

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

**A. 510(k) Number:**

k091884

**B. Purpose for Submission:**

New assay

**C. Analyte:**

Zonisamide

**D. Type of Test:**

Homogeneous enzyme immunoassay

**E. Applicant:**

ARK Diagnostics, Inc.

**F. Proprietary and Established Names:**

ARK™ Zonisamide Assay, Calibrators and Controls

**G. Regulatory Information:**

1. Regulation section: 21 CFR 862.3350, 862.3200, 862.3280
2. Classification: Class II – assay, calibrators; Class I reserved - controls
3. Product code: NWM (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™ Zonisamide Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of zonisamide in human serum or plasma on automated clinical chemistry analyzers. Zonisamide concentrations can be used as an aid in management of patients treated with zonisamide.

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

The ARK™ Zonisamide Control is intended for use in quality control of the ARK Zonisamide 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 Zonisamide Assay consists of reagents R1 anti-zonisamide polyclonal antibody with substrate and R2 zonisamide labeled with bacterial G6PDH enzyme. The ARK Zonisamide Calibrator consists of a six-level set (target values: 0.0, 2.5, 7.5, 20.0, 40.0, and 80 µg/mL ) to calibrate the assay, and the ARK Zonisamide Control consists of a three-level (target values 5.0, 25, 50 µg/mL) set used for quality control of the assay.

**J. Substantial Equivalence Information:**

1. Predicate device name(s): Seradyn QMS Zonisamide Assay
2. Predicate 510(k) number(s) k052511
3. Comparison with predicate:

| Characteristic      | Device   | Predicate  |
|---------------------|--|--|
| Intended Use        | The ARK™ Zonisamide Assay is intended for the quantitative determination of zonisamide in human serum or plasma on automated clinical chemistry analyzers.   | Same.  |
| Indications for Use | Zonisamide concentrations can be used as an aid in management of patients treated with zonisamide.   | Same   |
| Sample              | Serum or plasma  | Same   |
| Methodology         | Homogenous enzyme immunoassay (EIA)  | Same   |
| Reagent Components  | Two (2) reagent system:<br><br>Anti-zonisamide Antibody/Substrate Reagent (R1) containing rabbit polyclonal antibodies to zonisamide, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, preservatives, and stabilizers<br>Enzyme Reagent (R2) containing zonisamide labeled with bacterial G6PDH, buffer, bovine serum albumin, preservatives, and stabilizers | Two (2) reagent system containing rabbit polyclonal zonisamide antibodies in buffer and zonisamide-coated microparticles with azide preservatives. |

| Characteristic           | Device                                 | Predicate                             |
|--------------------------|--|---------------------------------------|
| Platform required        | Automated clinical chemistry analyzer  | Automated clinical chemistry analyzer |
| Calibrators and Controls | 6 calibrators and 3 levels of controls | Same                                  |

**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 Zonisamide Assay is a homogeneous immunoassay based on competition between drug in the specimen and zonisamide 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 coenzyme 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 Zonisamide Control (low, mid and high) 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:

| Sample               | N   | Mean<br>( $\mu\text{g/mL}$ ) | Within Run |           | Total |           |
|----------------------|-----|------------------------------|------------|-----------|-------|-----------|
|                      |     |                              | SD         | CV<br>(%) | SD    | CV<br>(%) |
| Control Low          | 160 | 5.0                          | 0.21       | 4.1       | 0.25  | 5.1       |
| Control Mid          | 160 | 24.4                         | 0.96       | 3.8       | 1.12  | 4.5       |
| Control High         | 160 | 50.6                         | 1.97       | 3.9       | 2.63  | 5.3       |
| Low Patient<br>Pool  | 160 | 7.0                          | 0.29       | 4.0       | 0.36  | 4.9       |
| Mid Patient<br>Pool  | 160 | 22.6                         | 0.81       | 3.5       | 1.01  | 4.4       |
| High Patient<br>Pool | 160 | 51.6                         | 2.47       | 4.9       | 2.96  | 5.9       |

***b. Linearity/assay reportable range:***

The manufacturer's claimed assay reportable range is from 2.0 to 50  $\mu\text{g/mL}$ , 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 below 2 to 48  $\mu\text{g/mL}$  were prepared from a gravimetrically prepared zonisamide stock solution and zonisamide-free serum pools. The dilutions were prepared so that zonisamide concentrations varied in increments of 1  $\mu\text{g/mL}$  within the range 2-8  $\mu\text{g/mL}$ ; and in increments of 8  $\mu\text{g/mL}$  within the range of 16  $\mu\text{g/mL}$  - 48  $\mu\text{g/mL}$ . 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 zonisamide and the nominal values for each dilution, using first order and second order polynomial determinations according to CLSI/NCCLS EP6-A. In the range below 3  $\mu\text{g/mL}$  deviations from linearity were within 15%; above 3  $\mu\text{g/mL}$  the deviations from linearity observed were within 5%. Linear regression yielded the equation: measured value = 0.97 (known gravimetric concentration) + 0.18,  $R^2 = 0.99$ .

Analytical recovery was performed by adding concentrated zonisamide drug (99% purity) into human serum negative for zonisamide. A stock concentrate of highly pure zonisamide was added volumetrically to human serum negative for zonisamide, representing drug concentrations across the assay range. Twenty replicates of each sample were assayed. The results were averaged and compared to the target concentration and percent recovery calculated. Results are shown below.

$\% \text{ Recovery} = 100 \times (\text{Mean recovered concentration} / \text{Theoretical concentration})$

| <b>Gravimetrically determined<br/>(theoretical)<br/>Concentration<br/>(µg/mL)</b> | <b>Mean Recovered<br/>Concentration<br/>(µg/mL)</b> | <b>Percent<br/>Recovery</b> |
|---|---|-----------------------------|
| 2.0   | 1.7   | 85.3                        |
| 3.0   | 3.0   | 100.0                       |
| 5.0   | 5.5   | 110.0                       |
| 15.0  | 15.7  | 104.5                       |
| 25.0  | 25.3  | 101.0                       |
| 35.0  | 35.0  | 100.0                       |
| 50.0  | 49.1  | 98.1                        |

Dilution of high samples:

The package insert recommends that patient samples with concentrations above the assay range of 50 µg/mL may be diluted with zero calibrator. Spiked samples with high levels of zonisamide (100.0 µg/mL) were diluted manually with Calibrator A of the ARK Zonisamide Calibrator. The mean result of 10 replicate measurements and recovery versus the target level is shown below. The mean recovery (n=10) was 102%, with percent CV = 3.8.

***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 zonisamide controls. No change in recovery or precision was observed in the real-time studies submitted in the 510(k). Real time studies are ongoing.

Calibrator value assignment:

Stock solutions are prepared by gravimetric dilution of pure zonisamide and then added to the synthetic calibrator/control matrix (calibrator A) to achieve the calibrator concentrations of 2.5, 7.5, 20, 40 and 80.0 µg/mL levels. Samples were assayed by the ARK™ Zonisamide 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 93-105% in the calibrator/control matrix and 93-104% 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 zonisamide level of each control was calculated. Control ranges were set at ± 10% 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 zonisamide levels were tested below the lowest positive calibrator concentration (2.5 µg/mL). Samples were prepared by gravimetric addition of pure zonisamide (USP) to stock solution, and addition of this stock solution to pooled human serum negative for zonisamide. Concentrations included 1.5, 2.0 and 2.5 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 zonisamide. Specifically, serum samples tested contained zonisamide at concentrations of approximately 15 µg/mL and 45 µg/mL. Each sample containing interferent was assayed, along with a serum control of zonisamide. Results for endogenous compounds and the metabolites N-acetyl zonisamide (NAZ) and non-glucuronidated 202-sulfamoyl acetyl phenol (SMAP) 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 Zonisamide                  | 50 µg/mL Zonisamide |
| Albumin                  | 12 g/dL                                  | 103.3                                | 97.9                |
| Bilirubin - conjugated   | 70 mg/dL                                 | 102.8                                | 101.0               |
| Bilirubin - unconjugated | 70 mg/dL                                 | 100.1                                | 98.8                |
| Cholesterol              | 651 mg/dL                                | 98.5                                 | 97.0                |

| Interfering Substance   | Highest Interferent Concentration tested | Percent recovery relative to control |                     |
|-------------------------|--|--------------------------------------|---------------------|
|                         |  | 15 µg/mL Zonisamide                  | 50 µg/mL Zonisamide |
| Gamma-Globulin          | 12 g/dL                                  | 97.3                                 | 101.4               |
| Hemoglobin              | 1000 mg/dL                               | 96.6                                 | 104.1               |
| Intralipid <sup>®</sup> | 1500 mg/dL                               | 94.8                                 | 94.7                |
| Rheumatoid Factor       | 1100 IU/mL                               | 98.4                                 | 100.2               |
| Triglycerides           | 1204 mg/dL                               | 96.5                                 | 96.9                |
| Uric Acid               | 30 mg/dL                                 | 98.5                                 | 99.4                |

Metabolites N-acetyl zonisamide (NAZ) and non-glucuronidated 202-sulfamoylacetyl phenol (SMAP)

| Metabolite | Conc. Tested (µg/mL) | 15 µg/mL Zonisamide% Cross-Reactivity | 45 µg/mL Zonisamide % Cross-Reactivity | 15 µg/mL Zonisamide % Interference | 45 µg/mL Zonisamide % Interference |
|------------|----------------------|---------------------------------------|--|------------------------------------|------------------------------------|
| NAZ        | 50.0                 | 1.7%                                  | 5.5%                                   | 5.4                                | 6.1                                |
|            | 10.0                 | 5.3%                                  | 3.3%                                   | 3.3                                | 0.7                                |
| SMAP       | 50.0                 | 18.2%                                 | 19.5%                                  | 57.1                               | 20.6                               |
|            | 10.0                 | 14.8%                                 | 27.3%                                  | 8.8                                | 5.8                                |

The complete list of potential endogenous interferents tested and observed recoveries are listed in the package insert

*f. Assay cut-off:*  
not applicable

2. Comparison studies:

*a. Method comparison with predicate device:*

Method comparison studies were performed using banked samples obtained from two laboratories. The method comparison was conducted with leftover human serum/plasma specimens that are not individually identifiable. 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 the predicate assay. Results of Passing-Bablok regression analysis for this study are shown below:

Method comparison summary:

| <b>Comparative Method</b> | <b>Number of Samples and range</b> | <b>Slope (95% CI)</b>         | <b>Intercept (95% CI)</b>   | <b>Correlation coefficient (r2)</b> |
|---------------------------|------------------------------------|-------------------------------|-----------------------------|-------------------------------------|
| Predicate                 | N= 176<br>Range:<br>5-45<br>ug/mL  | Y = 0.98<br>(0.94 to<br>1.02) | 0.25 (-<br>0.24 to<br>0.63) | 0.93 (0.91 to 0.95)                 |

*b. Matrix comparison:*

Two studies were performed for the matrix comparison study. In study number 1, assay recovery, as well as precision (within one run) was evaluated for zonisamide 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. Four samples spiked with zonisamide (7.5, 15, 30 and 60 µg/mL) were prepared from each specimen (for a total of 32 samples per anticoagulant). Replicate measurements for each of the 32 samples were averaged. Percent recoveries for all samples ranged from 95% to 113.4% relative to nominal spiked values; and from 91.3 to 109.0% 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 study number 2, matched specimens for serum and plasma were collected from four of the original eight subjects from study #1. Each matrix per subject was spiked with zonisamide for the target of 2.0 µg/mL. Each subject's matched matrices were tested as a group, performing two calibrated runs and tested in triplicate each run for a total of six replicates per serum or plasma matrix sample per subject. Percent recoveries for all samples ranged from 95% to 110 % relative to nominal spiked values; and from 99.2 to 109.0% 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.

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 zonisamide measurements.
4. Clinical cut-off: not applicable
5. Expected values/Reference range:

A therapeutic range for zonisamide has not been well established. A reference range between 10 to 40 µg/mL has been suggested. In one study, a 50% reduction in seizures was observed at serum concentrations ranging from 7 to 40 mg/L (Mimaki et al., 1992). Some studies indicate an increased incidence of adverse effects at serum concentrations in excess of 30 mg/L [Wilensky et al., 1985; Berent et al., 1987; Miura et al., 1993]. In general the relationship between these serum concentrations and clinical effect has not been well-defined, and considerable overlap in zonisamide concentrations has been observed between serum responders and non-responders as well as between serum levels associated with seizure control and adverse effects. Zonisamide concentrations should always be used in conjunction with information available from clinical evaluations and other diagnostic procedures.

Zonisamide metabolism can be influenced by enzyme inducing co-medications and polymorphisms. Pharmacokinetics may vary significantly, particularly with co-medication, and based on age. The half-life of zonisamide is 50-70 hours in patients on monotherapy and 25-35 hours in patients co-medicated with enzyme-inducing antiepileptic drugs.

The reference range of drug concentrations which is quoted should only imply a lower limit below which a therapeutic response is relatively unlikely and an upper limit above which toxicity is relatively likely to occur in the specific populations studied. Clinicians using these proposed ranges should be aware that because of individual variation patients may achieve therapeutic benefit with serum drug concentrations outside of these ranges or may experience toxicity with levels below the lower limit of the reference range.

The sponsor also includes the following in the limitations:

It is generally good practice to use the same method (as well as matrix) consistently for individual patient care due to the potential for method-to-method variabilities.

#### **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.