ARK Diagnostics, Inc.

ARK™ *Fentanyl 0.5 ng/mL Assay*

This ARK Diagnostics, Inc. package insert for the ARK Fentanyl 0.5 ng/mL 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
	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 0.5 ng/mL Assay*

2 INTENDED USE

The ARK Fentanyl 0.5 ng/mL Assay is an immunoassay intended for the qualitative detection of fentanyl in human urine at a cutoff concentration of 0.5 ng/mL. The assay is intended for use in laboratories with automated clinical chemistry analyzers.

This *in vitro* diagnostic device is intended solely for use in employment and insurance testing.

The ARK Fentanyl 0.5 ng/mL 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 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 determination of fentanyl in human urine aids the assessment of compliance for pain medication or for substance abuse. The ARK Fentanyl 0.5 ng/mL 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 from 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 0.5 ng/mL Assay is a homogeneous enzyme immunoassay technique used for the analysis of a specific compound in human urine. The assay is based on competition for antibody binding sites between drug in the specimen and drug labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH). As the latter is bound by antibody, enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly related to the drug concentration in the sample. 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
5052-0001-00	**ARK Fentanyl 0.5 ng/mL Assay** **Reagent R1 – Antibody/Substrate** rabbit polyclonal 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 0.5 ng/mL 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 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 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[9].
- 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 recommended pH range for urine specimens is 4.0 – 11.0[10].
- 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 0.5 ng/mL Assay – REF 5052-0001-00

Materials Required – Provided Separately

ARK Fentanyl Negative Calibrator (0.0 ng/mL) – REF 5052-0002-01
ARK Fentanyl Cutoff Calibrator (0.5 ng/mL) – REF 5052-0002-02
Quality Controls – ARK Fentanyl Control (0.25 ng/mL and 0.75 ng/mL) – REF 5052-0003-00

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 ARK Fentanyl 0.5 ng/mL Assay or contact Customer Support.

Assay Sequence

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

Qualitative Results

Use the 0.5 ng/mL Calibrator 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 absorbance (ΔA) value for the Cutoff Calibrator as Negative. Report results equal to or greater than the absorbance (ΔA) 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 0.5 ng/mL 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 (0.25 ng/mL and 0.75 ng/mL) is intended for use in quality control of the ARK Fentanyl 0.5 ng/mL Assay.

The Low Control should be Negative and the High Control should be Positive relative to the 0.5 ng/mL Cutoff Calibrator.

9 RESULTS AND EXPECTED VALUES

Qualitative Analysis - Negative Results

A specimen that gives an absorbance (ΔA) value less than the ARK Fentanyl 0.5 ng/mL Cutoff Calibrator absorbance (ΔA) 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 an absorbance (ΔA) value equal to or greater than the ARK Fentanyl 0.5 ng/mL Cutoff Calibrator absorbance (ΔA) 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 0.5 ng/mL Assay reagents, calibrators and controls were developed as companion products. Performance with substituted products cannot be assured.
- A positive result using the ARK Fentanyl 0.5 ng/mL Assay indicates only the presence of fentanyl and does not necessarily correlate with the extent of physiological and psychological effects.
- Boric acid is not recommended 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 0.5 ng/mL Assay.

Precision

Precision was determined by assaying fentanyl in human urine. Drug-free, negative human urine was supplemented with fentanyl (0.00 to 1.00 ng/mL). Testing was performed for 20 days, 2 runs per day in quadruplicate (N=160).

Human Urine (ng/mL)	Relative % Cutoff	# of Results	Qualitative Precision Results
0.00	-100	160	160 Negative
0.25	-50	160	160 Negative
0.38	-25	160	157 Negative; 3 Positive
0.50	Cutoff	160	33 Negative; 127 Positive
0.62	+25	160	160 Positive
0.75	+50	160	160 Positive
1.00	+100	160	160 Positive

Analytical Specificity

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

Metabolites and structural analogs of fentanyl were tested with the ARK Fentanyl 0.5 ng/mL Assay. Results are provided in the table below.

Compound	Concentration Tested (ng/mL)	Qualitative 0.5 ng/mL Cutoff
Acetyl fentanyl	0.60	Positive
Acetyl norfentanyl	1,000	Positive
Acrylfentanyl	0.80	Positive
Alfentanil	100,000	Negative
(±) β-hydroxythiofentanyl	1.40	Positive
Butyryl fentanyl	0.80	Positive
Carfentanil	150.00	Positive
Despropionyl fentanyl (4-ANPP)	65.00	Positive
4-Fluoro-isobutyryl fentanyl	2.00	Positive
Furanyl fentanyl	0.88	Positive
Isobutyryl fentanyl	0.75	Positive
(±)-3-cis-methyl fentanyl	2.50	Positive
Norcarfentanil	5,000	Negative
Norfentanyl	25.00	Positive
Ocfentanil	0.60	Positive
ω-1-Hydroxyfentanyl	0.80	Positive
Para-fluorobutyryl fentanyl (p-FBF)	2.50	Positive
Para-fluoro fentanyl	2.50	Positive
Remifentanil	10,000	Negative
Sufentanil	312.50	Positive
Valeryl fentanyl	1.00	Positive

The following opioids, structurally similar compounds, and functional analogs were negative at the concentrations tested.

Compound	Conc. Tested (µg/mL)	Compound	Conc. Tested (µg/mL)
6-Acetyl morphine	10	Naltrexone	50
Buprenorphine	100	Norbuprenorphine	50
Buprenorphine glucuronide	50	Norcodeine	50
Codeine	100	Normeperidine	100
Dextromethorphan	100	Normorphine	50
Dihydrocodeine	100	Noroxycodone	100
EDDP	100	Oxycodone	100
EMDP	50	Oxymorphone	50
Heroin	30	Pentazocine (Talwin)	10
Hydrocodone	100	Pipamperone	10
Hydromorphone	100	Risperidone	2
Levorphanol	50	Tapentadol	50
Meperidine	100	Tilidine	50
Methadone	100	Tramadol	100
Morphine	100	Tramadol-O-Desmethyl	100
Morphine-3-glucuronide	50	Tramadol-N-Desmethyl	100
Naloxone	50	Trazodone	10

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	Conc. Tested (µg/mL)	Result (-50% Cutoff)	Result (+50% Cutoff)
Acetaminophen	500	Negative	Positive
Acetylsalicylic acid	1000	Negative	Positive
Albuterol	100	Negative	Positive
Amitriptyline	15	Negative	Positive
Amobarbital	100	Negative	Positive
Amphetamine	30	Negative	Positive
Benzoylecgonine	100	Negative	Positive
Bupropion	50	Negative	Positive
Caffeine	100	Negative	Positive
Carbamazepine	100	Negative	Positive
Chlorpromazine	7	Negative	Positive
Clomipramine	20	Negative	Positive
Cyclobenzaprine	10	Negative	Positive
Desipramine	20	Negative	Positive
Doxepin	18	Negative	Positive
Ecgonine	75	Negative	Positive
Ephedrine	20	Negative	Positive
Fluoxetine	15	Negative	Positive
Fluphenazine	30	Negative	Positive
Ibuprofen	500	Negative	Positive
Imipramine	20	Negative	Positive
Ketamine	100	Negative	Positive
Lidocaine	50	Negative	Positive
Maprotiline	50	Negative	Positive
Methapyrilene	10	Negative	Positive
Methaqualone	50	Negative	Positive
Metronidazole	300	Negative	Positive
Nicotine	10	Negative	Positive
Norketamine	100	Negative	Positive
Nortriptyline	5	Negative	Positive
Oxazepam	100	Negative	Positive
Phencyclidine	75	Negative	Positive
Phenobarbital	100	Negative	Positive
Propoxyphene	50	Negative	Positive
Ranitidine	100	Negative	Positive
Secobarbital	100	Negative	Positive
Thioridazine	18	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). The results are presented below. No interference was observed.

Compound	Conc. Tested (mg/dL)	Result (-50% Cutoff)	Result (+50% Cutoff)
Acetone	1000	Negative	Positive
Ascorbic Acid	200	Negative	Positive
Bilirubin	2	Negative	Positive
Creatinine	400	Negative	Positive
Ethanol	1000	Negative	Positive
Galactose	10	Negative	Positive
Gamma Globulin	500	Negative	Positive
Glucose	3000	Negative	Positive
Hemoglobin	300	Negative	Positive
Human Albumin	500	Negative	Positive
Oxalic Acid	30	Negative	Positive
Riboflavin	3.75	Negative	Positive
Sodium Chloride	900	Negative	Positive
Urea	1000	Negative	Positive

Interference - Specific Gravity and pH

Urine samples with specific gravity values from 1.001 to 1.030 g/mL 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.

Comparative Analysis

One hundred (100) confirmed fentanyl-positive and fifty (50) confirmed fentanyl-negative clinical urine specimens were analyzed by ARK Fentanyl 0.5 ng/mL Assay. The LC-MS/MS confirmatory method was performed by a licensed reference laboratory and used a fentanyl cutoff of 0.2 ng/mL. The ARK Fentanyl 0.5 ng/mL Assay (cutoff 0.5 ng/mL) distinguished positive and negative results: overall agreement 100.0%, 100.0% clinical specificity and 100.0% clinical sensitivity.

		LC-MS/MS	
		(+)	(-)
ARK Fentanyl 0.5 ng/mL Assay	(+)	100	0
	(-)	0	50

12 REFERENCES

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13 TRADEMARKS

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