ARK Diagnostics, Inc.

For Export Only – Not For Sale in USA

ARK™ *Fentanyl Assay*

This ARK Diagnostics, Inc. package insert for the ARK Fentanyl 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		

Reagent Kit REF 5031-0001-00

Reagent Kit REF 5031-0001-01

1 NAME

ARK™ *Fentanyl Assay*

2 INTENDED USE

The ARK Fentanyl Assay is intended for the qualitative and/or semiquantitative determination of fentanyl in human urine at a cutoff concentration of 1.0 ng/mL. The assay provides a simple and rapid analytical screening procedure for detecting fentanyl in urine and is designated for professional use on automated clinical chemistry analyzers.

For prescription use only. Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.

The semiquantitative mode is for the purpose of (1) enabling laboratories to determine an appropriate dilution for the specimen for confirmation by a confirmatory method, or (2) permitting laboratories to establish quality control procedures.

The ARK Fentanyl Assay provides only preliminary analytical 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.

The confirmatory method should have a fentanyl cutoff ≤ 1.0 ng/mL.

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 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 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. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the specimen can be measured in terms of enzyme activity. 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 serum 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
5031-0001-00	**ARK Fentanyl 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 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

REF	Product Description	Quantity/Volume
5031-0001-01	**ARK Fentanyl 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 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

Reagent Handling and Storage

ARK Fentanyl 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.
- 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 can't be performed within 7 days, store the urine sample frozen.
- 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.
- 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 Assay – REF 5031-0001-00 or 5031-0001-01

Materials Required – Provided Separately

ARK Fentanyl Calibrator – REF 5031-0002-00
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. All qualitative testing results are expressed as enzymatic rate (mA/min). Report test results less than the rate for the Cutoff Calibrator as Negative. Report results equal to or greater than the rate for the Cutoff Calibrator as Positive.

Semiquantitative Results

To estimate the concentration of fentanyl, perform a 5-point calibration procedure; test calibrators in duplicate. Verify the calibration curve with ARK Low and High quality controls according to the established laboratory quality assurance plan. The semiquantitative mode of measurement is 0.3 to 10.0 ng/mL. Specimens may be diluted in ARK Calibrator A (Negative urine), and the result should fall within this range.

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
- A stored calibration curve was effective up to at least 15 days based on supporting data

Quality Control (QC)

Laboratories should establish QC procedures for the ARK Fentanyl 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. The ARK Fentanyl Control is an assayed control intended for quality control of the ARK Fentanyl Assay when run in either the qualitative or semiquantitative mode.

In Qualitative Mode 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 concentration cannot be determined. A confirmatory method is required.

Qualitative Analysis - Negative Results

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

Semiquantitative Analysis

The semiquantitation of positive results enables the laboratory to determine an appropriate dilution of the specimen for the confirmatory method. Semiquantitation also permits the laboratory to establish quality control procedures and assess reproducibility. Specimens may be diluted in ARK Calibrator A (Negative urine), and the result should fall within 0.3 to 10.0 ng/mL.

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 Assay reagents, calibrators and controls were developed as companion products. Performance with substituted products cannot be assured.
- A positive result using the ARK Fentanyl 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 investigated in the specificity study may interfere with the test and cause false results.
- To maintain sample stability store processed patient samples frozen at -20 °C.

11 SPECIFIC PERFORMANCE CHARACTERISTICS

The data appearing in this section were collected on the Beckman Coulter AU680® clinical chemistry analyzer using the ARK Fentanyl Assay.

Precision

Precision was determined by assaying fentanyl in human urine. Drug-free, negative human urine was supplemented with fentanyl (0.00 to 2.00 ng/mL), and both qualitative and semiquantitative protocols were performed for 20 days, 2 runs per day in quadruplicate (N=160).

Qualitative Precision

Fentanyl (ng/mL); N=160	Relative % Cutoff	Result
0.00	-100	160 Negative
0.50	-50	160 Negative
0.75	-25	160 Negative
1.00	0	158 Negative; 2 Positive
1.25	+25	160 Positive
1.50	+50	160 Positive
2.00	+100	160 Positive

Semiquantitative Precision

Fentanyl (ng/mL); N=160		Within-Run Precision		Total Precision	
Level Tested	Mean	SD	CV (%)	SD	CV (%)
0.00	0.00	0.01	NA	0.01	NA
0.50	0.44	0.04	8.8	0.05	11.3
0.75	0.72	0.04	5.8	0.05	7.0
1.00	0.95	0.04	4.1	0.05	5.5
1.25	1.12	0.05	4.7	0.07	5.8
1.50	1.34	0.07	5.0	0.08	6.0
2.00	1.80	0.06	3.2	0.08	4.4

Analytical Recovery

Analytical recovery for the ARK Fentanyl Assay was assessed using the semiquantitative mode. Drug-free, negative human urine was supplemented with fentanyl (0.40 to 10.00 ng/mL). Mean drug concentration observed for six (6) replicates and percentage recovery were calculated.

Concentration Tested (ng/mL)	Mean (N=6) (ng/mL)	Recovery (%)
0.40	0.40	100.4
0.75	0.69	91.6
1.75	1.68	96.0
3.00	2.94	97.8
4.00	4.11	102.6
6.00	6.28	104.7
10.00	9.59	95.9

Limit of Quantitation

The lowest concentration of fentanyl tested that met the criteria of recovery (± 0.1 ng/mL) and precision (≤ 0.1 SD) is 0.3 ng/mL.

Linearity

Linearity was assessed using the semiquantitative mode as suggested in CLSI/NCCLS Protocol EP6-A. Drug-free, negative human urine was supplemented with fentanyl (12.00 ng/mL) and dilutions were made proportionally with drug-free human urine. Fentanyl concentrations ranged from 0.00 to 10.00 ng/mL. Linearity at specific dilutions was considered acceptable if the percent difference was ±10% between the predicted 1st and 2nd order regressed values. A linear relationship was demonstrated between 0.00 and 10.00 ng/mL ($y = 0.9739x - 0.036$).

Estimated Value (ng/mL)	Measured Results (ng/mL)	Recovery (%)	1st Order Predicted Results	2nd Order Predicted Results	Difference (%)
0.00	0.01	NA	-0.04	-0.04	NA
0.50	0.52	104.0	0.45	0.45	-1.08
1.00	0.93	93.3	0.94	0.94	-0.14
2.00	1.78	89.2	1.91	1.92	0.24
4.00	3.71	92.7	3.86	3.87	0.28
6.00	5.89	98.2	5.81	5.82	0.17
8.00	8.01	100.1	7.76	7.76	0.02
10.00	9.54	95.4	9.70	9.69	-0.14

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 Assay in both the qualitative and semiquantitative modes. Results are provided in the table below.

Compound	Concentration Tested (ng/mL)	Semiquantitative Mean (ng/mL)	Qualitative 1.0 ng/mL Cutoff
Acetyl fentanyl	1.2	1.19	Positive
Acetyl norfentanyl	10,000	1.01	Positive
Alfentanil	100,000	0.20	Negative
Butyryl fentanyl	1.60	1.21	Positive
Carfentanil	500	1.07	Positive
Despropionyl fentanyl (4-ANPP)	75	1.30	Positive
Furanyl fentanyl	1.75	1.32	Positive
Isobutyryl fentanyl	1.50	1.27	Positive
(±)-3-cis-methyl fentanyl	5.00	1.34	Positive
Norcarfentanil	5,000	0.22	Negative
Norfentanyl	300	1.04	Positive
Ocfentanil	1.50	1.26	Positive
Para-fluorobutyryl fentanyl (p-FBF)	3.00	1.24	Positive
Para-fluoro fentanyl	3.00	1.25	Positive
Remifentanil	10,000	0.07	Negative
Sufentanil	625	1.01	Positive
Valeryl fentanyl	2.5	1.90	Positive

The following opioids, structurally similar compounds, and functional analogs were negative at the concentrations tested in both the qualitative and semiquantitative modes of the ARK Fentanyl Assay.

Compound	Conc. Tested (µg/mL)	Compound	Conc. Tested (µg/mL)
6-Acetyl morphine	10	Naloxone	50
Amphetamine	100	Naltrexone	50
Buprenorphine	100	Norbuprenorphine	50
Buprenorphine glucuronide	50	Norcodeine	50
Codeine	100	Norketamine	100
Dextromethorphan	100	Normeperidine	100
Dihydrocodeine	100	Normorphine	50
EDDP	100	Noroxycodone	100
EMDP	50	Oxycodone	100
Fluoxetine	50	Oxymorphone	50
Heroin	30	Pentazocine (Talwin)	10
Hydrocodone	100	Risperidone	2
Hydromorphone	100	Tapentadol	50
Ketamine	100	Thioridazine	50
Levorphanol	50	Tilidine	50
Meperidine	100	Tramadol	100
Methadone	100	Tramadol-O-Desmethyl	100
Morphine	100	Tramadol-N-Desmethyl	100
Morphine-3-glucuronide	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 in both qualitative and semiquantitative mode.

Compound	Conc. Tested (µg/mL)	Sample (-50% Cutoff)		Sample (+50% Cutoff)	
		Qualitative	Semi-Quant. (ng/mL)	Qualitative	Semi-Quant. (ng/mL)
Acetaminophen	500	Negative	0.54	Positive	1.40
Acetylsalicylic acid	1000	Negative	0.53	Positive	1.59
Albuterol	100	Negative	0.52	Positive	1.37
Amitriptyline	35	Negative	0.83	Positive	1.62
Amobarbital	100	Negative	0.55	Positive	1.38
Benzoylecgonine	100	Negative	0.68	Positive	1.44
Bupropion	50	Negative	0.83	Positive	1.62
Caffeine	100	Negative	0.54	Positive	1.40
Carbamazepine	100	Negative	0.47	Positive	1.37
Chlorpromazine	50	Negative	0.88	Positive	1.81
Clomipramine	50	Negative	0.82	Positive	1.47
Cyclobenzaprine	10	Negative	0.64	Positive	1.55
Desipramine	50	Negative	0.77	Positive	1.74
Doxepin	50	Negative	0.82	Positive	1.80
Ecgonine	100	Negative	0.75	Positive	1.80
Ephedrine	100	Negative	0.88	Positive	1.89
Fluphenazine	100	Negative	0.64	Positive	1.60
Ibuprofen	500	Negative	0.45	Positive	1.22
Imipramine	30	Negative	0.69	Positive	1.68
Lidocaine	50	Negative	0.52	Positive	1.37
Maprotiline	50	Negative	0.65	Positive	1.60
Methapyrilene	10	Negative	0.71	Positive	1.69
Methaqualone	50	Negative	0.53	Positive	1.36
Metronidazole	300	Negative	0.47	Positive	1.40
Nicotine	10	Negative	0.60	Positive	1.32
Nortriptyline	25	Negative	0.56	Positive	1.76
Oxazepam	100	Negative	0.65	Positive	1.46
Phencyclidine	100	Negative	0.71	Positive	1.93
Phenobarbital	100	Negative	0.49	Positive	1.37
Propoxyphene	50	Negative	0.48	Positive	1.35
Ranitidine	100	Negative	0.71	Positive	1.63
Secobarbital	100	Negative	0.65	Positive	1.47
Valproic acid	250	Negative	0.59	Positive	1.57
Venlafaxine	100	Negative	0.54	Positive	1.66

Interference - Endogenous Substances

High concentrations of the following endogenous substances were added into fentanyl-spiked urine (± 50% of the cutoff concentration). The results for both qualitative and semiquantitative mode are presented below. No interference was observed.

Compound	Conc. Tested (mg/dL)	Sample (-50% Cutoff)		Sample (+50% Cutoff)	
		Qualitative	Semi-Quant. (ng/mL)	Qualitative	Semi-Quant. (ng/mL)
Acetone	1000	Negative	0.45	Positive	1.31
Ascorbic Acid	200	Negative	0.50	Positive	1.37
Creatinine	400	Negative	0.47	Positive	1.35
Ethanol	1000	Negative	0.43	Positive	1.26
Galactose	10	Negative	0.47	Positive	1.37
Glucose	3000	Negative	0.43	Positive	1.27
Hemoglobin	300	Negative	0.51	Positive	1.43
Human Albumin	500	Negative	0.46	Positive	1.33
Oxalic Acid	30	Negative	0.51	Positive	1.36
Riboflavin	3.75	Negative	0.49	Positive	1.34
NaCl	900	Negative	0.51	Positive	1.34
Urea	1000	Negative	0.50	Positive	1.41

Interference - Specific Gravity and pH

Urine samples with specific gravity values from 1.004 to 1.026 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 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 Assay (cutoff 1.0 ng/mL) distinguished positive and negative results: overall agreement 98%, 100.0% clinical specificity and 97.0% clinical sensitivity.

		LC-MS/MS	
		(+)	(-)
ARK Fentanyl Assay	(+)	97	0
	(-)	3*	50

*Discordant Result Summary**

Sample ID	ARK Qualitative (Negative/Positive)	ARK Semiquantitative (ng/mL)	LC-MS/MS Fentanyl (ng/mL)
067	Negative	0.57	0.70
068	Negative	0.61	0.70
071	Negative	0.92	0.60

*Samples contained fentanyl with concentrations between the cutoffs for the ARK assay and LC-MS/MS.

12 REFERENCES

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13 TRADEMARKS

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Printed in USA
Revised September 2017
1600-0559-00 Rev 03