510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k182280

B. Purpose for Submission:

New device

C. Measurand:

Tramadol

D. Type of Test:

Homogenous enzyme immunoassay, qualitative and semi-quantitative

E. Applicant:

ARK Diagnostics, Inc.

F. Proprietary and Established Names:

ARK Tramadol Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.3650, Opiate test system

2. Classification:

   Class II

3. Product code:

   DJG

4. Panel:

   Toxicology (91)

H. Intended Use:

1. Intended use(s):

   See Indications for use below.

2. Indication(s) for 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. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   Beckman Coulter AU 680 chemistry analyzer

I. Device Description:

The ARK Tramadol Assay is a homogeneous enzyme immunoassay used for the detection of tramadol in human urine. ARK Tramadol Assay includes the following reagents:

Reagent R1 – Antibody/Substrate

Rabbit polyclonal antibodies to tramadol, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers

Reagent R2 – Enzyme

Tramadol derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), bovine serum albumin, buffer, sodium azide and stabilizers

All reagents are in liquid form and ready to use.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Immunalysis Tramadol Urine Enzyme Immunoassay

2. Predicate 510(k) number(s):

   k141803

3. Comparison with predicate:

Characteristic	**Predicate Device** Immunalysis Tramadol Urine Enzyme Immunoassay (k141803)	**Candidate Device** ARK Tramadol Assay
Similarities		
Intended Use	For the qualitative and semiquantitative determination of tramadol in human urine; For *in vitro* diagnostic use	Same

Characteristic	Predicate Device Immunalysis Tramadol Urine Enzyme Immunoassay (k141803)	Candidate Device ARK Tramadol Assay
Sample Matrix	Human urine	Same
Test System	Homogenous enzyme immunoassay (EIA)	Same
User Environment	Clinical laboratories; Prescription use only	Same
Reagents Form	Liquid – Ready to use	Same
Reagent Materials	Two reagent system: Antibody/substrate reagent and enzyme labeled conjugate Sodium azide preservative	Same
Storage	2-8˚C	Same
Antibody	Polyclonal antibodies to tramadol	Same
Detection	Absorbance change measured spectrophotometrically at 340 nm	Same

Characteristic	Predicate Device Immunalysis Tramadol Urine Enzyme Immunoassay (k141803)	Candidate Device ARK™ Tramadol Assay
Differences		
Cutoff Level	200 ng/mL	100 ng/mL

K. Standard/Guidance Document Referenced (if applicable):

- CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods.
- CLSI EP7-A2: Interference Testing in Clinical Chemistry
- CLSI EP12-A2: User Protocol for Evaluation of Qualitative Test Performance

L. Test Principle:

The ARK Tramadol Assay is based on competition between drug in the specimen and drug labeled with recombinant glucose-6-phosphate dehydrogenase (tramadol-rG6PDH), for antibody binding sites. When tramadol-rG6PDH is bound by antibody, enzyme activity decreases. In the presence of free tramadol from the specimen, the free tramadol competes for antibody binding to tramadol-rG6PDH, resulting in increased enzyme activity that is proportional to the free tramadol concentration. Active tramadol-rG6PDH 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision studies were performed using CLSI EP05-A3 as a guideline. 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 Mode:

Human Urine (ng/mL)	% Cutoff	# of Determination	Qualitative Precision
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 Mode:

Human Urine (ng/mL)	% Cutoff	# of Determinations	Semiquantitative 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	97 Negative/ 63 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

b. Linearity/assay reportable range:

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

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

ARK Tramadol Calibrators and Controls are traceable to high purity certified commercial tramadol.

d. *Detection limit:*

Not applicable.

e. *Analytical specificity:*

Cross-reactivity

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 yield 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 (%)
Tramadol	100	100
O-Desmethyltramadol	600	16.67
N-Desmethyltramadol	150	66.67

The following structurally related compounds do not cross-react with the ARK Tramadol Assay in both qualitative and semiquantitative modes at the concentration tested:

Compound	Concentration Tested (µg/mL)
6-Acetyl Morphine	10
Amitriptyline	100
Amphetamine	100
Chlorpromazine	50
Clomipramine	50
Cyclobenzaprine	10
Desipramine	50
Dextromethorphan	100
Diphenhydramine	500
Doxepin	50
EDDP	100
EMDP	50
Fentanyl	100
Fluoxetine	50
Imipramine	30
Ketamine	100
MDEA	75
Meperidine	100
Methadone	100
Methapyrilene	10
Methylphenidate	100
Methylphenidate Metabolite	100
Morphine	50
Morphine-3-glucuronide	50
N-Desmethyltapentadol	100
Norcodeine	100
NorFentanyl	100
Norketamine	100

Normeperidine	50
Normorphine	50
Noroxycodone	25
Nortriptyline	10
Pentazocine (Talwin)	100
PCP	100
Propranolol	15
Quinine	450
Risperidone	50
Tapentadol	100
Thioridazine	100
Trazodone	10
Venlafaxine	100

Interference

High concentrations of the structurally unrelated compounds or endogenous 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 listed at the concentrations 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-Acetylcodine	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
S-(+) Amphetamine	100,000	Negative	Positive
Benzoylecgonine	500,000	Negative	Positive
Benzylpiperazine	100,000	Negative	Positive
Bromazepam	100,000	Negative	Positive
4-Bromo-	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

Compound	Concentration (ng/mL)	75 ng/mL (-25% Cutoff)	125 ng/mL (+25% Cutoff)
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
Desakylflurazepam	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
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
Hexobarital	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

Compound	Concentration (ng/mL)	75 ng/mL (-25% Cutoff)	125 ng/mL (+25% Cutoff)
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
Meprotiline	50,000	Negative	Positive
Methadone	500,000	Negative	Positive
S(+)-methamphetamine	500,000	Negative	Positive
Methaqualone	100,000	Negative	Positive
Methylphenidate	25,000	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
Norbuprenorphine	100,000	Negative	Positive
Norcodeine	100,000	Negative	Positive
Nordiazepam	100,000	Negative	Positive
Normorphine	100,000	Negative	Positive
Norproproxyphene	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

Compound	Concentration (ng/mL)	75 ng/mL (-25% Cutoff)	125 ng/mL (+25% Cutoff)
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
Ritalinic Acid	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
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

Endogenous Substances	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

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.

Com-pound	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	1% w/v	Negative	Positive	Negative	Negative

The following statement is listed under the Limitations section of the labeling, "Do not use Boric Acid as a preservative."

pH and Specific Gravity

For potential interference from the pH of urine, device performance in the qualitative and semi-quantitative modes was tested using a range of urine pH values (3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0). All test samples were prepared in drug free

urine containing Tramadol at ± 25% of the cutoff (75 ng/mL and 125 ng/mL tramadol concentrations). No positive or negative interference was observed at urine pH values ranging from 3.0 to 11.0 for each test mode.

For potential interference from the specific gravity of urine, device performance in the qualitative and semi-quantitative modes was tested using a range of urine specific gravity values (1.000, 1.002, 1.005, 1.010, 1.015, 1.020, 1.025 and 1.030). All test samples were prepared in drug free urine containing Tramadol at ± 25% of the cutoff (75 ng/mL and 125 ng/mL tramadol concentrations). No positive or negative interference was observed at urine specific gravity values ranging from 1.000 to 1.030 for each test mode.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

A total of 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**	**Near Cutoff Negative Between 50% below the Cutoff and the Cutoff (50 – 99**	**Near Cutoff Positive Between the Cutoff and 50% above the Cutoff**	**High Positive Greater than 50% above the Cutoff**
Negative	50	0	0	0
Positive	0	5*	4	56

*Discordant Results: O-desmethyltramadol was detected in the below samples and contributed to the positive result obtained with the ARK Tramadol Assay.

Discordant Sample ID #	Tramadol by LC/MS- MS (ng/mL)	Candidate Device (Positive/Negative)
01	74	Positive
05	98.7	Positive
06	98.9	Positive

Discordant Sample ID #	Tramadol by LC/MS- MS (ng/mL)	Candidate Device (Positive/Negative)
51	75.0	Positive
52	79.0	Positive

b. Matrix comparison:

Not applicable. Urine is the only claimed matrix for the candidate device.

3. Clinical studies:

a. Clincal Sensitivity:

Not applicable.

b. Clinical specificity:

Not applicable.

c. Other clinical supportive data (when a. and b. are not applicable):

The sponsor provided published, peer-reviewed articles and reports which, in addition to data provided by the sponsor, supported that for doses of tramadol anticipated in the intended use population, a 100 ng/mL cutoff would detect the analyte for approximately 3-4 days (60-100 hours).

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

Not applicable.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.