ARK™ Tramadol Assay

This ARK Diagnostics, Inc. package insert for the ARK Tramadol 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 tramadol 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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EC REP

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CH REP

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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 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 5040-0001-00

Reagent Kit REF 5040-0001-01

Reagent Kit REF 5040-0001-02

1 Name

ARK™ Tramadol Assay

2 Intended Use

The ARK Tramadol Assay is an immunoassay intended for the qualitative and/or semiquantitative determination of tramadol in human urine at a cutoff concentration of 100 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 Tramadol 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

Tramadol [(±)cis-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl) cyclohexanol hydrochloride] is a centrally acting opioid analgesic that is prescribed for the management of moderate to moderately severe pain in adults. Tramadol is an opioid agonist and inhibitor of norepinephrine and serotonin re-uptake. The analgesic effect of tramadol may be due to both binding to μ-opioid receptors and weak inhibition of re-uptake of norepinephrine and serotonin. Analgesia in humans begins approximately within one hour after administration and reaches a peak in approximately two to three hours.[1]

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

Following oral administration, approximately 90% of tramadol is excreted in urine, of which 25-30% is excreted as unchanged drug and the rest as metabolites, glucuronides and sulfates. Tramadol is primarily metabolized in the liver by *O*- and *N*-demethylation to form *O*-desmethyltramadol and *N*-desmethyltramadol, respectively, followed by conjugation reactions to form glucuronides and sulfates. The main analgesic effective metabolite, *O*-desmethyltramadol, is known to have a higher affinity for opioid receptors than the parent drug.[3,4,5]

4 Principles of the Procedure

The ARK Tramadol Assay is a homogeneous enzyme immunoassay technique used for the analysis of tramadol 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
5040-0001-00	**ARK Tramadol Assay** **Reagent R1 – Antibody/Substrate** rabbit polyclonal antibodies to tramadol, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 28 mL
	Reagent R2 – Enzyme Tramadol 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
5040-0001-01	**ARK Tramadol Assay** **Reagent R1 – Antibody/Substrate** rabbit polyclonal antibodies to tramadol, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 115 mL
	Reagent R2 – Enzyme Tramadol 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
5040-0001-02	**ARK Tramadol Assay** **Reagent R1 – Antibody/Substrate** rabbit polyclonal antibodies to tramadol, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 500 mL
	Reagent R2 – Enzyme Tramadol 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 Tramadol 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 Tramadol 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.[6,7]
- 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. The recommended pH range for urine specimens is 4.0 – 11.0.[8]
- Obtain another sample for testing if adulteration of the sample is suspected. Adulteration of urine specimens can affect the test result.

- Boric acid interferes with results from this device. Do not use boric acid as a preservative.

8 Procedure

Materials Provided

ARK Tramadol Assay – REF 5040-0001-00, 5040-0001-01 or 5040-0001-02

Materials Required – Provided Separately

ARK Tramadol Calibrator – REF 5040-0002-00
ARK Tramadol Calibrator A (Negative) – REF 5040-0002-01
ARK Tramadol Calibrator B (Cutoff) – REF 5040-0002-02
Quality Controls – ARK Tramadol Control – REF 5040-0003-00

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 Tramadol 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 as a Cutoff Calibrator to distinguish negative and positive samples. Run the ARK Tramadol Low (75 ng/mL) and High (125 ng/mL) Controls as Negative and Positive respectively. Report test results less than the response value for the Cutoff Calibrator as Negative. Report test results equal to or greater than the response value for the Cutoff Calibrator as Positive.

Semiquantitative Results

Perform a 5-point calibration procedure; test calibrators in duplicate. Verify the calibration curve with the ARK Tramadol Low (75 ng/mL) and High (125 ng/mL) quality controls according to the established laboratory quality assurance plan. Specimens with sample results above the highest ARK Tramadol calibrator level (1000 ng/mL) may be diluted in ARK Tramadol 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 30 days based on supporting data

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

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

9 Results and Expected Values

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

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

Qualitative Analysis – Positive Results
A specimen that gives a response value equal to or greater than the ARK Tramadol Calibrator B Cutoff response value is interpreted as positive, indicating that tramadol 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 Tramadol calibrator level (1000 ng/mL) may be diluted in ARK Tramadol 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 Tramadol Assay reagents, calibrators and controls were developed as companion products. Performance with substituted products cannot be assured.
- A positive result using the ARK Tramadol Assay indicates only the presence of tramadol and does not necessarily correlate with the extent of physiological and psychological effects.

- **Boric acid interferes with results from this device. Do not test samples that have 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 Tramadol Assay.

Precision

Drug-free, negative human urine was supplemented with tramadol (0.0 to 200.0 ng/mL). Each level was assayed in quadruplicate twice a day for 20 days (N=160) in both qualitative and semiquantitative modes. Results are summarized in the tables below.

Qualitative Precision

Human Urine (ng/mL)	% Cutoff	# of Determinations	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	83 Negative/ 77 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

Human Urine (ng/mL)	Relative % Cutoff	# of Results	Mean (ng/mL)	Semiquantitative Precision Results
0.0	-100	160	1.7	160 Negative
25.0	-75	160	29.2	160 Negative
50.0	-50	160	53.7	160 Negative
75.0	-25	160	76.7	160 Negative
100.0	Cutoff	160	98.5	97 Negative/ 63 Positive
125.0	+25	160	120.5	160 Positive
150.0	+50	160	142.6	160 Positive
175.0	+75	160	165.3	160 Positive
200.0	+100	160	189.0	160 Positive

Analytical Recovery

Recovery across the assay range was assessed using the semiquantitative mode. Drug-free, negative human urine was supplemented with tramadol (1100.0

ng/mL) and dilutions were made proportionally with drug-free human urine. Tramadol 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	52.8	105.6
100.0	107.3	107.3
200.0	191.2	95.6
300.0	277.1	92.4
400.0	361.5	90.4
500.0	490.7	98.1
600.0	654.8	109.1
700.0	724.4	103.5
800.0	872.5	109.1
900.0	917.9	102.0
1000.0	984.1	98.4

Analytical Specificity

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

Tramadol Metabolites

The cross-reactivity of the following metabolites of tramadol 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 tramadol cutoff. 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

Compound	Lowest Concentration Tested That Produced a Response Approximately Equivalent to the Cutoff (ng/mL)	Percent Cross-reactivity (%)
O-Desmethyltramadol	600	16.67
N-Desmethyltramadol	150	66.67

Structurally Related Compounds

The following structurally related compounds were negative at the concentrations tested with the ARK Tramadol Assay in both qualitative and semiquantitative modes.

Compound	Concentration Tested (ng/mL)
6-Acetylmorphine	100,000
Amitriptyline	100,000
Amphetamine	100,000
Chlorpromazine	100,000
Clomipramine	100,000
Cyclobenzaprine	100,000
Desipramine	100,000
Dextromethorphan	100,000
Diphenhydramine	500,000
Doxepin	100,000
EDDP	100,000
EMDP	50,000
Fentanyl	100,000
Fluoxetine	100,000
Imipramine	100,000
Ketamine	100,000
MDEA	75,000
Meperidine	100,000
Methadone	500,000
Methapyrilene	10,000
Methylphenidate	100,000
Methylphenidate Metabolite (Ritalinic Acid)	100,000
Morphine	100,000
Morphine-3-beta-glucuronide	100,000
N-Desmethyltapentadol	100,000
Norcodeine	100,000
Norfentanyl	100,000
Norketamine	100,000
Normeperidine	50,000
Normorphine	100,000
Noroxycodone	25,000
Nortriptyline	100,000
PCP	100,000
Pentazocine	100,000
Propranolol	15,000
Quinine	450,000
Risperidone	50,000
Tapentadol	100,000
Thioridazine	100,000
Trazodone	100,000
Venlafaxine	100,000

Interference – Exogenous Substances

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

Compound	Concentration (ng/mL)	75 ng/mL (-25% Cutoff)	125 ng/mL (+25% Cutoff)
6-Acetylcodeine	100,000	Negative	Positive
6-Acetylmorphine	100,000	Negative	Positive
7-Aminoclonazepam	100,000	Negative	Positive
7-Aminoflunitrazepam	100,000	Negative	Positive
7-Aminonitrazepam	100,000	Negative	Positive
Albuterol	100,000	Negative	Positive
Acetaminophen	500,000	Negative	Positive
Acetylsalicylic Acid	500,000	Negative	Positive
Alprazolam	50,000	Negative	Positive
Amitriptyline	100,000	Negative	Positive
Amobarbital	100,000	Negative	Positive
Amphetamine	100,000	Negative	Positive
Benzoylecgonine	500,000	Negative	Positive
Benzylpiperazine	100,000	Negative	Positive
Bromazepam	100,000	Negative	Positive
4-Bromo-2,5-Dimethoxyphenethylamine	100,000	Negative	Positive
Buprenorphine	100,000	Negative	Positive
Buprenorphine Glucuronide	50,000	Negative	Positive
Bupropion	25,000	Negative	Positive
Butabarbital	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
Chlorpromazine	100,000	Negative	Positive
Clobazam	100,000	Negative	Positive
Clomipramine	100,000	Negative	Positive
Clonazepam	100,000	Negative	Positive
Cocaine	100,000	Negative	Positive
Codeine	100,000	Negative	Positive
Cotinine	100,000	Negative	Positive
Cyclobenzaprine	100,000	Negative	Positive
Delta-9-THC	100,000	Negative	Positive
Demoxepam	100,000	Negative	Positive
Desalkylflurazepam	100,000	Negative	Positive
Desipramine	100,000	Negative	Positive
Dextromethorphan	100,000	Negative	Positive
Diazepam	50,000	Negative	Positive
Dihydrocodeine	100,000	Negative	Positive
Diphenhydramine	100,000	Negative	Positive
Doxepin	100,000	Negative	Positive
Ecgonine	100,000	Negative	Positive

Compound	Concentration (ng/mL)	75 ng/mL (-25% Cutoff)	125 ng/mL (+25% Cutoff)
Ecgonine Methyl Ester	100,000	Negative	Positive
EDDP	100,000	Negative	Positive
1R, 2S(-)-Ephedrine	100,000	Negative	Positive
1S, 2R(+)-Ephedrine	100,000	Negative	Positive
EtG	100,000	Negative	Positive
Ethylmorphine	100,000	Negative	Positive
R-Fenfluramine	100,000	Negative	Positive
S-Fenfluramine	100,000	Negative	Positive
Fentanyl	100,000	Negative	Positive
Flunitrazepam	100,000	Negative	Positive
Fluoxetine	50,000	Negative	Positive
Fluphenazine	100,000	Negative	Positive
Flurazepam	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-delta-9-THC	100,000	Negative	Positive
Ibuprofen	100,000	Negative	Positive
Imipramine	100,000	Negative	Positive
Ketamine	100,000	Negative	Positive
Lamotrigine	100,000	Negative	Positive
Levorphanol	75,000	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
MDA	100,000	Negative	Positive
MDEA	10,000	Negative	Positive
MDMA	50,000	Negative	Positive
Meperidine	100,000	Negative	Positive
Meprobamate	100,000	Negative	Positive
Methadone	500,000	Negative	Positive
S(+)-methamphetamine	500,000	Negative	Positive
Methaqualone	100,000	Negative	Positive
Methylphenidate	25,000	Negative	Positive
Methylphenidate Metabolite (Ritalinic Acid)	100,000	Negative	Positive
Metronidazole	300,000	Negative	Positive
Midazolam	100,000	Negative	Positive
Morphine	100,000	Negative	Positive
Morphine-3-beta-glucuronide	100,000	Negative	Positive
Morphine-6-beta-glucuronide	100,000	Negative	Positive
Nalorphine	100,000	Negative	Positive
Naloxone	100,000	Negative	Positive
Naltrexone	100,000	Negative	Positive
Naproxen	100,000	Negative	Positive
N-desmethyltapentadol	25,000	Negative	Positive
Nicotine	10,000	Negative	Positive
Nitrazepam	100,000	Negative	Positive

Compound	Concentration (ng/mL)	75 ng/mL (-25% Cutoff)	125 ng/mL (+25% Cutoff)
Norbuprenorphine	100,000	Negative	Positive
Norcodeine	100,000	Negative	Positive
Nordiazepam	100,000	Negative	Positive
Normorphine	100,000	Negative	Positive
Norpropoxyphene	100,000	Negative	Positive
Norpseudoephedrine	100,000	Negative	Positive
Nortriptyline	100,000	Negative	Positive
Oxazepam	100,000	Negative	Positive
Oxazepam Glucuronide	10,000	Negative	Positive
Oxycodone	100,000	Negative	Positive
Oxymorphone	100,000	Negative	Positive
PCP	10,000	Negative	Positive
Pentazocine	50,000	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
Propoxyphene	100,000	Negative	Positive
Propranolol	2,000	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
Salicylic Acid	100,000	Negative	Positive
Secobarbital	100,000	Negative	Positive
Sertraline	50,000	Negative	Positive
Sufentanil Citrate	10,000	Negative	Positive
Tapentadol	25,000	Negative	Positive
Temazepam	100,000	Negative	Positive
11-nor-9-carboxy THC	100,000	Negative	Positive
Theophylline	100,000	Negative	Positive
Thioridazine	25,000	Negative	Positive
Tilidine	50,000	Negative	Positive
Trazodone	100,000	Negative	Positive
Triazolam	100,000	Negative	Positive
Trifluoromethylphenylpiperazine	100,000	Negative	Positive
Trimipramine	100,000	Negative	Positive
Valproic Acid	250,000	Negative	Positive
Zolpidem Tartrate	100,000	Negative	Positive

Interference – Endogenous Substances

High concentrations of the following endogenous substances were added into urine spiked with tramadol (± 25% of the cutoff concentration). No interference was observed when tested with the ARK Tramadol Assay in both qualitative and semiquantitative modes.

Compound	Concentration Tested	75 ng/mL (-25% Cutoff)	125 ng/mL (+25% Cutoff)
Acetone	1000 mg/dL	Negative	Positive
Ascorbic Acid	1500 mg/dL	Negative	Positive
Bilirubin	2 mg/dL	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	3000 mg/dL	Negative	Positive
Hemoglobin	300 mg/dL	Negative	Positive
Human Albumin	500 mg/dL	Negative	Positive
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

Interference – Boric Acid

One percent (1%) w/v of boric acid was added into urine spiked with tramadol (± 25% of the cutoff concentration) and tested with the ARK Tramadol Assay in both qualitative and semiquantitative modes. Results are provided in the table below.

Compound	Concentration Tested	Semiquantitative Mode		Qualitative Mode	
		75 ng/mL (-25% Cutoff)	125 ng/mL (+25% Cutoff)	75 ng/mL (-25% Cutoff)	125 ng/mL (+25% Cutoff)
Boric Acid	1% w/v	Negative	Positive	Negative	Negative

Boric acid interferes with results from this device. Do not test samples that have boric acid as a preservative.

Interference – Specific Gravity and pH

Urine samples with specific gravity values from 1.000 to 1.030 and pH values ranging from 3.0 to 11.0 were tested in the presence of the two levels of tramadol at ± 25% of the cutoff concentration. No interference was observed when tested with the ARK Tramadol Assay in both qualitative and semiquantitative modes.

Method Comparison

A total of one hundred fifteen (115) unaltered clinical human urine specimens that are not individually identifiable were analyzed for tramadol with the ARK Tramadol Assay in both qualitative and semiquantitative modes and the results were compared to LC-MS/MS. The LC-MS/MS confirmatory method was performed by a licensed reference laboratory. Results are summarized in the tables below.

ARK Immunoassay Result	Low Negative Less than 50% below the Cutoff (< 50 ng/mL by LC-MS/MS)	Near Cutoff Negative Between 50% below the Cutoff and the Cutoff (50 – 99 ng/mL by LC-MS/MS)	Near Cutoff Positive Between the Cutoff and 50% above the Cutoff (100 – 150 ng/mL by LC-MS/MS)	High Positive Greater than 50% above the Cutoff (> 150 ng/mL by LC-MS/MS)
Negative	50	0	0	0
Positive	0	5*	4	56

*Discordant Results

Sample ID Number	ARK Immunoassay Result	Tramadol (ng/mL by LC-MS/MS)
01	Positive	74.0
05	Positive	98.7
06	Positive	98.9
51	Positive	75.0
52	Positive	79.0

O-desmethyltramadol was detected in these samples and contributed to the positive result obtained with the ARK Tramadol Assay.

12 References

1. Prescribing Information. 2017. ULTRAM®. Janssen Pharmaceuticals, Inc. (Titusville, New Jersey).

2. Department of Justice, Drug Enforcement Administration. Schedules of Controlled Substances: Placement of Tramadol Into Schedule IV. Federal Register / Vol. 79, No. 127 / Wednesday, July 2, 2014 (Effective Date: August 18, 2014) / Rules and Regulations.

3. Gong, L. et al. 2014. PharmGKB Summary: Tramadol Pathway. *Pharmacogenet Genomics*. **24(7)**:374-380.

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13 Trademarks

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