510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K232522

B Applicant

ARK Diagnostics, Inc.

C Proprietary and Established Names

ARK Levetiracetam II Assay

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
ORI	Class II	21 CFR 862.3350 - Diphenylhydantoin Test System	TX - Clinical Toxicology

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

Levetiracetam

C Type of Test:

Homogeneous enzyme immunoassay (EIA)

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

ARK Levetiracetam II Assay: The ARK Levetiracetam II Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of levetiracetam in human serum or plasma on automated clinical chemistry analyzers. Levetiracetam concentrations can be used as an aid in management of patients treated with levetiracetam.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D Special Instrument Requirements:

The assay has been validated on the Beckman Coulter AU680.

IV Device/System Characteristics:

A Device Description:

The ARK Levetiracetam II Assay consists of reagents R1 anti-levetiracetam monoclonal antibody with substrate and R2 levetiracetam labeled with bacterial G6PDH enzyme.

B Principle of Operation:

The ARK Levetiracetam II Assay is a homogeneous immunoassay based on competition between drug in the specimen and levetiracetam labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. 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 the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance. Endogenous serum G6PDH does not interfere with the results because the coenzyme NAD functions only with the bacterial enzyme used in the assay.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Ark Levetiracetam Assay

B Predicate 510(k) Number(s):
K091653

C Comparison with Predicate(s):

Device & Predicate Device(s):	K232522	K091653
Device Trade Name	ARK Levetiracetam II	ARK Levetiracetam

	Assay	Assay
General Device Characteristic Similarities		
Intended Use/Indications For Use	For the quantitative determination of levetiracetam in human serum or plasma on automated clinical chemistry analyzers.	Same
Specimen	Serum or plasma	Same
Methodology	Homogeneous enzyme immunoassay (EIA)	Same
Platform required	Automated clinical chemistry analyzer	Same
Accessary reagents	Calibrators (six levels) and controls (three levels)	Same
Measurement range	2.0 to 100.0 µg/mL	Same
General Device Characteristic Differences		
Reagent components	Anti-levetiracetam Antibody/Substrate Reagent (R1) containing rabbit monoclonal antibodies to levetiracetam	Anti-levetiracetam Antibody/Substrate Reagent (R1) containing rabbit polyclonal antibodies to levetiracetam

VI Standards/Guidance Documents Referenced:

CLSI Guideline EP 17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures
CLSI Guideline EP5-A3: Evaluation of Precision Performance of Quantitative Measurement Methods
CLSI Guideline EP6-Ed2: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach
CLSI Guideline EP7-Ed3: Interference Testing in Clinical Chemistry
CLSI Guideline EP37: Supplemental Tables for Interference Testing in Clinical Chemistry

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision studies were performed based upon recommendations in the CLSI EP05-A3 guideline. Tri-level controls and three samples of levetiracetam in three pooled human serum samples were used in the study. Data were collected on a single analyzer over twenty (20) non-consecutive days. Each level was assayed in quadruplicate twice a day. Each of the runs per day was separated by at least two hours. The within run, between day, and total imprecision estimates were calculated. Results are shown below.

			Within Run		Between Day		Total	
Sample	N	Mean (µg/mL)	SD	CV (%)	SD	CV (%)	SD	CV (%)
ARK Levetiracetam II Control								
Low	160	7.6	0.16	2.1	0.09	1.2	0.18	2.3
Mid	160	30.5	0.45	1.5	0.34	1.1	0.60	2.0
High	160	75.7	1.41	1.9	0.99	1.3	2.19	2.9
Human Serum								
Low	160	7.7	0.11	1.4	0.06	0.8	0.12	1.6
Mid	160	32.6	0.47	1.4	0.34	1.1	0.61	1.9
High	160	80.5	1.62	2.0	0.80	1.0	1.79	2.2

2. Linearity:

Linearity studies were performed based upon recommendations in CLSI EP06-Ed2. Ten (10) levels of samples ranging from 2 to 100 µg/mL were prepared from a gravimetrically prepared levetiracetam stock solution and levetiracetam-free serum pools. The averaged results of multiple runs and replicates (n=6) for each sample using the ARK Levetiracetam II Assay were used in the calculations. Regression analyses were performed between the measured mean levetiracetam and the nominal values for each dilution, using weighted linear regression analysis according to CLSI/NCCLS EP6-Ed2 and results are shown below. The regression equation calculated according to EP06-Ed2 is y=1.003x.

Estimated Value (µg/mL)	Results (µg/mL)	Predicted Results	% Difference
0.0	NA	NA	NA
2.0	1.88	2.01	11.2
3.0	3.2	3.04	5.9
4.0	3.9	4.07	3.3
6.0	6.1	6.13	0.7
10.0	11.0	10.25	-1.5
20.0	20.0	20.56	-3.5
40.0	42.0	41.18	-5.2
60.0	62.1	61.80	-6.4

80.0	78.9	82.42	-7.5
100.0	105.4	103.03	-8.6

Analytical recovery:
Serum samples across the assay range were prepared containing levetiracetam (sourced from USP). The results of the six replicates were averaged and compared to the theoretical target concentration and the percentage recovery was calculated. Results are shown below.

(Percent Recovery = 100 x Mean recovered concentration/Theoretical concentration)

Theoretical Concentration (µg/mL)	Mean Recovered Concentration (µg/mL)	Percent Recovery
2.0	1.9	95.0
4.0	3.9	97.5
10.0	9.8	98.0
20.0	20.1	100.5
45.0	46.2	102.6
80.0	77.8	97.3
100.0	100.3	100.3

A manual dilution protocol has not been validated to enable measurements of concentrations above the measurement range.

3. Analytical Specificity/Interference:

Interference studies were carried out based upon recommendations in CLSI EP7-A3. Interference was tested using serum pools at two levels of levetiracetam, 15 and 50 µg/mL. Each sample was assayed using the ARK Levetiracetam II Assay, along with a serum control of levetiracetam. Bias exceeding 10% is considered significant interference. The highest concentration of interferent tested that did not cause significant interference is summarized in the table below.

		Percentage Recovery	
Interfering Substance	Interferent Concentration	15 µg/mL Levetiracetam	50 µg/mL Levetiracetam
Human Albumin	12 g/dL	98.7	99.9
Bilirubin - conjugated	72 mg/dL	100.2	100.9
Bilirubin - unconjugated	72 mg/dL	101.7	97.1
Cholesterol	620 mg/dL	94.9	100.8
Human Gamma-Globulin	12 g/dL	102.7	98.7
Hemoglobin	1050 mg/dL	100.9	95.8
Intralipid	2000 mg/dL	98.7	100.4

Rheumatoid Factor	1080 IU/mL	98.8	94.5
Triglycerides	1670 mg/dL	98.6	95.9
Uric Acid	30 mg/dL	91.0	98.8

Specificity:
Metabolite 2-pyrrolidone-N-butyric acid (ucb L057) was also tested for cross-reactivity.

Metabolite	Concentration (µg/mL)	Percent Recovery	
		Levetiracetam 15 µg/mL	Levetiracetam 50 µg/mL
2-pyrrolidone-*N*-butyric acid	250.0	101.4	100.2

Drug interference:
Brivaracetam (Briviact) interferes with measurements of levetiracetam (Keppra) in the ARK Levetiracetam II Assay. Patients undergoing a switch in drug therapy involving Keppra and Briviact should not be monitored for levetiracetam using the ARK assay if there is a possibility these drugs are co-present in circulation.

The device was not impacted by other structurally unrelated drugs tested. A high concentration of each compound was spiked into normal human serum with known levels of levetiracetam (approximately 15 and 50 µg/mL) and assayed along with a serum control of levetiracetam. Measurement of levetiracetam resulted in ≤10% error in the presence of drug compounds at the levels tested.

Compound	Concentration (µg/mL)	Compound	Concentration (µg/mL)
Acetaminophen	500	Nortriptyline	20
Acetylsalicylic acid	1000	Oxcarbazepine	50
Amitriptyline	20	Phenobarbital	200
Caffeine	100	Phenytoin	200
Carbamazepine	120	Primidone	100
Clonazepam	50	Probenecid	600
Cyclosporin A	40	Salicylic Acid	500
Diazepam	50	Sulfamethoxazole	400
Digoxin	40	Sulfisoxazole	400
Erythromycin	200	Theophylline	250
Ethosuximide	250	Tiagabine	200
Felbamate	250	Topiramate	250
Gabapentin	100	Trimethoprim	40
Heparin	200 units/mL	Valproic Acid	500
Hydrochlorothiazide	20	Verapamil	100
Ibuprofen	500	Vigabatrin	150

Lamotrigine	250	Warfarin	250
Naproxen	500	Zonisamide	250

4. Assay Reportable Range:

   The manufacturer's claimed assay reportable range is from 2.0 to 100 µg/mL.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   The device is traceable to commercially available reference materials.

6. Detection Limit:

   The LOQ of the ARK Levetiracetam II Assay was determined using three reagent lots and the results supported an LOQ of 2.0 µg/mL with the manufacturer's acceptance criteria of CV within 20% and recovery within +/-15%.

7. Assay Cut-Off:

   Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Method comparison studies were performed to evaluate the performance of the ARK Levetiracetam II Assay. 104 serum patient samples spanning the range of 3.4 µg/mL to 98.3 µg/mL were tested on one assay lot with each sample tested in singlicate. Measurements obtained with the ARK Levetiracetam II Assay were made on the Beckman Coulter AU680 analyzer, while measurements of levetiracetam by the predicate assay were made on the Roche Hitachi 917 analyzer. Results from the candidate device were compared to the predicate device and results of the Passing- Bablok regression analysis for the study are shown below.

   $Y = 1.04X – 0.30$, $R^2 = 0.99$

2. Matrix Comparison:

   The sponsor provided studies that were adequate to support the use of the following anticoagulants: lithium heparin, sodium heparin, and potassium EDTA with the ARK II Levetiracetam II Assay.

C Clinical Studies:

1. Clinical Sensitivity:

   Not applicable.

2. Clinical Specificity:

Not applicable.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

D Clinical Cut-Off:

See expected values.

E Expected Values/Reference Range:

The following statement is included in the package insert:
A reference range for levetiracetam has not been well established. Reference ranges for seizure control have been proposed, which include serum/plasma trough concentrations from 6 to 46 µg/mL (35 to 270 µmol/L) or 10 to 40 µg/mL (59 to 235 µmol/L) including a laboratory alert level at 50 µg/mL (294 µmol/L). However, these ranges have not been validated by adequate controlled trials, and in general the relationship between these serum concentrations and clinical effect has not been well-defined. Levetiracetam drug concentrations should be used in conjunction with information available from clinical evaluations and other diagnostic procedures. Circulating levels of levetiracetam (serum blood concentrations) may be affected by compliance, renal function, pregnancy, drug-drug interactions and timing of the sample draw. Furthermore, the clinical effect of these serum blood concentrations may be further altered by changes in progression in the severity of the disease and the addition or withdrawal of concomitant drugs which may interact pharmacodynamically with circulating levels of levetiracetam.

The reference range of drug concentrations which is quoted should only imply a lower limit below which a therapeutic response is relatively unlikely to occur, and an upper limit above which toxicity is relatively likely to occur in the specific patient populations studied. Generally, clinicians using reference ranges such as these should be aware that, because of individual variation, patients may achieve therapeutic benefit with serum drug concentrations outside of these ranges and may experience toxicity with levels below the lower limit of the reference range. Sampling time should be standardized such that trough serum concentrations are measured just before the next dosage, preferably in the morning.

VIII Proposed Labeling:

The labeling support the finding of substantial equivalence for this device.

IX Conclusion:

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