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

A. 510(k) Number:
k091653

B. Purpose for Submission:
New assay

C. Analyte:
Levetiracetam

D. Type of Test:
Homogeneous enzyme immunoassay

E. Applicant:
ARK Diagnostics, Inc.

F. Proprietary and Established Names:
ARK™ Levetiracetam Assay, Calibrators and Controls

G. Regulatory Information:

1. Regulation section: 21 CFR 862.3350, 862.3200, 862.3280
2. Classification: Class II
3. Product code: ORI (assay), LAS (controls), DLJ (calibrator)
4. Panel: 91

H. Intended Use:

1. Intended use(s):

See indications for use.

2. Indication(s) for use:

The ARK™ Levetiracetam 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.

The ARK™ Levetiracetam Calibrator is intended for use in calibration of the ARK Levetiracetam Assay.

The ARK™ Levetiracetam Control is intended for use in quality control of the ARK Levetiracetam Assay.

3. Special conditions for use statement(s):

For prescription use only.

See information in **Expected Range** Section below for special conditions for use.

4. Special instrument requirements:

The assay has been validated on the Hitachi 917.

I. Device Description:

The ARK Levetiracetam Assay consists of reagents R1 anti-levetiracetam polyclonal antibody with substrate and R2 levetiracetam labeled with bacterial G6PDH enzyme. The ARK Levetiracetam Calibrator consists of a six-level set (target values: 0.0, 5.0, 12.5, 25.0, 50.0, and 100 µg/mL) to calibrate the assay, and the ARK Levetiracetam Control consists of a three-level (target values 7.5, 30, 75 µg/mL) set used for quality control of the assay.

J. Substantial Equivalence Information:

1. Predicate device name(s): ARK Topiramate Assay

2. Predicate 510(k) number(s) k083799

3. Comparison with predicate:

Characteristic	**Device** ARK™ Levetiracetam Assay	**Predicate** ARK™ Topiramate Assay K083799
Intended Use	The ARK™ Levetiracetam Assay is intended for the quantitative determination of levetiracetam in human serum or plasma on automated clinical chemistry analyzers.	The ARK™ Topiramate Assay is intended for the quantitative determination of topiramate in human serum or plasma on automated clinical chemistry analyzers.
Indications for Use	Levetiracetam concentrations can be used as an aid in management of patients treated with levetiracetam.	The results obtained are used in the diagnosis and treatment of topiramate overdose and in monitoring levels of topiramate to help ensure appropriate therapy.
Sample	Serum or plasma	Serum or plasma
Methodology	Homogenous enzyme immunoassay (EIA)	Homogenous enzyme immunoassay (EIA)
Reagent Components	Two (2) reagent system: Anti-levetiracetam Antibody/Substrate Reagent (R1) containing rabbit polyclonal antibodies to levetiracetam, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, preservatives, and stabilizers Enzyme Reagent (R2) containing	Two (2) reagent system: Anti-topiramate Antibody/Substrate Reagent (R1) containing rabbit polyclonal antibodies to an epitope of topiramate, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, preservatives, and stabilizers Enzyme Reagent (R2) containing

	levetiracetam labeled with bacterial G6PDH, buffer, bovine serum albumin, preservatives, and stabilizers	topiramate epitope labeled with bacterial G6PDH, buffer, bovine serum albumin, preservatives, and stabilizers
Platform required	Automated clinical chemistry analyzer	Automated clinical chemistry analyzer
Testing environment	Routine clinical laboratory	Routine clinical laboratory

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

CLSI documents:

"Evaluation of Precision Performance of Clinical Chemistry Devices", EP5;

"Evaluation of the Linearity of Quantitative Measurement", EP6;

"Interference Testing in Clinical Chemistry", EP7;

"Method Comparison and Bias Estimation Using Patient Samples", EP9;

"Protocols for Determination of Limits of Detection and Limits of Quantitation", EP 17-A.

L. Test Principle:

The ARK Levetiracetam 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 proportional 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 co-enzyme NAD functions only with the bacterial enzyme used in the assay.

M. Performance Characteristics (if/when applicable): Performance was validated on the Hitachi 917 instrument.

1. Analytical performance:

a. Precision

Samples evaluated included the ARK Levetiracetam Control and three pooled human serum samples. Data were collected on a single analyzer over twenty non-consecutive days. Five calibrations were performed (Days 1, 5, 10, 12 and 17) during this interval to provide variation (calibration was performing in a stable manner). Each sample was assayed in quadruplicate twice a day, with each run separated by at least two hours. Calculations were conducted according to CLSI Guideline EP5-A2. Results are summarized below:

			Within Run		Between Day		Total	
Sample	**N**	**Mean (µg/mL)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
Control Low	160	7.5	0.25	3.4	0.23	3.2	0.34	4.5
Control Mid	160	29.4	0.85	2.9	0.83	2.8	1.08	3.7
Control High	160	73.4	2.14	2.9	2.03	2.8	3.08	4.2
Low Patient Pool	160	6.9	0.26	3.8	0.22	3.1	0.33	4.8
Mid Patient Pool	160	30.2	0.87	2.9	1.10	3.7	1.23	4.1
High Patient Pool	160	75.5	2.19	2.9	2.35	3.1	3.31	4.4

b. Linearity/assay reportable range:

The manufacturer's claimed assay reportable range is from 2.0 to 100 µg/mL, based on linearity, recovery, lower limit of quantitation and method comparison results submitted in the 510(k). (See respective sections below for specific information on performance).

Linearity:
Samples ranging from 2 to 100 µg/mL were prepared from a gravimetrically prepared levetiracetam stock solution and levetiracetam-free serum pools. The dilutions were prepared so that levetiracetam concentrations varied in increments of 1 ug/mL within the range 2-10 µg/mL; and in increments of 10 ug/mL within the range of 10 µg/mL - 100 µg/mL. The averaged results of multiple runs and replicates (n=6) for each sample using the ARK assay were used in the calculations. Regression analyses were performed between the measured mean levetiracetam and the nominal values for each dilution, using first order and second order polynomial determinations according to CLSI/NCCLS EP6-A. In the range from 7-100 ug/mL deviations from linearity were < 2%. In the range from 4-7 µg/mL, deviations were less than 5%, and in the range from 2-3 µg/mL, deviations ranged from 8 -13%. Regression equation was:
(measured) = 1.0309(gravimetrically determined) + 0.0264, $r^2 = 0.9996$

Trueness/Recovery:
Serum samples across the assay range were prepared containing pure levetiracetam (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 $\frac{\text{Mean recovered concentration}}{\text{Theoretical concentration}}$

Theoretical Concentration (µg/mL)	**Mean Recovered Concentration (µg/mL)**	**Percent Recovery**
2.0	1.9	95.8
4.0	3.8	94.6

10.0	10.0	100.0
20.0	19.2	95.9
45.0	44.1	98.0
80.0	79.3	99.1
100.0	105.3	105.3

Dilution of high samples:
The package insert recommends that patient samples with concentrations above the assay range may be diluted with zero calibrator. To support this, both spiked samples, and high patient samples were diluted 4-fold with zero calibrator (calibrator A) before pipetting the sample into the sample cup. The average of multiple measurements was used to determine recovery (relative to gravimetric concentration) for spiked samples and (relative to LC/MS/MS) for patient samples. Results are shown below.

Spiked Sample Dilution

Spiked Level	Mean (µg/mL)	Dilution Factor	Recovery (%)
200.0 µg/mL	50.6 (n = 10)	4	101.2

Patient Specimen Dilution

LC/MS/MS (µg/mL)	ARK Result x Dilution Factor 4 (µg/mL)	Diff (%)
144.2	136.4	-5.4
104.6	102.0	-2.5
108.4	128.8	18.8
107.6	104.8	-2.6

To further examine the dilution procedure (recommended in the package insert) for any matrix effects, patient samples with concentrations in the assay range were diluted 4-fold with both serum and calibrator. The percent recovery for both are shown below.

Diluted Patient Specimen: Comparison to LC/MS/MS

	Diluted with Cal A Result x factor 4		Diluted with serum Result x factor 4	
LC/MS/MS (µg/mL)	(µg/mL)	% Difference	(µg/mL)	% Difference
86.4	82.4	-4.6	86.4	0.0
55.5	56.8	2.3	56.0	0.9
42.8	39.6	-7.5	38.8	-9.3
41.1	38.4	-6.6	40.4	-1.7
30.2	28.8	-4.6	28.4	-6.0

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

Stability:

Stability is evaluated using both accelerated and real-time protocols. For real-time stability testing, performance of materials refrigerated at 2-8 degrees C are compared to a reference set of materials that are stored frozen. Results are evaluated at multiple time points during the expiration dating period for accuracy and precision in measuring levetiracetam controls. No change in recovery or precision was observed in the real-time studies submitted in the 510(k). Real time studies (to support the expiration dating of 25 months) are ongoing.

Calibrator value assignment:

Stock solutions of pure levetiracetam (USP) were prepared and then added to both the synthetic calibrator/control matrix (calibrator A) to achieve the calibrator concentrations of 4.0 µg/mL, 10.0 µg/mL, 20.0 µg/mL, 45.0 µg/mL and 80.0 µg/mL levels. Samples were assayed by the ARK™ Levetiracetam Assay. Multiple runs and replicates were evaluated to determine recovery in the two matrices. The same spiking was performed with serum, so that any matrix effects may be detected. Analytical recoveries observed were 92-98% in the calibrator/control matrix and 95-100% in the serum matrix.

Control value assignment:
Quality control (QC) ranges were established using three runs with four replicates tested per run (n=12 for each control level) and the mean levetiracetam level of each control was calculated. Control ranges were set at ± 15% around the mean level tested. The package insert notes that each laboratory should establish its own ranges for each new lot of controls.

d. Detection limit:

Accuracy and precision studies near the low range of the assay were conducted to determine the manufacturer's claimed lower limit of quantitation (LOQ). Studies generally followed CLSI Guideline EP-17 guideline. Three levetiracetam levels were tested below the lowest positive calibrator concentration (5.0 µg/mL). Samples were prepared by gravimetric addition of pure levetiracetam (USP) to stock solution, and addition of this stock solution to pooled human serum negative for levetiracetam. Concentrations included 1.0, 2.0 and 3.0 µg/mL. Eight replicates of each sample were tested in each of five runs to give 40 replicates of each sample. Testing was performed on a total of 3 lots. Each run was performed on a separate day, with a separate calibration to enhance variability (i.e., challenge the assay). Results for all lots supported that performance met the manufacturer's acceptance criteria of the LOQ of 2.0 µg/mL having CV within 20% and recovery within +/-15%.

e. Analytical specificity:

Studies included testing for interference from endogenous compounds, metabolite, and commonly co-administered, and other anti-epileptic drugs.

Serum samples with clinically high concentrations of the potential interfering substances were tested by the assay in the presence of varying amounts of levetiracetam.

Specifically, serum samples tested contained levetiracetam at concentrations of 50 ug/mL, 15 ug/mL, and in some cases also 6 ug/mL. In addition, metabolite cross-reactivity was also tested in the absence of levetiracetam. Each sample containing interferent was assayed, along with a serum control of levetiracetam. Results for endogenous compounds and the metabolite ucb L057 are shown below. The complete list of interferents tested and results are included in the package insert.

Interfering Substance	Highest Interferent Concentration tested	Percent recovery relative to control	
		15 µg/mL Levetiracetam	50 µg/mL Levetiracetam
Albumin	12 g/dL	99.8	102.6
Bilirubin	70 mg/dL	100.4	102.1
Bilirubin	70 mg/dL	99.3	107.9
Cholesterol	535 mg/dL	105.3	94.0
Gamma-Globulin	12 g/dL	99.8	109.5
Hemoglobin	1000 mg/dL	98.6	100.9
Intralipid®	1500 mg/dL	97.1	99.8
Rheumatoid Factor	1100 IU/mL	98.1	106.4
Triglycerides	1033 mg/dL	96.8	100.2
Uric Acid	30 mg/dL	99.6	102.5

Metabolite (2-pyrrolidone-N-butyric acid) ucb L057:

Percent recovery of levetiracetam in presence of 250 ug ucb L057				
Conc. Tested (µg/mL)	**0 µg/mL Levetiracetam**	**6 µg/mL Levetiracetam**	**15 µg/mL Levetiracetam**	**50 µg/mL Levetiracetam**
250.0	**0.0**	**95.1**	**97.0**	**106.6**

(Results correspond to cross-reactivity calculations of 1.3% at 50 µg/mL, and -0.2% at 15 ug/mL in this evaluation.)

f. Assay cut-off:

See limit of quantitation section.

2. Comparison studies:

a. Method comparison with predicate device:

Method comparison studies were performed using banked samples obtained from two laboratories. Specimens were mostly derived from a wide geographic area in the U.S., from patients across a wide range of ages, including both male and female. Samples were selected to be within the assay concentration range. No other selection criteria were applied.

Results of samples obtained with the ARK assay were compared to those obtained with three different reference methods. Assay descriptions and summary validation information for the comparator methods were included in the 510(k). In the largest of the three evaluations (n=305) results were compared to those of an LC/MS/MS assay. Results of Passing-Bablok regression analysis for this study are shown below:

Method comparison summary:

Comparative Method	**Number of Samples and range**	**Slope (95% CI)**	**Intercept (95% CI)**	**Mean bias of ARK relative to LCMS (SD)**	**Correlation coefficient (r^2)**
LC/MS/MS	N= 305 Range: 2-86 ug/mL	Y = 1.01 (0.99 to 1,03)	0.25 (-0.24 to 0.63)	0.76 (0.35 to 1.16)	0.97 (0.96 to 0.97)

In addition there were smaller method comparison studies with two additional reference methods. The averaged bias (reference minus ARK) was within 10%. Additional testing of patient samples, with levetiracetam concentrations close to 100 ug/mL, by the ARK assay and the reference method demonstrated agreement. Differences were all within +/- 10%.

b. Matrix comparison:

Assay recovery, as well as precision (within one run) was evaluated for levetiracetam spiked into the following plasma types: Lithium heparin, Potassium, EDTA, Sodium heparin and serum. Matched specimens for serum and plasma were collected from eight subjects. Five samples spiked with levetiracetam (2, 10, 20, 40, 80 μg/mL) were prepared from each specimen (for a total of 40 samples per anticoagulant). Replicate measurements for each of the forty samples were averaged. Percent recoveries for all forty samples ranged from 95.3% to 109.6% relative to nominal spiked values; and from 95.0 to 107.2 for plasma relative to serum values. For the large majority of samples recoveries well-within these ranges were observed, and no specific trends (of differences in recovery relative to concentration) were observed.

In addition, the sponsor submitted a summary of literature suggesting equivalence between serum and heparinized plasma for measuring levetiracetam in patient samples, as

well as literature cautioning that levetiracetam is hydrolyzed in blood (Patsalos et al, **Epilepsia 47** 1818-21, 2006). To address this, the package insert advises users to process the blood as soon as possible after collection to prepare serum or plasma, since hydrolysis of levetiracetam may occur in the prolonged presence of whole blood.

3. Clinical studies:
   a. *Clinical Sensitivity:* NA. Not typically submitted for this type of assay.
   b. *Clinical specificity:* NA. Not typical for this type of assay.
   c. Other clinical supportive data (when a. and b. are not applicable): The sponsor provided a discussion with balanced and representative literature discussing clinical use of levetiracetam measurements.
4. Clinical cut-off: see expected values.
5. Expected values/Reference range:

The following is included in the package insert:

A reference range for levetiracetam has not been well established. Tentative reference ranges for seizure control have been proposed, which include concentrations from 6 to 46 μg/mL (35 to 270 μmol/L; trough samples). 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.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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