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

A. 510(k) Number:
k083799

B. Purpose for Submission:
New device

C. Measurand:
Topiramate

D. Type of Test:
Quantitative enzyme immunoassay

E. Applicant:
ARK Diagnostics, Inc.

F. Proprietary and Established Names:
ARK Topiramate Assay
ARK Topiramate Calibrator
ARK Topiramate Control

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
NWM	Diphenylhydantoin test system	862.3350	Toxicology
DLJ	Clinical Toxicology Calibrator	862.3200	Toxicology
LAS	Clinical Toxicology Control Material	862.3280	Toxicology

H. Intended Use:

1. Intended use(s):
   See Indications for use below.

2. Indication(s) for use:
   The ARK™ Topiramate Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of topiramate in human serum or plasma on automated clinical chemistry analyzers.

   The results obtained are used in the diagnosis and treatment of topiramate overdose and in monitoring levels of topiramate to help ensure appropriate therapy.

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

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

3. Special conditions for use statement(s):
   Topiramate drug concentrations should be used in conjunction with information available from clinical evaluations and other diagnostic procedures. Clinicians should carefully monitor patients during therapy and dosage adjustments. Pharmacokinetics may vary widely, particularly with co-medications, age, and/or compromised renal function. Multiple samples over time may be needed to determine steady-state concentrations for individual patients.

4. Special instrument requirements:
   Performance was evaluated on the Roche Hitachi 917.

I. Device Description:

The ARK Topiramate Assay is a two reagent system: Anti-topiramate Antibody/Substrate Reagent (R1) containing rabbit polyclonal antibodies to topiramate, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, preservatives and stabilizers and Enzyme Reagent (R2) containing topiramate epitope labeled with bacterial G6PDH, buffer, bovine serum albumin, preservatives and stabilizers.
ARK Topiramate Calibrator is comprised of a synthetic protein matrix (buffer, bovine serum albumin, and preservatives) with the following concentrations (µg/mL) of topiramate: CAL A (0.0), CAL B (2.0), CAL C (4.0), CAL D (8.0), CAL E (24.0) and CAL F (60.0). ARK Topiramate Control is comprised of a synthetic protein matrix (buffer, bovine serum albumin, and preservatives) with the following nominal concentrations (µg/mL) of topiramate: LOW (2.5), MID (10.0) and HIGH (40.0).

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Seradyn QMS® Topiramate Assay, INNOFLUOR Calibrator Reagent Set,

INNOFLUOR Topiramate Control Set

2. Predicate K number(s):
k070645, k970509, k9705173.

3. Comparison with predicate:

Similarities		
Item	Predicate Device (k070645)	Proposed Device
Intended Use	Intended for the quantitative determination of topiramate in human serum or plasma on automated clinical chemistry analyzers. The results obtained are used in the diagnosis and treatment of topiramate overdose and in monitoring levels of topiramate to help ensure appropriate therapy.	Same
Sample type	Serum and plasma	Same
Number of calibrators	Six	Same
Number of controls	Three	Same
Reagent condition and storage	Liquid, 2-8˚C	Same

Differences		
Item	Predicate Device (k070645)	Proposed Device
Test principle	Homogenous particle-enhanced turbidometric immunoassay (particle agglutination) (PETIA)	Homogenous enzyme immunoassay (EIA)
Measuring range	1.5 – 32 ug/mL	1.5 – 54.0 ug/mL

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

- CLSI Guideline EP5-A2: *Evaluation of Precision Performance of Quantitative Measurement Methods*
- CLSI Guideline EP 17-A: *Protocols for Determination of Limits of Detection and Limits of Quantitation*
- CLSI Guideline EP6-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach*
- CLSI Guideline EP9-A2*: Method Comparison and Bias Estimation Using Patient Samples*

- CLSI Protocol EP7-A2: *Interference Testing in Clinical Chemistry*

L. Test Principle:

The ARK Topiramate Assay is a homogeneous immunoassay based on competition between drug in the specimen and topiramate epitope 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision was evaluated using CLSI Guideline EP5-A2: *Evaluation of Precision Performance of Quantitative Measurement Methods* as a guideline. Tri-level controls containing topiramate were used in the study. Each level of control was assayed in quadruplicate twice a day for 20 days. Each of the runs per day was separated by at least two hours. Each run was calibrated separately. Two lots of reagents were used.

Sample	N	Mean (µg/mL)	Within run		Between day		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)
1	160	2.4	0.08	3.5	0.05	2.0	0.10	4.3
2	160	10.2	0.24	2.4	0.14	1.4	0.28	2.7
3	160	40.2	1.19	2.9	0.64	1.6	1.29	3.2

Three patient sample pools were evaluated in a 5-day precision study. A high patient pool was supplemented with topiramate stock prepared by gravimetric addition of purified USP topiramate in methanol. The serum samples were assayed in quadruplicate twice a day for 5 days. Each of the runs per day was separated by at least two hours. One calibration curve was used to quantitate all five days. A total of 40 determinations were made for each sample.

Sample	N	Mean (µg/mL)	Within run		Between day		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)
Low	40	2.7	0.1	3.8	0.1	3.6	0.1	5.1
Med	40	11.2	0.3	2.7	0.2	1.8	0.4	3.1
High	40	39.5	1.2	3.1	1.6	4.2	2.0	5.2

b. Linearity/assay reportable range:

Linearity: Linearity was evaluated using CLSI Guideline EP6-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach* as a guideline.

The claimed measuring range for this device is 1.5 to 54.0 µg/mL. The study to evaluate linearity was performed on the ROCHE Hitachi 917. A 60.0 µg/mL serum sample was prepared by adding USP topiramate gravimetrically into pooled human serum. This high sample was mixed with human serum negative for topiramate in varying proportions to create a dilution series of 19 samples with varying topiramate concentrations between across the assay range. The topiramate value of the high sample was confirmed by measurement with a reference method (gas chromatography). Two analytical runs of three replicates of each sample were assayed on an automated clinical chemistry analyzer. The results of the six replicates were averaged. The data was analyzed using linear regression as well as non-linear fitted polynomial regression. The linear bias was estimated by the predicted results according to 1^{st} order and 2^{nd} order mathematical regressions and the percentage difference between the predicted values according to CLSI EP6-A. Percentage difference between the predicted values of the 1^{st} and 2^{nd} order regressed values was considered acceptable if it was ± 10%. A linear relationship was demonstrated between 1.2 and 54.0 µg/mL.

Dilution/Carry-over: High sample carryover and accuracy of dilution were evaluated. Pooled human serum was supplemented gravimetrically with USP topiramate to contain 80.0 µg/mL of topiramate (High Sample) and 2.0 µg/mL (Low Sample). A single replicate was tested and five sets of the following sequential arrangement were tested: "$H_1H_2H_3L_1L_2L_3$". The mean result for 5 replicates of L_1 represents the possible carryover effect compared to the mean result for 5 replicates of L_3 (unaffected by a preceding high sample). No carry-over was observed from a high topiramate sample to a low topiramate sample.
Accuracy of dilution of a high sample was accomplished by diluting the High Sample manually with the ARK Topiramate Calibrator "A" (zero calibrator as diluent). A four-fold dilution was made to achieve an expected value of 20.0 µg/mL. The mean of 10 replicate measurements of diluted topiramate was calculated to determine the percentage recovery. Two lots of assay were used for these studies. Recovery of all diluted samples was acceptable (98.8%).

Recovery**:** Analytical recovery was evaluated by adding concentrated topiramate drug (USP) into human serum negative for topiramate. The stock solution was added volumetrically to human serum negative for topiramate, representing drug concentrations across the assay range. Test sample concentrations were 1.5, 2.5, 4.0, 5.0, 6.0, 10.0, 15.0, 30.0, 45.0, and 55.0 µg/mL.
Two analytical runs of three replicates of each sample were assayed on an automated clinical chemistry analyzer. The results of the six replicates were averaged and compared to the theoretical target concentration and the percentage recovery was calculated. Recovery at all samples was within ± 10% of the expected sample concentration.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Freezing and Thawing: Three human serum pools were created to evaluate time of storage at refrigerator temperature (2 to 8 °C) and the effects of freezing and thawing. The topiramate levels of the serum pools were approximately 4, 10 and 30 µg/mL, which spans the suggested therapeutic range of 2 to 25 µg/mL. One aliquot of each specimen was refrigerated for the duration of the study, 28 days. Three additional aliquots were frozen, with one aliquot each to evaluate 1, 2 and 3 freeze/thaw cycles. Freeze/thaw (F/T) cycles included a minium of 1 day for each period of freezing before thawing. All specimens were tested together on Day 5. After testing, the three aliquots were refrozen, and on Day 28 all specimens were tested again. This caused the three aliquots to have undergone an additional freeze/thaw cycle each to give 2, 3, and 4 freeze/thaw cycles for the respective specimens. The original refrigerated aliquot was maintained as the control throughout the testing period for each specimen, and Day 0 measurements were also made. Each test point included triplicate measurements. The mean topiramate levels were calculated.
Tri-level quality controls for the ARK Topiramate Control were used to evaluate freezing and thawing of the calibrator matrix. The nominal concentrations are 2.5 (LOW), 10.0 (MID) and 40.0 (HIGH) µg/mL. Three sets of controls were frozen, with 1, 2 or 3 freeze/thaw cycles respectively. One set was refrigerated under normal storage and served as the control. Two analytical runs were performed with 5 replicate determinations of topiramate per run. The mean topiramate concentration was calculated for 10 replicates each. Human serum specimens endured up to 28 days refrigeration and up to 4 freeze/thaw (F/T) cycles. No substantial differences either from the Day 0 measurement prior to challenge or in comparison with the measured topiramate in refrigerated aliquots were observed for the three specimens studied. The sponsor recommends that clarified specimens be stored up to one week at 2 to 8°C. If testing will be delayed more than one week, specimens should be stored frozen (≤ -10°C) up to four weeks prior to being tested. Users should limit the number of freeze-thaw cycles.

Expected values: ARK Topiramate Calibrator is comprised of a synthetic protein matrix (buffer, bovine serum albumin, and preservatives) with the following concentrations (µg/mL) of topiramate: CAL A (0.0), CAL B (2.0), CAL C (4.0), CAL D (8.0), CAL E (24.0) and CAL F (60.0). ARK Topiramate Control is comprised of a synthetic protein matrix (buffer, bovine serum albumin, and preservatives) with the following nominal concentrations (µg/mL) of topiramate: LOW (2.5), MID (10.0) and HIGH (40.0). Topiramate is traceable to United States Pharmacopeia (USP) topiramate. There is no internationally recognized standard for topiramate.

Value Assignment: Calibrators and controls are prepared gravimetrically and using USP topiramate. New lots must have topiramate levels ±5% compared to an internal reference calibrator or control lot.

Stability: Accelerated and real time stability studies are being performed to evaluate the stability of the ARK Topiramate Assay, Calibrator and Control. Stability testing protocols and acceptance criteria were described and found to be acceptable. Multiple lots are tested; precision and accuracy is evaluated after testing multiple replicates at each testing time point.

In addition, avoid prolonged exposure to temperatures above 32˚C.

d. Detection limit:

Limit of Quantitation (LOQ) studies were conducted using CLSI Guideline EP 17-A: *Protocols for Determination of Limits of Detection and Limits of Quantitation*.

Pooled human serum representative of the patient specimen matrix was supplemented with topiramate to give concentrations of 0.5, 1.0 and 1.5 µg/mL. Eight replicates of each sample were tested in each of five runs to give a minimum of 40 replicates of each LOQ sample tested. The LOQ of the ARK Topiramate Assay is defined as the lowest concentration for which acceptable inter-assay precision (≤ 20% CV) and recovery (± 15%) is observed.

Nominal (µg/mL)	Mean (µg/mL)	SD	% CV	% Recovery	N
0.5	0.3	0.03	8.8	62.0	40
1.0	1.0	0.05	5.3	96.0	40
1.5	1.4	0.05	3.7	94.2	40

The sponsor's criteria of LOQ were met at 1.0 µg/mL, but the claimed LoQ for the ARK Topiramate Assay is 1.5 µg/mL.

Results below the measuring range are reported as <1.5 µg/mL.

e. Analytical specificity:

A study was performed on the ROCHE Hitachi 917 to evaluate potential interferents of the assay.

Interference studies were conducted using CLSI Protocol EP7-A2: *Interference Testing in Clinical Chemistry* as a guideline. Clinically high concentrations of potentially interfering substances in serum with known levels of topiramate (approximately 5 and 20 µg/mL) were evaluated. Each sample was assayed using the ARK Topiramate Assay, along with a serum control of topiramate. Multiple replicates of each sample and their respective serum controls were tested. The mean results of topiramate were calculated and the percentage recovery relative to the serum control mean result was determined. Interference was indicated when addition of a compound led to a > 10% change in measuring topiramate levels above the results seen in the control.

i. Endogenous levels: The following endogenous compounds were evaluated and found not to interfere with the ARK Topiramate Assay:

Substance	**Concentration**
Albumin	12 g/dL
Bilirubin	60 mg/dL
Cholesterol	301 mg/dL
Gamma globulin	10 g/dL
Hemoglobin	1000 mg/dL
Heparin	200 units/mL
Triglycerides	1105 mg/dL
Uric Acid	25 mg/dL

ii. Cross-reactivity: Cross-reactivity was tested for a known metabolite of topiramate, 9-Hydroxy-topiramate (results below). It does not appear that this metabolite causes significant cross-reactivity with the ARK Topiramate Assay. Percent cross-reactivity was calculated as:

$$\% \text{ Cross-reactivity} = 100 \times \frac{(\text{mean value TEST} - \text{mean value CONTROL})}{(\text{conc. of cross-reactant})}$$

Percent interference was calculated as:

$$\% \text{ Interference} = 100 \times \frac{(\text{mean value TEST} - \text{mean value CONTROL})}{(\text{mean value CONTROL})}$$

Metabolite	Metabolite Conc. ug/mL	% Cross-reactivity		% Interference	
		5 µg/mL Topiramate	20 µg/mL Topiramate	5 µg/mL Topiramate	20 µg/mL Topiramate
9-Hydroxy-topiramate	40.0	1.2	1.6	8.6	3.2

iii. Co-administered drugs and common OTC drugs: A list of available drugs (with concentrations) tested is included in the package insert. At the concentrations tested, the drugs did not cause significant interference with the assay.

f. Assay cut-off:
Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:
Method comparison was conducted using CLSI Guideline EP9-A2*: Method Comparison and Bias Estimation Using Patient Samples*. Method comparison was conducted with leftover human serum specimens that are not individually identifiable. Human serum specimens from patients receiving topiramate therapy were collected during routine practices at an external site. The study

was performed with the proposed device on the ROCHE Hitachi 917 and on an FDA-cleared device (predicate). One instrument and two lots of reagents were used. 113 specimens were tested; unaltered patient samples up to 54.0 µg/mL were included. In addition, 15 specimens were supplemented with USP topiramate to create samples with topiramate levels that more fully spanned the assay range.
Specimens were diluted in the zero calibrator for the predicate method in order to obtain a quantitative measurement at concentrations above its reportable range.

Results of the Passing-Bablok regression analysis for the study are shown below.

Slope:	0.99 [95% CI: 0.97, 1.01]
y-intercept	-0.17
Correlation coefficient (r2)	0.99
N	113

b. Matrix comparison:
A study was performed on the ROCHE Hitachi 917 to study the equivalency of plasma and serum. Individual plasma and serum samples negative for topiramate were obtained and these samples were supplemented with topiramate at four concentrations spanning the proposed measuring range. Four anticoagulants studied included sodium heparin, lithium heparin, sodium EDTA (ethylenediaminetetraacetate), and potassium EDTA.
Six replicates of each sample were tested and the mean result was calculated. The mean percentage recovery in various plasma and serum was determined relative to a serum control. All of the plasma types evaluated were found to be equivalent to serum for the measurement of topiramate.

The use of serum separator tubes was not evaluated.

3. Clinical studies:
   a. Clinical Sensitivity:
   Not applicable.
   b. Clinical specificity:
   Not applicable.
   c. Other clinical supportive data (when a. and b. are not applicable):
   Not applicable.

4. Clinical cut-off:
   Not applicable.

5. Expected values/Reference range:

   A therapeutic range for topiramate has not been well established. The proposed therapeutic range (trough sample) for seizure control is 2 to 25 µg/mL and an

inconsistent correlation exists between levels of circulating topiramate to toxicity, adverse affect or clinical efficacy[1]. Topiramate levels above 25 µg/mL may occur infrequently. Therefore, monitoring topiramate concentration in patients is warranted.

[1]Johannessen, S. I. et al. 2003. Therapeutic Drug Monitoring of the Newer Antiepileptic Drugs. *Ther Drug Monit*. **25**:347-63.

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 a substantial equivalence decision.