ARK Diagnostics, Inc.

ARK™ *Fentanyl II Assay*

This ARK Diagnostics, Inc. package insert for the ARK Fentanyl II Assay must be read prior to use. Package insert instructions must be followed accordingly. The assay provides a simple and rapid analytical screening procedure for detecting Fentanyl in urine. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

CUSTOMER SERVICE

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KEY TO SYMBOLS USED

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
	Consult Instructions for Use	R1 / R2	Reagent 1/ Reagent 2
	Temperature limitation	IVD	*In Vitro* Diagnostic Medical Device
Rx Only	For Prescription Use Only		

1 NAME

ARK™ *Fentanyl II Assay*

2 INTENDED USE

The ARK Fentanyl II Assay is an immunoassay intended for the qualitative detection 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 II Assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed positive 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 SUMMARY AND EXPLANATION OF THE TEST

Fentanyl [N-(1-(2-phenylethyl)-4-piperidinyl)-N-phenylpropanamide] is a synthetic opioid narcotic analgesic similar to morphine.[1] Fentanyl is 50-100 times more potent than morphine. It is prescribed for patients with chronic pain and is used to manage pain after surgery or for treatment of breakthrough pain in cancer patients.[2] Fentanyl is prescribed in various forms: by injection (intravenous or intramuscular), transdermal patch[3], and orally (transmucosal lozenge or film). Fentanyl such as the transdermal system can be abused in a manner similar to other opioid agonists, legal or illicit. All patients receiving opioids should be routinely monitored for signs of misuse, abuse and addiction.

Fentanyl has high potency and short duration of action, and it is abused for its intense euphoric effects. It is very dangerous when substituted illicitly for other opioids because of its potency and overdoses can lead to respiratory depression and death.[4,5] It is a Schedule II substance under the U.S. Controlled Substances Act.

The ARK Fentanyl II Assay detects fentanyl in human urine. The test is not intended to differentiate between drugs of abuse and prescription use of fentanyl. There are no uniformly recognized drug levels for fentanyl in urine.

The primary metabolism of fentanyl leads to the time-dependent urinary excretion of fentanyl and norfentanyl.[6-8] The half-life of fentanyl may range 3 - 12 hours. Fentanyl is exclusively metabolized by N-dealkylation and hydroxylation. More than 90% of the dose is eliminated as norfentanyl and hydroxylated metabolites. Less than 7% of the dose is excreted unchanged in the urine.

4 PRINCIPLES OF THE PROCEDURE

The ARK Fentanyl II Assay is a homogeneous enzyme immunoassay technique used for the analysis of a specific compound in human urine. The assay is based on competition between drug in the specimen and drug labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH) for antibody binding sites. As the latter binds antibody, enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly related to the drug 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. Endogenous G6PDH does not interfere because the coenzyme NAD functions only with the bacterial enzyme used in the assay.

5 REAGENTS

REF	Product Description	Quantity/Volume
5069-0001-00	**ARK Fentanyl II Assay** **Reagent R1 – Antibody/Substrate** Rabbit monoclonal antibodies to fentanyl, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 28 mL
	Reagent R2 – Enzyme Fentanyl derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), bovine serum albumin, buffer, sodium azide and stabilizers	1 X 28 mL

Reagent Kit REF 5069-0001-00

Reagent Kit REF 5069-0001-01

Reagent Kit REF 5069-0001-02

REF	Product Description	Quantity/Volume
5069-0001-01	**ARK Fentanyl II Assay** **Reagent R1 – Antibody/Substrate** Rabbit monoclonal antibodies to fentanyl, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 115 mL
	Reagent R2 – Enzyme Fentanyl derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), bovine serum albumin, buffer, sodium azide and stabilizers	1 X 115 mL

REF	Product Description	Quantity/Volume
5069-0001-02	**ARK Fentanyl II Assay** **Reagent R1 – Antibody/Substrate** Rabbit monoclonal antibodies to fentanyl, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 500 mL
	Reagent R2 – Enzyme Fentanyl derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), bovine serum albumin, buffer, sodium azide and stabilizers	1 X 500 mL

Reagent Handling and Storage

ARK Fentanyl II Assay reagents are provided liquid, ready to use and may be used directly from the refrigerator. When not in use, reagents must be stored at 2–8°C (36–46°F), upright and with screw caps tightly closed. If stored as directed, reagents are stable until the expiration date printed on the label. Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°C (90°F). **Improper storage of reagents can affect assay performance.**

ARK Fentanyl II products contain ≤0.09% sodium azide. As a precaution, affected plumbing including instrumentation should be flushed adequately with water to mitigate the potential accumulation of explosive metal azides. No special handling is required regarding other assay components.

6 WARNINGS AND PRECAUTIONS

- For *In Vitro* Diagnostic Use. For prescription use only. *Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.*
- Reagents R1 and R2 are provided as a matched set and should not be interchanged with reagents from different lot numbers.
- Do not use reagents after the expiration date.
- Reagents contain ≤0.09% sodium azide.

7 SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Human urine is required. Treat as potentially infectious material.
- Collect urine using standard sampling cups and procedures. Care should be taken to preserve the chemical and physical integrity of the urine sample from the time it is collected until the time it is assayed, including during transport. Fresh urine specimens are suggested.
- Cap the urine sample immediately after collection, store refrigerated at 2-8°C (36–46°F) and assay within 7 days after collection. If the assay cannot be performed within 7 days, store the urine sample frozen at -20°C for up to 6 months prior to analysis.[9,10,11,12]
- To protect the integrity of the sample, do not induce foaming and avoid repeated freezing and thawing.
- Frozen specimens must be thawed and mixed thoroughly prior to analysis.
- Centrifuge specimens with high turbidity or visible particulate matter before testing.
- The presence of bubbles or foam on specimens may lead to short samples and erroneous results.
- The recommended pH range for urine specimens is 4.0 – 11.0[13].
- Obtain another sample for testing if adulteration of the sample is suspected. Adulteration of urine specimens can affect the test result.

8 PROCEDURE

Materials Provided

ARK Fentanyl II Assay – REF 5069-0001-00, 5069-0001-01 or 5069-0001-02

Materials Required – Provided Separately

ARK Fentanyl Calibrator A (Negative) – REF 5031-0002-01
ARK Fentanyl Calibrator B (Cutoff) – REF 5031-0002-02
Quality Controls – ARK Fentanyl Control – REF 5031-0003-00

Instruments

Reagents R1 and R2 may need to be transferred to analyzer-specific reagent containers prior to use. Avoid cross-contamination of R1 and R2. Refer to the instrument-specific operator's manual for daily maintenance. Consult the analyzer-specific application sheet for programming the fentanyl assay or contact Customer Support.

Assay Sequence

To run or calibrate the assay, see the instrument-specific operator's manual.

Qualitative Results

Use the 1.0 ng/mL Calibrator B as a Cutoff Calibrator to distinguish negative and positive samples. Run the Low and High Controls as Negative and Positive respectively. Report test results less than the rate (mA/min) value for the Cutoff Calibrator as Negative. Report results equal to or greater than the rate (mA/min) value for the Cutoff Calibrator as Positive.

When to Re-Calibrate

- Whenever a new lot number of reagents is used
- Whenever indicated by quality control results
- Whenever required by standard laboratory protocols

Quality Control (QC) and Calibration

Laboratories should establish QC procedures for the ARK Fentanyl II Assay. All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Each laboratory should establish its own ranges for each new lot of controls. Control results should fall within established ranges as determined by laboratory procedures and guidelines. The ARK Fentanyl Control is intended for use in quality control of the ARK Fentanyl II Assay.

The Low Control should be Negative and the High Control should be Positive relative to the 1.0 ng/mL Cutoff Calibrator.

9 RESULTS AND EXPECTED VALUES

The actual fentanyl concentration cannot be determined. A confirmatory method is required.

Qualitative Analysis - Negative Results

A specimen that gives a rate (mA/min) value less than the ARK Fentanyl Calibrator B Cutoff rate (mA/min) value is interpreted as negative; either the specimen does not contain fentanyl or fentanyl is present in a concentration below the cutoff level of this assay.

Qualitative Analysis - Positive Results

A specimen that gives a rate (mA/min) value equal to or greater than the ARK Fentanyl Calibrator B Cutoff rate (mA/min) value is interpreted as positive, indicating that fentanyl is present.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

10 LIMITATIONS

- The assay is designated for use with human urine only.
- ARK Fentanyl II Assay reagents, and ARK Fentanyl calibrators and controls were developed as companion products. Performance with substituted products cannot be assured.
- A positive result using the ARK Fentanyl II Assay indicates only the presence of fentanyl and does not necessarily correlate with the extent of physiological and psychological effects.
- **Do not use Boric Acid as a preservative.**
- Interpretation of results must take into account that urine concentrations can vary extensively with fluid intake and other biological variables.

- It is possible that substances other than those tested in the specificity study may interfere with the test and cause false results.

11 SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance characteristics were collected on the Beckman Coulter AU680® automated clinical chemistry analyzer using the ARK Fentanyl II Assay.

Precision

Drug-free, negative human urine was supplemented with fentanyl (0.00 to 2.00 ng/mL). Each level was assayed in quadruplicate twice a day for 20 days (N=160). Results are summarized in the table 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	84 Negative; 76 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

Analytical Specificity

All compounds tested were added to drug-free, negative human urine.

The cross-reactivity of the following metabolites and structural analogs of fentanyl was evaluated by spiking these compounds into drug-free, negative human urine and evaluated by dose-response to determine the approximate equivalence to the 1.0 ng/mL fentanyl cutoff. These concentrations were used to determine the percent cross-reactivity according to the formula:

% Cross-reactivity = (Cutoff concentration / Concentration approximately equivalent to the 1.0 ng/mL cutoff) X 100

For the compounds Alfentanil, Norcarfentanil, and Remifentanil that did not produce a positive result, the highest concentration tested was used to calculate percent cross-reactivity.

Cross-reactivity

For the major metabolite, norfentanyl, the lowest concentration capable of producing a positive result is shown below.

Norfentanyl (Major Metabolite)

Compound	Concentration Approximately Equivalent to the Cutoff (ng/mL)	Percent Cross-reactivity (%)
Norfentanyl	15	7

Other Metabolites and Structural Analogs of Fentanyl

Compound	Concentration Approximately Equivalent to the Cutoff (ng/mL)	Percent Cross-reactivity (%)
Acetyl fentanyl	1.1	90.91
Isobutyryl fentanyl	1.1	90.91
ω-1-Hydroxyfentanyl	1.2	83.33
Acrylfentanyl	1.3	76.92
Butyryl fentanyl	1.4	71.43
Furanyl fentanyl	1.5	66.67
Para-fluoro fentanyl	1.5	66.67
Ocfentanil	1.6	62.50
4-Fluoro-isobutyryl fentanyl	1.9	52.63
Para-fluorobutyryl fentanyl (p-FBF)	1.9	52.63
Valeryl fentanyl	2.3	43.48
β-hydroxyfentanyl	9.5	10.53
Acetyl norfentanyl	12.1	8.26
(±) β-hydroxythiofentanyl	32.7	3.06
(±)-3-cis-methyl fentanyl	144.1	0.69
Carfentanil	448.2	0.22
Despropionyl fentanyl (4-ANPP)	471.8	0.21
Sufentanil	2,362	0.04
Remifentanil	>10,000	<0.01
Norcarfentanil	>50,000	<0.002
Alfentanil	>100,000	<0.001

The following opioids, structurally similar compounds, and functional analogs were negative at the concentrations tested with the ARK Fentanyl II Assay.

Compound	Concentration Tested (µg/mL)	Compound	Concentration Tested (µg/mL)
6-Acetyl morphine	100	Naltrexone	100
Buprenorphine	100	Norbuprenorphine	100
Buprenorphine glucuronide	100	Norcodeine	100
Codeine	100	Normeperidine	100
Dextromethorphan	100	Normorphine	100
Dihydrocodeine	100	Noroxycodone	100
EDDP	100	Oxycodone	100
EMDP	100	Oxymorphone	100
Heroin	100	Pentazocine (Talwin)	100
Hydrocodone	100	Pipamperone	90
Hydromorphone	100	Quinine	100
9-Hydroxyrisperidone	100	Quinidine	100
Labetalol	100	Risperidone	100
Levorphanol	100	Tapentadol	100
m-Chlorophenylpiperazine (m-CPP)	100	Thioridazine	100
		Tilidine	100
Meperidine	100	Tramadol	100
Methadone	100	Tramadol-O-Desmethyl	100
Morphine	100	Tramadol-N-Desmethyl	100
Morphine-3-glucuronide	100	Trazodone	100
Naloxone	100		

Interference – Structurally Unrelated Compounds

High concentrations of the following structurally unrelated compounds were added into fentanyl-spiked urine (± 50% of the cutoff concentration). The substances listed below did not yield a false result relative to the cutoff.

Compound	Concentration Tested (µg/mL)	0.5 ng/mL (-50% Cutoff)	1.5 ng/mL (+50% Cutoff)
Acetaminophen	500	Negative	Positive
Acetylsalicylic acid	1000	Negative	Positive
Albuterol	100	Negative	Positive
Amitriptyline	100	Negative	Positive
Amobarbital	100	Negative	Positive
Amphetamine	100	Negative	Positive
Benzoylecgonine	100	Negative	Positive
Bupropion	100	Negative	Positive
Caffeine	100	Negative	Positive
Carbamazepine	100	Negative	Positive
Chlorpromazine	100	Negative	Positive
Clomipramine	100	Negative	Positive
Cyclobenzaprine	100	Negative	Positive
Desipramine	100	Negative	Positive
Doxepin	100	Negative	Positive
Ecgonine	100	Negative	Positive
Ephedrine	100	Negative	Positive
Fluoxetine	100	Negative	Positive
Fluphenazine	100	Negative	Positive
Ibuprofen	500	Negative	Positive
Imipramine	100	Negative	Positive
Ketamine	100	Negative	Positive
Lidocaine	100	Negative	Positive
Maprotiline	100	Negative	Positive
Methapyrilene	100	Negative	Positive
Methaqualone	100	Negative	Positive
Metronidazole	300	Negative	Positive
Nicotine	100	Negative	Positive
Norketamine	100	Negative	Positive
Nortriptyline	60	Negative	Positive
Oxazepam	100	Negative	Positive
Phencyclidine	100	Negative	Positive
Phenobarbital	100	Negative	Positive
Propoxyphene	100	Negative	Positive
Ranitidine	100	Negative	Positive
Secobarbital	100	Negative	Positive
Valproic acid	250	Negative	Positive
Venlafaxine	100	Negative	Positive

Interference – Endogenous Substances

High concentrations of the following endogenous substances were added into fentanyl-spiked urine (± 50% of the cutoff concentration). No interference was observed when tested with the ARK Fentanyl II Assay.

Compound	Concentration Tested (mg/dL)	0.5 ng/mL (-50% Cutoff)	1.5 ng/mL (+50% Cutoff)
Acetone	1000	Negative	Positive
Ascorbic Acid	560	Negative	Positive
Bilirubin	2	Negative	Positive
Creatinine	500	Negative	Positive
Ethanol	1000	Negative	Positive
Galactose	10	Negative	Positive
Gamma Globulin	500	Negative	Positive
Glucose	3000	Negative	Positive
Hemoglobin	500	Negative	Positive
Human Albumin	500	Negative	Positive
Oxalic Acid	100	Negative	Positive
Riboflavin	7.5	Negative	Positive
Sodium Chloride	4000	Negative	Positive
Urea	2000	Negative	Positive

Interference – Boric Acid

One percent (1%) w/v of boric acid was added into fentanyl-spiked urine (± 50% of the cutoff concentration). Results are provided in the table below.

Compound	Concentration Tested	0.5 ng/mL (-50% Cutoff)	1.5 ng/mL (+50% Cutoff)
Boric Acid	1% w/v	Negative	Negative

Interference – Specific Gravity and pH

Urine samples with specific gravity values from 1.002 to 1.030 and pH values ranging from 3.0 to 11.0 were tested in the presence of the two levels of fentanyl at ± 50% of the cutoff concentration. No interference was observed when tested with the ARK Fentanyl II Assay.

Method Comparison

A total of one hundred forty seven (147) unaltered clinical urine specimens that are not individually identifiable were analyzed for fentanyl with the ARK Fentanyl II Assay and by LC-MS/MS. The LC-MS/MS confirmatory method was performed by a licensed reference laboratory and used a fentanyl cutoff of 0.2 ng/mL.

Specimens were tested with the ARK Fentanyl II Assay in singleton on a Beckman Coulter AU680 analyzer and compared to results obtained by LC-MS/MS. Groups of up to 31 specimens were assayed per run. Each run was verified by assaying the bi-level ARK Fentanyl Controls (0.5 ng/mL and 1.5 ng/mL) as quality control samples.

Results are summarized as follows:

ARK Immunoassay 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*	21	11	62
Negative	50	2	0	0

Discordant Results

*Norfentanyl was detected in this discordant sample (Sample ID #052) and contributed to the positive result obtained with the ARK Fentanyl II Assay for this sample.

Sample ID Number	ARK Immunoassay Result	Fentanyl (ng/mL by LC-MS/MS)	Norfentanyl (ng/mL by LC-MS/MS)
052*	Positive	0.4	7.6
065	Positive	0.5	5.2
058	Positive	0.5	7.9
069	Positive	0.5	31.2
060	Positive	0.5	425.4
056	Positive	0.6	3.7
072	Positive	0.6	13.8
062	Positive	0.6	14.5
074	Positive	0.6	14.6
055	Positive	0.6	16.9
071	Positive	0.6	19.0
070	Positive	0.6	161.7
051	Positive	0.7	2.1
066	Positive	0.7	3.1
064	Positive	0.8	15.9
073	Positive	0.8	45.8
063	Positive	0.9	2.2
061	Positive	0.9	6.5
057	Positive	0.9	12.3
053	Positive	0.9	14.0
059	Positive	0.9	62.6
054	Positive	0.9	63.4

12 REFERENCES

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13 TRADEMARKS

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