ARK™ EDDP Assay

This ARK Diagnostics, Inc. package insert for the ARK EDDP 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 EDDP in urine. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Report any serious incident that has occurred in relation to the device to the manufacturer and the appropriate competent authority as applicable.

Customer Service

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CE 2797

EC REP

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CH REP

MedEnvoy Switzerland
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Switzerland

Key to Symbols Used

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE 2797	CE Mark with notified body number
	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 5051-0001-00

Reagent Kit REF 5051-0001-01

Reagent Kit REF 5051-0001-02

1 Name

ARK™ EDDP Assay

2 Intended Use

The ARK EDDP Assay is an immunoassay intended for the qualitative and/or semiquantitative determination of EDDP in human urine at cutoff concentrations of 100 ng/mL and 300 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 semiquantitative mode is for the purpose of (1) enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method, such as Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/tandem Mass Spectrometry (LC-MS/MS), or (2) permitting laboratories to establish quality control procedures.

The ARK EDDP 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 Test

Methadone (DOLOPHINE®) is a synthetic opioid, a compound that is capable of binding to opioid receptors and creates many of the same effects seen with natural opiates, including analgesia and sedation. Methadone is indicated for the management of pain severe enough to require daily, around-the-clock, long-term opioid treatment and for which alternative treatment options are inadequate.[1]

Methadone is a Schedule II substance under the United States Controlled Substances Act for its potential for abuse and risk of dependence.[2]

Methadone is metabolized through hepatic N-demethylation to a variety of inactive metabolites, including the primary metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and the secondary metabolite 2-ethyl-5-methyl-3,3-diphenylpyraline (EMDP) at lower concentrations. Important enzymes in methadone metabolism are CYP3A4, CYP2C19 and CYP2B6. CYP2D6 appears to have a minor role and CYP1A2 may possibly be involved in methadone metabolism. Excretion of methadone and its metabolites (including EDDP) occurs primarily through the kidneys.[3-6]

4 Principles of the Procedure

The ARK EDDP Assay is a homogeneous enzyme immunoassay technique used for the analysis of EDDP in human urine. The assay is based on competition between EDDP in the specimen and EDDP labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH) for antibody binding sites. As the latter binds antibody, enzyme activity decreases. In the presence of EDDP from the specimen, enzyme activity increases and is directly related to the EDDP 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
5051-0001-00	**ARK EDDP Assay** **Reagent R1 – Antibody/Substrate** rabbit antibodies to EDDP, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 28 mL
	Reagent R2 – Enzyme EDDP derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), bovine serum albumin, buffer, sodium azide and stabilizers	1 X 14 mL

REF	Product Description	Quantity/Volume
5051-0001-01	**ARK EDDP Assay** **Reagent R1 – Antibody/Substrate** rabbit antibodies to EDDP, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 115 mL
	Reagent R2 – Enzyme EDDP derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), bovine serum albumin, buffer, sodium azide and stabilizers	1 X 58 mL

REF	Product Description	Quantity/Volume
5051-0001-02	**ARK EDDP Assay** **Reagent R1 – Antibody/Substrate** rabbit antibodies to EDDP, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 500 mL
	Reagent R2 – Enzyme EDDP derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), bovine serum albumin, buffer, sodium azide and stabilizers	1 X 250 mL

Reagent Handling and Storage
ARK EDDP 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 EDDP 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

- Each laboratory is responsible for supplying a valid specimen for analysis according to their quality procedures.
- 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.[7,8]
- Do not induce foaming and avoid repeated freezing and thawing to preserve the integrity of the specimen from the time it is collected until the time it is assayed.
- The presence of bubbles or foam on specimens can lead to short sample delivery and erroneous results.
- Frozen specimens must be thawed and mixed thoroughly prior to analysis.
- Centrifuge specimens with high turbidity or visible particulate matter before testing.
- Each laboratory should consult available literature and internal data regarding specimen stability.
- 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 EDDP Assay – REF 5051-0001-00, 5051-0001-01 or 5051-0001-02

Materials Required – Provided Separately

ARK EDDP Calibrator – REF 5051-0002-00
ARK EDDP Calibrator A (Negative) – REF 5051-0002-01
ARK EDDP Calibrator B (Cutoff) – REF 5051-0002-02
ARK EDDP Calibrator C (Cutoff) – REF 5051-0002-03
Quality Controls – ARK EDDP Control – REF 5051-0003-00

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. Many automated clinical chemistry analyzers with photometric rate determination at 340 nm are suitable. Consult the analyzer-specific application sheet for programming the ARK EDDP Assay, available from your distributor or ARK Customer Service. Application Protocol Sheets which have been CLIA categorized or bear the CE Mark have been verified by the manufacturer. It is the responsibility of the laboratory to perform all appropriate validation for use of the assay with other settings or analyzers.

Refer to the instrument-specific operator's manual for daily maintenance.

Assay Sequence

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

Qualitative Results

Use the 100 ng/mL Calibrator B or the 300 ng/mL Calibrator C as a Cutoff Calibrator to distinguish negative and positive samples. Run the ARK EDDP Low (75 ng/mL) and High (125 ng/mL) Controls with Cutoff Calibrator B or the ARK EDDP Low (225 ng/mL) and High (375 ng/mL) Controls with Cutoff Calibrator C, as Negative and Positive respectively. Report test results less than the response value for the applicable Cutoff Calibrator as Negative. Report test results equal to or greater than the response value for the applicable Cutoff Calibrator as Positive.

Semiquantitative Results

Perform a 5-point calibration procedure; run calibrators in duplicate. Verify the calibration curve with the ARK EDDP Low and High quality controls according to the established laboratory quality assurance plan. Specimens with sample results above the highest ARK EDDP calibrator level (1000 ng/mL) may be diluted in ARK EDDP Calibrator A (Negative urine) and retested.

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) and Calibration
Laboratories should establish QC procedures for the ARK EDDP 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 EDDP Control is intended for use in quality control of the ARK EDDP Assay.

In Qualitative Mode, the Low Control should be Negative and the High Control should be Positive relative to the applicable Cutoff Calibrator (100 ng/mL or 300 ng/mL).

9 Results and Expected Values

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

Qualitative Analysis – Negative Results
A specimen that gives a response value less than the ARK EDDP Calibrator B or ARK EDDP Calibrator C Cutoff response value is interpreted as negative; either the specimen does not contain EDDP or EDDP is present in a concentration below the applicable cutoff level of this assay.

Qualitative Analysis – Positive Results
A specimen that gives a response value equal to or greater than the ARK EDDP Calibrator B or ARK EDDP Calibrator C Cutoff response value is interpreted as positive, indicating that EDDP is present.

Semiquantitative Analysis

Semiquantitative results for positive specimens enable the laboratory to determine an appropriate dilution of the specimen for the confirmatory method. Semiquantitative results also permit the laboratory to establish quality control procedures and assess reproducibility. Specimens with sample results above the highest ARK EDDP calibrator level (1000 ng/mL) may be diluted in ARK EDDP Calibrator A (Negative urine) and retested.

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 EDDP Assay reagents, calibrators and controls were developed as companion products. Performance with substituted products cannot be assured.

- A positive result using the ARK EDDP Assay indicates only the presence of EDDP and does not necessarily correlate with the extent of physiological and psychological effects.
- 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 EDDP Assay.

Precision

Drug-free, negative human urine was supplemented with EDDP (0.0 to 200.0 ng/mL for the 100 ng/mL cutoff and 0.0 to 600.0 for the 300 ng/mL cutoff). Each level was assayed in quadruplicate twice a day for 20 days (N=160) and evaluated qualitatively and semiquantitatively. Results are summarized in the tables below.

Qualitative Precision – 100 ng/mL Cutoff

Human Urine (ng/mL)	Relative % Cutoff	# of Results	Qualitative Precision Results
0.0	-100	160	160 Negative
25.0	-75	160	160 Negative
50.0	-50	160	160 Negative
75.0	-25	160	160 Negative
100.0	Cutoff	160	123 Negative; 37 Positive
125.0	+25	160	160 Positive
150.0	+50	160	160 Positive
175.0	+75	160	160 Positive
200.0	+100	160	160 Positive

Semiquantitative Precision – 100 ng/mL Cutoff

Human Urine (ng/mL)	Relative % Cutoff	# of Results	Mean (ng/mL)	Semiquantitative Precision Results
0.0	-100	160	0.3	160 Negative
25.0	-75	160	22.6	160 Negative
50.0	-50	160	47.7	160 Negative
75.0	-25	160	72.2	160 Negative
100.0	Cutoff	160	98.1	114 Negative; 46 Positive
125.0	+25	160	125.3	160 Positive
150.0	+50	160	145.1	160 Positive

Human Urine (ng/mL)	Relative % Cutoff	# of Results	Mean (ng/mL)	Semiquantitative Precision Results
175.0	+75	160	169.4	160 Positive
200.0	+100	160	190.7	160 Positive

Qualitative Precision – 300 ng/mL Cutoff

Human Urine (ng/mL)	Relative % Cutoff	# of Results	Qualitative Precision Results
0.0	-100	160	160 Negative
75.0	-75	160	160 Negative
150.0	-50	160	160 Negative
225.0	-25	160	160 Negative
300.0	Cutoff	160	57 Negative; 103 Positive
375.0	+25	160	160 Positive
450.0	+50	160	160 Positive
525.0	+75	160	160 Positive
600.0	+100	160	160 Positive

Semiquantitative Precision – 300 ng/mL Cutoff

Human Urine (ng/mL)	Relative % Cutoff	# of Results	Mean (ng/mL)	Semiquantitative Precision Results
0.0	-100	160	0.3	160 Negative
75.0	-75	160	72.2	160 Negative
150.0	-50	160	145.1	160 Negative
225.0	-25	160	205.9	160 Negative
300.0	Cutoff	160	298.8	85 Negative; 75 Positive
375.0	+25	160	381.4	160 Positive
450.0	+50	160	461.0	160 Positive
525.0	+75	160	539.8	160 Positive
600.0	+100	160	620.0	160 Positive

Analytical Recovery

Recovery across the assay range was assessed using the semiquantitative mode. Drug-free, negative human urine was supplemented with EDDP (1100.0 ng/mL) and dilutions were made proportionally with drug-free human urine. EDDP concentrations ranged from 50.0 to 1000.0 ng/mL. At each level, percentage recovery was calculated based on the mean concentration (N=6) compared to the expected concentration. Results are summarized in the table below.

Theoretical Concentration (ng/mL)	Mean Concentration (ng/mL)	Recovery (%)
50.0	47.6	95.1
75.0	72.1	96.1
100.0	97.1	97.1
200.0	189.1	94.6
300.0	286.6	95.5
400.0	414.5	103.6
500.0	506.6	101.3
600.0	647.4	107.9
700.0	722.7	103.2
800.0	800.6	100.1
900.0	880.8	97.9
1000.0	955.8	95.6

Analytical Specificity

All compounds tested were added to drug-free, negative human urine and tested with the ARK EDDP Assay in both qualitative and semiquantitative modes.

The cross-reactivity of the following structurally related compounds was evaluated by spiking these compounds into drug-free, negative human urine to determine the minimum concentration that would give a positive result approximately equivalent to the 100 ng/mL and 300 ng/mL EDDP cutoffs. These concentrations were used to determine the percent cross-reactivity according to the formula:

% Cross-reactivity = (Cutoff concentration / Lowest concentration of cross-reactant causing a positive result) X 100

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

Structurally Related Compounds – 100 ng/mL Cutoff

Compound	Concentration Tested (ng/mL)	Semiquantitative Mode Result (Positive/Negative)	Qualitative Mode Result (Positive/Negative)	Cross-reactivity (%)
EDDP	100	Positive	Positive	100
Methadone	2,000,000	Negative	Negative	<0.005
EMDP	400,000	Negative	Negative	<0.025
Chlorpromazine	100,000	Negative	Negative	<0.1
Diphenhydramine	100,000	Negative	Negative	<0.1
Methylphenidate	100,000	Negative	Negative	<0.1
Doxylamine	100,000	Negative	Negative	<0.1

Structurally Related Compounds – 300 ng/mL Cutoff

Compound	Concentration Tested (ng/mL)	Semiquantitative Mode Result (Positive/Negative)	Qualitative Mode Result (Positive/Negative)	Cross-reactivity (%)
EDDP	300	Positive	Positive	100
Methadone	4,500,000	Negative	Negative	<0.007
EMDP	1,000,000	Negative	Negative	<0.03
Chlorpromazine	100,000	Negative	Negative	<0.3
Diphenhydramine	100,000	Negative	Negative	<0.3
Methylphenidate	100,000	Negative	Negative	<0.3
Doxylamine	100,000	Negative	Negative	<0.3

Interference

Structurally Unrelated Compounds – 100 ng/mL Cutoff

High concentrations of the following structurally unrelated compounds were added into urine spiked with EDDP (± 25% of the 100 ng/mL cutoff concentration) and tested with the ARK EDDP Assay in both qualitative and semiquantitative modes. The substances listed at the concentrations below did not yield a false result relative to the 100 ng/mL cutoff.

Compound	Concentration Tested (ng/mL)	Spiked EDDP Level	
		75 ng/mL (-25% Cutoff)	125 ng/mL (+25% Cutoff)
4-Bromo-2,5-dimethoxyphenethylamine	100,000	Negative	Positive
Acetaminophen	500,000	Negative	Positive
Acetylsalicylic Acid	500,000	Negative	Positive
6-Acetylcodeine	100,000	Negative	Positive
6-Acetylmorphine	100,000	Negative	Positive
Alprazolam	100,000	Negative	Positive
7-Aminoclonazepam	100,000	Negative	Positive
7-Aminoflunitrazepam	100,000	Negative	Positive
7-Aminonitrazepam	100,000	Negative	Positive
Amitriptyline	100,000	Negative	Positive
Amobarbital	100,000	Negative	Positive
S-(+)-Amphetamine	100,000	Negative	Positive
Benzylpiperazine	100,000	Negative	Positive
Bromazepam	100,000	Negative	Positive
Buprenorphine	100,000	Negative	Positive
Bupropion	100,000	Negative	Positive
Butabarbital	100,000	Negative	Positive
Butalbital	100,000	Negative	Positive
Caffeine	500,000	Negative	Positive
Cannabidiol	100,000	Negative	Positive
Cannabinol	100,000	Negative	Positive
Carbamazepine	100,000	Negative	Positive

Compound	Concentration Tested (ng/mL)	Spiked EDDP Level	
		75 ng/mL (-25% Cutoff)	125 ng/mL (+25% Cutoff)
Carisoprodol	100,000	Negative	Positive
Chlordiazepoxide	100,000	Negative	Positive
Cis-Tramadol	100,000	Negative	Positive
Clobazam	100,000	Negative	Positive
Clomipramine	100,000	Negative	Positive
Clonazepam	100,000	Negative	Positive
Clozapine	100,000	Negative	Positive
Codeine	100,000	Negative	Positive
Cotinine	100,000	Negative	Positive
Cyclobenzaprine	100,000	Negative	Positive
Dehydronorketamine	100,000	Negative	Positive
Demoxepam	100,000	Negative	Positive
Desipramine	100,000	Negative	Positive
Desalkylflurazepam	100,000	Negative	Positive
Dextromethorphan	100,000	Negative	Positive
Diazepam	100,000	Negative	Positive
Digoxin	100,000	Negative	Positive
Dihydrocodeine	100,000	Negative	Positive
Δ9 THC	100,000	Negative	Positive
Doxepin	100,000	Negative	Positive
1R,2S (-) Ephedrine	100,000	Negative	Positive
1S,2R (+) Ephedrine	100,000	Negative	Positive
Ethyl-β-D-Glucuronide	100,000	Negative	Positive
Ethylmorphine	100,000	Negative	Positive
(S-)-Fenfluramine	100,000	Negative	Positive
(R+)-Fenfluramine	100,000	Negative	Positive
Fentanyl	100,000	Negative	Positive
Flunitrazepam	100,000	Negative	Positive
Fluoxetine	100,000	Negative	Positive
Flurazepam	100,000	Negative	Positive
Haloperidol	100,000	Negative	Positive
Heroin	100,000	Negative	Positive
Hexobarbital	100,000	Negative	Positive
Hydrocodone	100,000	Negative	Positive
Hydromorphone	100,000	Negative	Positive
11-hydroxy-Δ9 THC	100,000	Negative	Positive
Ibuprofen	500,000	Negative	Positive
Imipramine	100,000	Negative	Positive
Ketamine	100,000	Negative	Positive
Lamotrigine	100,000	Negative	Positive
Levorphanol Tartrate	100,000	Negative	Positive
Lidocaine	100,000	Negative	Positive
Lorazepam	100,000	Negative	Positive
Lorazepam Glucuronide	50,000	Negative	Positive
Lormetazepam	100,000	Negative	Positive
LSD	100,000	Negative	Positive
Maprotiline	100,000	Negative	Positive

Compound	Concentration Tested (ng/mL)	Spiked EDDP Level	
		75 ng/mL (-25% Cutoff)	125 ng/mL (+25% Cutoff)
(+)-MDA	100,000	Negative	Positive
MDEA	100,000	Negative	Positive
MDMA	100,000	Negative	Positive
Meperidine	100,000	Negative	Positive
Meprobamate	100,000	Negative	Positive
S(+)-Methamphetamine	100,000	Negative	Positive
Methaqualone	100,000	Negative	Positive
Methoxetamine	100,000	Negative	Positive
Methylone	100,000	Negative	Positive
Midazolam	100,000	Negative	Positive
Morphine	100,000	Negative	Positive
Morphine-3β-D-Glucuronide	100,000	Negative	Positive
Morphine-6β-D-Glucuronide	50,000	Negative	Positive
N-Desmethyltapentadol	100,000	Negative	Positive
Nalorphine	100,000	Negative	Positive
Naloxone	100,000	Negative	Positive
Naltrexone	100,000	Negative	Positive
Naproxen	100,000	Negative	Positive
Nitrazepam	100,000	Negative	Positive
11-nor-9-carboxy-Δ9-THC	100,000	Negative	Positive
Norbuprenorphine	50,000	Negative	Positive
Norcodeine	100,000	Negative	Positive
Nordiazepam	100,000	Negative	Positive
Norketamine	100,000	Negative	Positive
Normorphine	100,000	Negative	Positive
Norpropoxyphene	100,000	Negative	Positive
Norpseudoephedrine	100,000	Negative	Positive
Nortriptyline	100,000	Negative	Positive
Olanzapine	100,000	Negative	Positive
Oxazepam	100,000	Negative	Positive
Oxycodone	100,000	Negative	Positive
Oxymorphone	100,000	Negative	Positive
PCP	100,000	Negative	Positive
Pentazocine	100,000	Negative	Positive
Pentobarbital	100,000	Negative	Positive
Phenobarbital	100,000	Negative	Positive
Phentermine	100,000	Negative	Positive
Phenylephrine	100,000	Negative	Positive
Phenylpropanolamine	100,000	Negative	Positive
Phenytoin	100,000	Negative	Positive
PMA	100,000	Negative	Positive
Prazepam	100,000	Negative	Positive
Propoxyphene	100,000	Negative	Positive
Propranolol	100,000	Negative	Positive
Protriptyline	100,000	Negative	Positive
R,R (+)- Pseudoephedrine	100,000	Negative	Positive
S,S (-)- Pseudoephedrine	100,000	Negative	Positive

Compound	Concentration Tested (ng/mL)	Spiked EDDP Level	
		75 ng/mL (-25% Cutoff)	125 ng/mL (+25% Cutoff)
Ranitidine	100,000	Negative	Positive
Ritalinic Acid	100,000	Negative	Positive
Salicylic Acid	100,000	Negative	Positive
Secobarbital	100,000	Negative	Positive
Sertraline	100,000	Negative	Positive
Sufentanil Citrate	50,000	Negative	Positive
Tapentadol	100,000	Negative	Positive
Temazepam	100,000	Negative	Positive
Theophylline	100,000	Negative	Positive
Thioridazine	100,000	Negative	Positive
Trazodone	100,000	Negative	Positive
Triazolam	100,000	Negative	Positive
Trifluoromethylphenylpiperazine	100,000	Negative	Positive
Trimipramine	100,000	Negative	Positive
Venlafaxine	100,000	Negative	Positive
Verapamil	100,000	Negative	Positive
Zolpidem Tartrate	100,000	Negative	Positive

Structurally Unrelated Compounds – 300 ng/mL Cutoff

High concentrations of the following structurally unrelated compounds were added into urine spiked with EDDP (± 25% of the 300 ng/mL cutoff concentration) and tested with the ARK EDDP Assay in both qualitative and semiquantitative modes. The substances listed at the concentrations below did not yield a false result relative to the 300 ng/mL cutoff.

Compound	Concentration Tested (ng/mL)	Spiked EDDP Level	
		225 ng/mL (-25% Cutoff)	375 ng/mL (+25% Cutoff)
4-Bromo-2,5-dimethoxyphenethylamine	100,000	Negative	Positive
Acetaminophen	500,000	Negative	Positive
Acetylsalicylic Acid	500,000	Negative	Positive
6-Acetylcodeine	100,000	Negative	Positive
6-Acetylmorphine	100,000	Negative	Positive
Alprazolam	100,000	Negative	Positive
7-Aminoclonazepam	100,000	Negative	Positive
7-Aminoflunitrazepam	100,000	Negative	Positive
7-Aminonitrazepam	100,000	Negative	Positive
Amitriptyline	100,000	Negative	Positive
Amobarbital	100,000	Negative	Positive
S-(+)-Amphetamine	100,000	Negative	Positive
Benzylpiperazine	100,000	Negative	Positive
Bromazepam	100,000	Negative	Positive
Buprenorphine	100,000	Negative	Positive
Bupropion	100,000	Negative	Positive
Butabarbital	100,000	Negative	Positive

Compound	Concentration Tested (ng/mL)	Spiked EDDP Level	
		225 ng/mL (-25% Cutoff)	375 ng/mL (+25% Cutoff)
Butalbital	100,000	Negative	Positive
Caffeine	500,000	Negative	Positive
Cannabidiol	100,000	Negative	Positive
Cannabinol	100,000	Negative	Positive
Carbamazepine	100,000	Negative	Positive
Carisoprodol	100,000	Negative	Positive
Chlordiazepoxide	100,000	Negative	Positive
Cis-Tramadol	100,000	Negative	Positive
Clobazam	100,000	Negative	Positive
Clomipramine	100,000	Negative	Positive
Clonazepam	100,000	Negative	Positive
Clozapine	100,000	Negative	Positive
Codeine	100,000	Negative	Positive
Cotinine	100,000	Negative	Positive
Cyclobenzaprine	100,000	Negative	Positive
Dehydronorketamine	100,000	Negative	Positive
Demoxepam	100,000	Negative	Positive
Desipramine	100,000	Negative	Positive
Desalkylflurazepam	100,000	Negative	Positive
Dextromethorphan	100,000	Negative	Positive
Diazepam	100,000	Negative	Positive
Digoxin	100,000	Negative	Positive
Dihydrocodeine	100,000	Negative	Positive
Δ9 THC	100,000	Negative	Positive
Doxepin	100,000	Negative	Positive
1R,2S (-) Ephedrine	100,000	Negative	Positive
1S,2R (+) Ephedrine	100,000	Negative	Positive
Ethyl-β-D-Glucuronide	100,000	Negative	Positive
Ethylmorphine	100,000	Negative	Positive
(S-)-Fenfluramine	100,000	Negative	Positive
(R+)-Fenfluramine	100,000	Negative	Positive
Fentanyl	100,000	Negative	Positive
Flunitrazepam	100,000	Negative	Positive
Fluoxetine	100,000	Negative	Positive
Flurazepam	100,000	Negative	Positive
Haloperidol	100,000	Negative	Positive
Heroin	100,000	Negative	Positive
Hexobarbital	100,000	Negative	Positive
Hydrocodone	100,000	Negative	Positive
Hydromorphone	100,000	Negative	Positive
11-hydroxy-Δ9 THC	100,000	Negative	Positive
Ibuprofen	500,000	Negative	Positive
Imipramine	100,000	Negative	Positive
Ketamine	100,000	Negative	Positive
Lamotrigine	100,000	Negative	Positive
Levorphanol Tartrate	100,000	Negative	Positive
Lidocaine	100,000	Negative	Positive

Compound	Concentration Tested (ng/mL)	Spiked EDDP Level	
		225 ng/mL (-25% Cutoff)	375 ng/mL (+25% Cutoff)
Lorazepam	100,000	Negative	Positive
Lorazepam Glucuronide	50,000	Negative	Positive
Lormetazepam	100,000	Negative	Positive
LSD	100,000	Negative	Positive
Maprotiline	100,000	Negative	Positive
(+)-MDA	100,000	Negative	Positive
MDEA	100,000	Negative	Positive
MDMA	100,000	Negative	Positive
Meperidine	100,000	Negative	Positive
Meprobamate	100,000	Negative	Positive
S(+)-Methamphetamine	100,000	Negative	Positive
Methaqualone	100,000	Negative	Positive
Methoxetamine	100,000	Negative	Positive
Methylone	100,000	Negative	Positive
Midazolam	100,000	Negative	Positive
Morphine	100,000	Negative	Positive
Morphine-3β-D-Glucuronide	100,000	Negative	Positive
Morphine-6β-D-Glucuronide	50,000	Negative	Positive
N-Desmethyltapentadol	100,000	Negative	Positive
Nalorphine	100,000	Negative	Positive
Naloxone	100,000	Negative	Positive
Naltrexone	100,000	Negative	Positive
Naproxen	100,000	Negative	Positive
Nitrazepam	100,000	Negative	Positive
11-nor-9-carboxy-Δ9-THC	100,000	Negative	Positive
Norbuprenorphine	50,000	Negative	Positive
Norcodeine	100,000	Negative	Positive
Nordiazepam	100,000	Negative	Positive
Norketamine	100,000	Negative	Positive
Normorphine	100,000	Negative	Positive
Norpropoxyphene	75,000	Negative	Positive
Norpseudoephedrine	100,000	Negative	Positive
Nortriptyline	100,000	Negative	Positive
Olanzapine	100,000	Negative	Positive
Oxazepam	100,000	Negative	Positive
Oxycodone	100,000	Negative	Positive
Oxymorphone	100,000	Negative	Positive
PCP	50,000	Negative	Positive
Pentazocine	100,000	Negative	Positive
Pentobarbital	100,000	Negative	Positive
Phenobarbital	100,000	Negative	Positive
Phentermine	100,000	Negative	Positive
Phenylephrine	100,000	Negative	Positive
Phenylpropanolamine	100,000	Negative	Positive
Phenytoin	100,000	Negative	Positive
PMA	100,000	Negative	Positive
Prazepam	100,000	Negative	Positive

Compound	Concentration Tested (ng/mL)	Spiked EDDP Level	
		225 ng/mL (-25% Cutoff)	375 ng/mL (+25% Cutoff)
Propoxyphene	100,000	Negative	Positive
Propranolol	100,000	Negative	Positive
Protriptyline	100,000	Negative	Positive
R,R (+)- Pseudoephedrine	100,000	Negative	Positive
S,S (-)- Pseudoephedrine	100,000	Negative	Positive
Ranitidine	100,000	Negative	Positive
Ritalinic Acid	100,000	Negative	Positive
Salicylic Acid	100,000	Negative	Positive
Secobarbital	100,000	Negative	Positive
Sertraline	100,000	Negative	Positive
Sufentanil Citrate	50,000	Negative	Positive
Tapentadol	100,000	Negative	Positive
Temazepam	100,000	Negative	Positive
Theophylline	100,000	Negative	Positive
Thioridazine	100,000	Negative	Positive
Trazodone	100,000	Negative	Positive
Triazolam	100,000	Negative	Positive
Trifluoromethylphenylpiperazine	100,000	Negative	Positive
Trimipramine	100,000	Negative	Positive
Venlafaxine	100,000	Negative	Positive
Verapamil	100,000	Negative	Positive
Zolpidem Tartrate	100,000	Negative	Positive

Endogenous Substances

High concentrations of the following endogenous substances were added into urine spiked with EDDP at ± 25% of the cutoff concentrations (75 ng/mL and 125 ng/mL for the 100 ng/mL cutoff, 225 ng/mL and 375 ng/mL for the 300 ng/mL cutoff). No interference was observed when tested with the ARK EDDP Assay in both qualitative and semiquantitative modes.

Compound	Concentration Tested	Spiked EDDP Level	
		-25% Cutoff	+25% Cutoff
Acetone	1000 mg/dL	Negative	Positive
Ascorbic Acid	1500 mg/dL	Negative	Positive
Bilirubin – Conjugated	2 mg/dL	Negative	Positive
Bilirubin – Unconjugated	2 mg/dL	Negative	Positive
Boric Acid	1% w/v	Negative	Positive
Creatinine	500 mg/dL	Negative	Positive
Ethanol	1000 mg/dL	Negative	Positive
Galactose	10 mg/dL	Negative	Positive
Gamma Globulin	500 mg/dL	Negative	Positive
Glucose	2000 mg/dL	Negative	Positive
Hemoglobin	300 mg/dL	Negative	Positive
Human Albumin	500 mg/dL	Negative	Positive

Compound	Concentration Tested	Spiked EDDP Level	
		-25% Cutoff	+25% Cutoff
Oxalic Acid	100 mg/dL	Negative	Positive
Riboflavin	7.5 mg/dL	Negative	Positive
Sodium Azide	1% w/v	Negative	Positive
Sodium Chloride	6000 mg/dL	Negative	Positive
Sodium Fluoride	1% w/v	Negative	Positive
Urea	6000 mg/dL	Negative	Positive

Specific Gravity and pH

Urine samples with specific gravity values ranging 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 EDDP at ± 25% of the cutoff concentrations (75 ng/mL and 125 ng/mL for the 100 ng/mL cutoff, 225 ng/mL and 375 ng/mL for the 300 ng/mL cutoff). No interference was observed when tested with the ARK EDDP Assay in both qualitative and semiquantitative modes.

Method Comparison

A total of one hundred nine (109) unaltered clinical human urine specimens that are not individually identifiable were analyzed for EDDP at the two cutoff levels with the ARK EDDP Assay in both qualitative and semiquantitative modes and the results were compared to GC/MS. The GC/MS confirmatory method was performed by a licensed reference laboratory. Results are summarized in the tables below.

Method Comparison – 100 ng/mL Cutoff

ARK Immunoassay Result	Low Negative Less than 50% below the Cutoff (< 50 ng/mL by GC/MS)	Near Cutoff Negative Between 50% below the Cutoff and the Cutoff (50 – 99 ng/mL by GC/MS)	Near Cutoff Positive Between the Cutoff and 50% above the Cutoff (100 – 150 ng/mL by GC/MS)	High Positive Greater than 50% above the Cutoff (> 150 ng/mL by GC/MS)
Negative	40	5	0	0
Positive	0	0	4	60

Method Comparison – 300 ng/mL Cutoff

ARK Immunoassay Result	Low Negative Less than 50% below the Cutoff (< 150 ng/mL by GC/MS)	Near Cutoff Negative Between 50% below the Cutoff and the Cutoff (150 – 299 ng/mL by GC/MS)	Near Cutoff Positive Between the Cutoff and 50% above the Cutoff (300 – 450 ng/mL by GC/MS)	High Positive Greater than 50% above the Cutoff (> 450 ng/mL by GC/MS)
Negative	49	4	0	0
Positive	0	1*	3	52

*Discordant Result

Sample ID Number	ARK Immunoassay Result	EDDP (ng/mL by GC/MS)
51	Positive	294

12 References

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Revised May 2026
1600-0870-00 Rev 05