desmethyl | 100,000 |
| Trazodone | 10,000 |

The sponsor performed a study to evaluate whether common endogenous compounds cause negative or positive interference. These compounds were spiked, at the concentrations listed below, into urine samples containingfentanyl at concentrations corresponding to -50% and +50% of the cutoff. None of the compounds tested caused a positive result at -50% of the cutoff or a negative result at +50% of the cutoff.

| Compound | Concentration Tested (mg/dL) | Candidate Device Result | |
|---|---|---|---|
| | | 0.5 ng/mL Fentanyl | 1.5 ng/mL Fentanyl |
| Acetone | 1000 | NEG | POS |
| Ascorbic Acid | 560 | NEG | POS |
| Bilirubin | 2.0 | NEG | POS |
| Creatinine | 500 | NEG | POS |
| Ethanol | 1000 | NEG | POS |
| Galactose | 10 | NEG | POS |
| Gamma globulin | 500 | NEG | POS |
| Glucose | 3000 | NEG | POS |
| Hemoglobin | 500 | NEG | POS |
| Human Albumin | 500 | NEG | POS |
| Oxalic Acid | 100 | NEG | POS |
| Riboflavin | 7.5 | NEG | POS |
| Sodium Chloride | 4000 | NEG | POS |
| Urea | 2000 | NEG | POS |

The sponsor performed a study to evaluate whether low or high pH causes negative or positive interference with the candidate device. Samples containing fentanyl concentrations corresponding to -50% and +50% of the cutoff were adjusted to pH levels ranging from 3.0 to 11.0. None of the pH levels tested caused a positive result at -50% of the cutoff or a negative result at +50% of the cutoff.

| Compound Tested | Candidate Device Result | |
|---|---|---|
| | 0.5 ng/mL Fentanyl (POS/NEG) | 1.5 ng/mL Fentanyl (POS/NEG) |
| Urine pH 3.0 | NEG | POS |
| Urine pH 4.0 | NEG | POS |
| Urine pH 5.0 | NEG | POS |
| Urine pH 6.0 | NEG | POS |
| Urine pH 7.0 | NEG | POS |
| Urine pH 8.0 | NEG | POS |
| Urine pH 9.0 | NEG | POS |
| Urine pH 10.0 | NEG | POS |
| Urine pH 11.0 | NEG | POS |

The sponsor performed a study to evaluate whether low or high specific gravity causes negative or positive interference. Samples containing

fentanyl concentrations corresponding to -50% and +50% of the cutoff were adjusted to specific gravity levels ranging from 1.001 to 1.030. None of the specific gravity levels tested caused a positive result at -50% of the cutoff or a negative result at +50% of the cutoff.

| Specific Gravity Tested | Candidate Device Result | |
|---|---|---|
| | 0.5 ng/ml Fentanyl N=3 (POS/NEG) | 1.5 ng/ml Fentanyl N=3 (POS/NEG) |
| 1.001 | NEG | POS |
| 1.002 | NEG | POS |
| 1.004 | NEG | POS |
| 1.010 | NEG | POS |
| 1.018 | NEG | POS |
| 1.024 | NEG | POS |
| 1.026 | NEG | POS |
| 1.028 | NEG | POS |
| 1.030 | NEG | POS |

The sponsor evaluated boric acid and determined that it causes false negative results. The use of boric acid as a preservative is contraindicated in the device labeling.

| Compound | Concentration Tested | Candidate Device Result | |
|---|---|---|---|
| | | 0.5 ng/ml Fentanyl Mean of N=3 (ng/mL) | 1.5 ng/ml Fentanyl Mean of N=3 (ng/mL) |
| Boric Acid | 1% w/v | NEG | NEG |

The sponsor performed a study to evaluate whether structurally unrelated compounds cause negative or positive interference with the candidate device. These compounds at the concentrations listed in the table below were spiked into urine samples containing fentanyl concentrations corresponding to -50% and +50% of the cutoff. None of the compounds tested caused a positive result at -50% of the cutoff or a negative result at +50% of the cutoff.

| Compound | Concentration Tested (µg/mL) | Candidate Device Result | |
|---|---|---|---|
| | | 0.5 ng/mL Fentanyl (POS/NEG) | 1.5 ng/mL Fentanyl (POS/NEG) |
| Acetaminophen | 500 | NEG | POS |
| Acetylsalicylic acid | 1000 | NEG | POS |
| Albuterol | 100 | NEG | POS |
| Amitriptyline | 35 | NEG | POS |
| Amobarbital | 100 | NEG | POS |
| Benzoylecgonine | 100 | NEG | POS |
| Bupropion | 50 | NEG | POS |
| Caffeine | 100 | NEG | POS |
| Carbamazepine | 100 | NEG | POS |
| Chlorpromazine | 50 | NEG | POS |
| Clomipramine | 50 | NEG | POS |
| Cyclobenzaprine | 10 | NEG | POS |
| Desipramine | 50 | NEG | POS |
| Doxepin | 50 | NEG | POS |
| Ecgonine | 100 | NEG | POS |
| Ephedrine | 100 | NEG | POS |
| Fluphenazine | 100 | NEG | POS |
| Ibuprofen | 500 | NEG | POS |
| Imipramine | 30 | NEG | POS |
| Lidocaine | 50 | NEG | POS |
| Maprotiline | 50 | NEG | POS |
| Methapyrilene | 10 | NEG | POS |
| Methaqualone | 50 | NEG | POS |
| Metronidazole | 300 | NEG | POS |
| Nicotine | 10 | NEG | POS |
| Nortriptyline | 25 | NEG | POS |
| Oxazepam | 100 | NEG | POS |
| Phencyclidine | 100 | NEG | POS |
| Phenobarbital | 100 | NEG | POS |
| Propoxyphene | 50 | NEG | POS |
| Ranitidine | 100 | NEG | POS |
| Secobarbital | 100 | NEG | POS |
| Valproic acid | 250 | NEG | POS |
| Venlafaxine | 100 | NEG | POS |

*f. Assay cut-off:*

Characterization of how the device performs analytically around the claimed cutoff concentration of 1 ng/mL fentanyl is described in the

precision section, M.1.a. above.

2. Comparison studies:

*a. Method comparison with predicate device:*

100 confirmed fentanyl-positive and 50 confirmed fentanyl-negative unaltered clinical urine specimens were analyzed with the ARK Fentanyl Assay in single replicates on a Beckman Coulter AU680 analyzer and compared to results obtained by LC-MS/MS. Results are summarized in the following table:

| Candidate Device Result | Low Negative Less than 50% below the Cutoff (< 0.5 ng/mL by LC-MS/MS) | Near Cutoff Negative Between 50% below the Cutoff and the Cutoff (0.5 – 0.9 ng/mL by LC-MS/MS) | Near Cutoff Positive Between the Cutoff and 50% above the Cutoff (1.0 – 1.5 ng/mL by LC-MS/MS) | High Positive Greater than 50% above the Cutoff (> 1.5 ng/mL by LC-MS/MS) |
|---|---|---|---|---|
| **Positive** | 1* | 20 | 12 | 64 |
| **Negative** | 50 | 3 | 0 | 0 |

Discordant results:

*Norfentanyl was detected in this discordant sample and contributed to the positive result obtained by the candidate device for this sample.

| Sample Number | Immunoassay result | Fentanyl (ng/mL by LC-MS/MS) | Sample Number | Immunoassay result | Fentanyl (ng/mL by LC-MS/MS) |
|---|---|---|---|---|---|
| 051 | Positive | 0.7 | 062 | Positive | 0.6 |
| 052* | Positive | 0.4 | 063 | Positive | 0.9 |
| 053 | Positive | 0.9 | 064 | Positive | 0.8 |
| 054 | Positive | 0.9 | 065 | Positive | 0.5 |
| 055 | Positive | 0.6 | 066 | Positive | 0.7 |
| 056 | Positive | 0.6 | 069 | Positive | 0.5 |
| 057 | Positive | 0.9 | 070 | Positive | 0.6 |
| 058 | Positive | 0.5 | 072 | Positive | 0.6 |

| Sample Number | Immunoassay result | Fentanyl (ng/mL by LC-MS/MS) | Sample Number | Immunoassay result | Fentanyl (ng/mL by LC-MS/MS) |
|---|---|---|---|---|---|
| 059 | Positive | 0.9 | 073 | Positive | 0.8 |
| 060 | Positive | 0.5 | 074 | Positive | 0.6 |
| 061 | Positive | 0.9 | | | |

*b. Matrix comparison:*

Not applicable. The device is intended to be used with human 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.