ARK™ *Zonisamide Assay*

This ARK Diagnostics, Inc. package insert for the ARK Zonisamide Assay must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Report any serious incident that has occurred in relation to the device to the manufacturer and the appropriate competent authority as applicable. A Summary of Safety and Performance is available through Eudamed (European database on medical devices), SRN: US-MF-000023925.

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Key to Symbols Used

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE 2797	CE Mark with notified body number
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	R1 R2	Reagent 1/ Reagent 2
Rx Only	For Prescription Use Only		

Reagent Kit REF 5022-0001-00

1 Name

ARK™ ***Zonisamide Assay***

2 Intended 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.

3 Summary and Explanation of the Test

Zonisamide (1,2-benzisoxazole-3-methanesulfonamide, ZONEGRAN®) is an anti-convulsant drug approved for use as adjunctive therapy in the treatment of partial seizures in adults with epilepsy.[1]

4 Principles of the Procedure

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 coenyzme NAD functions only with the bacterial enzyme used in the assay.

5 Reagents

REF	Product Description	Quantity/Volume
5022-0001-00	**ARK Zonisamide Assay** **Reagent R1 – Antibody/Substrate** rabbit polyclonal antibodies to zonisamide, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, preservatives, and stabilizers	1 X 28 mL
	Reagent R2 – Enzyme Zonisamide labeled with bacterial G6PDH, buffer, bovine serum albumin, preservatives, and stabilizers	1 X 14 mL

Reagent Handling and Storage

ARK Zonisamide Assay reagents are provided liquid, ready to use and may be used directly from the refrigerator. When not in use, reagents must be stored at 2–8°C (36–46°F), upright and with screw caps tightly closed. If stored as directed, reagents are stable until the expiration date printed on the label. Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°C (90°F). **Improper storage of reagents can affect assay performance.**

6 Warnings and Precautions

- For **In Vitro Diagnostic** Use**.** For prescription use only.
- Reagents R1 and R2 are provided as a matched set and should not be interchanged with reagents from different lot numbers.

7 Specimen Collection and Preparation for Analysis

- Each laboratory is responsible for supplying a valid specimen for analysis according to their quality procedures.
- Serum or plasma is required. For consistency, using the same specimen matrix for individual patients is a good practice. A steady state, trough (pre-dose) sample is generally accepted as most consistent for therapeutic drug monitoring of zonisamide. Time of blood draw since last dose should be noted.
- Whole blood cannot be used. The following anticoagulants may be used with this assay.
    - Sodium heparin
    - Lithium heparin
    - Potassium EDTA
- **Avoid hemolyzed samples. Zonisamide distributes into erythrocytes.**[2-3,13]
- Blood collection must be performed with collection tubes compatible for use with therapeutic drug monitoring (TDM).
- Follow the collection tube manufacturer's recommendations for collection, processing and centrifugation.
- CLSI document GP44-A4 outlines procedures for minimizing artifacts due to specimen collection and handling for common laboratory tests.[15]

- Do not induce foaming and avoid repeated freezing and thawing to preserve the integrity of the specimen from the time it is collected until the time it is assayed.
- Fibrin, red blood cells, and other particulate matter may cause an erroneous result. Ensure adequate centrifugation.

- The presence of bubbles or foam on specimens can lead to short sample delivery and erroneous results.
- Each laboratory should consult available literature and internal data regarding specimen stability.
- Clarified specimens may 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. Care should be taken to limit the number of freeze-thaw cycles.
- **Handle all patient specimens as if they were potentially infectious.**

8 Procedure

Materials Provided
ARK Zonisamide Assay – **REF** 5022-0001-00

Materials Required – Provided Separately
ARK Zonisamide Calibrator – **REF** 5022-0002-00
Quality Controls – ARK Zonisamide Control – **REF** 5022-0003-00

Instruments
Reagents R1 and R2 may need to be transferred to analyzer-specific reagent containers prior to use. Avoid cross-contamination of R1 and R2.

Many automated clinical chemistry analyzers with photometric rate determination at 340 nm are suitable. Consult the analyzer-specific application sheet for programming the ARK Zonisamide Assay, available from your distributor or ARK Customer Service. Application Protocol Sheets which have been CLIA categorized or bear the CE Mark have been verified by the manufacturer. It is the responsibility of the laboratory to perform all appropriate validation for use of the assay with other settings or analyzers.

Refer to the instrument-specific operator's manual for daily maintenance.

Assay Sequence
To run or calibrate the assay, see the instrument-specific operator's manual.

Calibration
Perform a full calibration (6- point) procedure using the ARK Zonisamide Calibrators A, B, C, D, E, and F; run calibrators in duplicate. Calibration is required with each new reagent kit lot number. Verify the calibration curve with at least two levels of quality controls according to the established laboratory quality assurance plan.

When to Re-Calibrate

- Whenever a new lot number of reagents is used
- Whenever indicated by quality control results
- Whenever required by standard laboratory protocols

Quality Control (QC)

Laboratories should establish QC procedures for the ARK Zonisamide Assay. All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Good laboratory practice suggests that at least two levels (low and high medical decision points) of quality control be tested each day patient samples are assayed and each time a calibration is performed. Monitor the control values for any trends or shifts. If any trends or shifts are detected, or if the control does not recover within the specified range, review all operating parameters according to your clinical laboratory quality procedures. Contact Customer Service for further assistance.

Manual Dilution Protocol

To estimate drug levels in specimens exceeding the upper limit of quantitation, manually dilute the specimen with zero calibrator (CAL A). The concentration after dilution must exceed the limit of quantitation and fall within the measuring range. Multiply the assayed result by the dilution factor.

$$\text{Manual Dilution Factor} = \frac{\text{(Volume of Specimen + Volume of CAL A)}}{\text{Specimen Volume}}$$

9 Results

Report result units as µg/mL or µmol/L. To convert results from µg/mL zonisamide to µmol/L zonisamide, multiply µg/mL by 4.71. Refer to the instrument specific operator's manual for any result error codes.

10 Limitations of Procedure

This assay is designed for use with serum or plasma only; refer to the section **Specimen Collection and Preparation for Analysis**. 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. See the section **Expected Values** below.

11 Expected Values

A therapeutic range for zonisamide has not been well established. A reference range between 10 to 40 µg/mL has been suggested. [4-6] In one study, a 50% reduction in seizures was observed at serum concentrations ranging from 7 to 40 mg/L.[7] Some studies indicate an increased incidence of adverse effects at serum concentrations in excess of 30 mg/L.[8-10] 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.[3,11-13] Pharmacokinetics may vary significantly, particularly with co-medication, and based on age.[6] 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.

12 Specific Performance Characteristics

Each laboratory is responsible for verification of performance using instrument parameters established for their analyzer. The following performance characteristics were obtained on the Roche/Hitachi 917 System.

Sensitivity

Limit of Quantitation (LOQ)

The LOQ of the ARK Zonisamide Assay was determined according to CLSI EP17-A and is defined as the lowest concentration for which acceptable inter-assay precision and recovery is observed (≤20% CV with ±15% recovery). The LOQ was determined to be 2.0 µg/mL.

Assay Range

The range of the assay is 2.0 to 50.0 µg/mL. Report results below this range as <2.0 µg/mL. Report results above this range as >50.0 µg/mL.

Recovery

Analytical recovery was performed by adding concentrated zonisamide drug 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.

$$\% \text{ Recovery} = 100 \times \frac{\text{Mean recovered concentration}}{\text{Theoretical concentration}}$$

Theoretical Concentration (µg/mL)	Mean Recovered Concentration (µg/mL)	Percent Recovery
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

Linearity

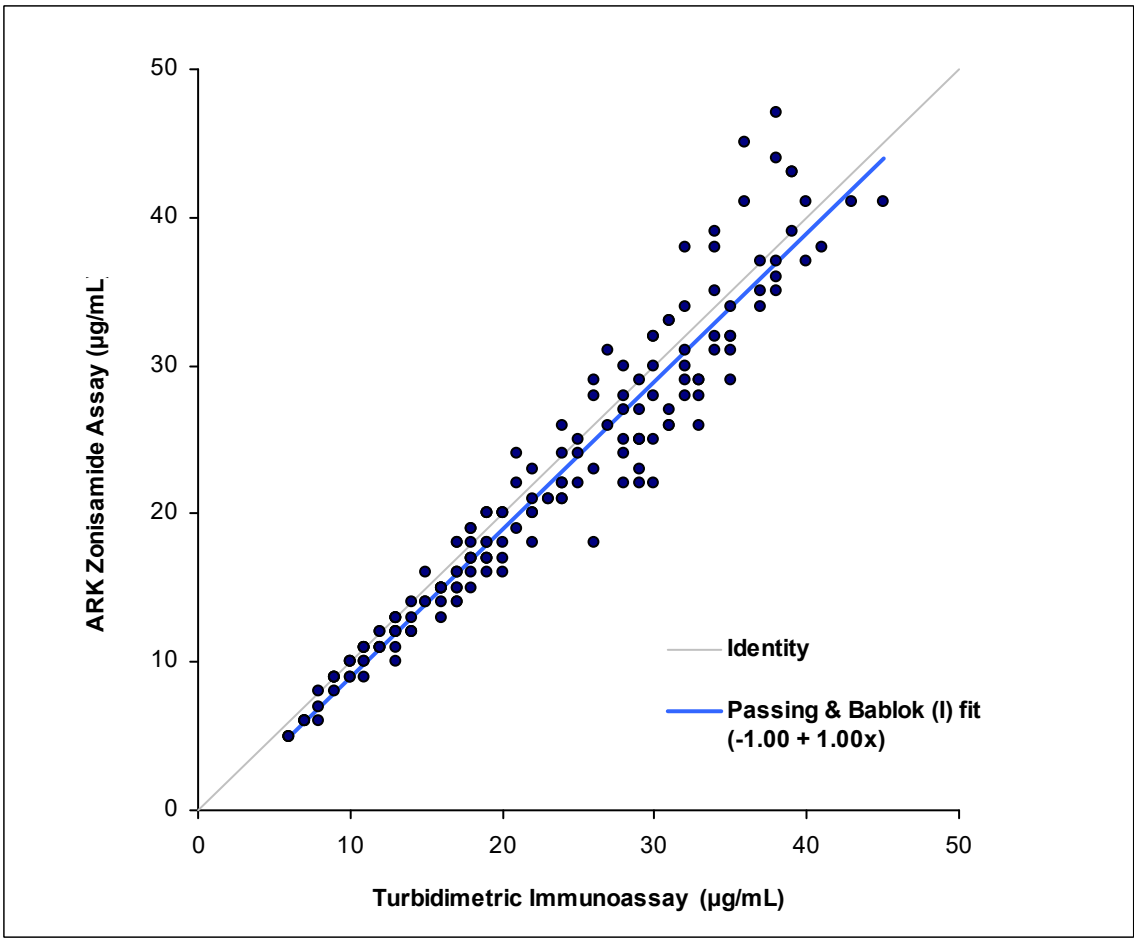
Linearity studies were performed as suggested in CLSI/NCCLS Protocol EP6-A. An 80.0 µg/mL serum sample was prepared and dilutions were made proportionally with human serum negative for zonisamide. Zonisamide concentrations ranged from 0.8 to 80.0 µg/mL. Linearity at specific dilutions was considered acceptable if the percent difference was ±10% between the predicted 1st and 2nd order regressed values or ±15% below 3.0 µg/mL. A linear relationship between 2.4 and 48.0 µg/mL is shown below.

Estimated Value (µg/mL)	Results (µg/mL)	1st Order Predicted Results	2nd Order Predicted Results	% Difference
2.4	2.3	2.5	2.3	-7.0
3.2	3.2	3.3	3.2	-3.8
4.0	4.1	4.1	4.0	-1.8
4.8	4.8	4.8	4.8	-0.6
5.6	5.8	5.6	5.6	0.3
6.4	6.7	6.4	6.5	1.0
7.2	7.4	7.2	7.3	1.4
8.0	8.2	8.0	8.1	1.8
16.0	16.2	15.7	16.2	2.7
24.0	23.4	23.5	24.0	2.3
32.0	32.0	31.3	31.7	1.4
40.0	39.7	39.1	39.2	0.4
48.0	45.8	46.8	46.5	-0.7

Method Comparison

Correlation studies were performed using CLSI/NCCLS Protocol EP9-A2. Results from the ARK Zonisamide assay were compared with results from a turbidimetric immunoassay. The zonisamide concentrations ranged from 6 µg/mL to 45 µg/mL. Results of the Passing-Bablok[14] regression analysis for the study are shown below.

Slope	1.00	(0.96 to 1.00)
y-intercept	- 1.00	(- 1.00 to - 0.46)
Correlation Coefficient (r^2)	0.93	(0.91 to 0.95)
Number of Samples	176	



Precision

Precision was determined as described in CLSI/NCCLS Protocol EP5-A2. Tri-level controls containing zonisamide and pooled human serum specimens 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. The within run, between day, total SD, and percent CVs were calculated. Results are shown below. Acceptance criteria: <10% total CV.

Sample	N	Mean (µg/mL)	Within Run SD	Within Run CV (%)	Between Day SD	Between Day CV (%)	Total SD	Total CV (%)
ARK Zonisamide Control								
LOW	160	5.0	0.21	4.1	0.16	3.2	0.25	5.1
MID	160	24.4	0.96	3.8	0.56	2.3	1.12	4.5
HIGH	160	50.6	1.97	3.9	1.33	2.6	2.63	5.3
Human Serum								
LOW	160	7.0	0.29	4.0	0.21	3.0	0.36	4.9
MID	160	22.6	0.81	3.5	0.59	2.6	1.01	4.4
HIGH	160	51.6	2.47	4.9	1.66	3.2	2.96	5.9

Interfering Substances

Interference studies were conducted using CLSI/NCCLS Protocol EP7-A2 as a guideline. Clinically high concentrations of the following potentially interfering substances in serum with known levels of zonisamide (approximately 15 and 45 µg/mL) were evaluated. Each sample was assayed using the ARK Zonisamide Assay, along with a serum control of zonisamide. Measurement of zonisamide resulted in ≤10% error in the presence of interfering substances at the levels tested.

		Percentage Recovery	
Interfering Substance	Interferent Concentration	15 µg/mL Zonisamide	45 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
Gamma-Globulin	12 g/dL	97.3	101.4
Hemoglobin	1000 mg/dL	96.6	104.1
Intralipid®	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

Specificity

Cross-reactivity was tested for available metabolites of zonisamide. Other medications routinely administered with zonisamide and anti-epileptic drugs were also tested to determine whether these compounds affect the quantitation of zonisamide concentrations using the ARK Zonisamide Assay. High levels of these compounds were spiked into serum pools containing Low (15 µg/mL) and High (45 µg/mL) therapeutic levels of zonisamide. The samples were analyzed and the zonisamide concentrations of samples containing interferent were compared to the serum control.

Metabolites

N-acetyl zonisamide (NAZ) and the non-glucuronidated 2-sulfamoylacetyl phenol (SMAP) were evaluated. Metabolites NAZ and SMAP-glucuronide are found primarily in urine of patients administered zonisamide therapy.[3,7,13] They have not been detected in plasma. Crossreactivity was evaluated in the presence of Low (15 µg/mL) and High (45 µg/mL) zonisamide.

Metabolite	Metabolite Conc (µg/mL)	Percentage Cross-Reactivity		Percentage Interference	
		Low Zonisamide	High Zonisamide	Low Zonisamide	High Zonisamide
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

Drug Interference

Zonisamide-selective antibody did not crossreact with other anti-epileptic or coadministered drugs tested. A high concentration of each compound was spiked into normal human serum with known levels of zonisamide (approximately 15 and 45 µg/mL) and assayed along with a serum control of zonisamide. Measurement of zonisamide resulted in ≤10% error in the presence of drug compounds at the levels tested.

Compound	Concentration (µg/mL)	Percentage Recovery	
		15 µg/mL Zonisamide	45 µg/mL Zonisamide
2-Ethyl-2-phenylmalonamide	1000	98.4	100.2
Acetaminophen	200	98.7	98.7
Acetyl Salicylic Acid	1000	100.3	102.3
Caffeine	100	97.0	97.5
Carbamazepine-10, 11-epoxide	120	99.9	100.9
Carbamazepine	120	101.7	100.8
10-Hydroxy Carbamazepine	100	96.6	93.5
Clonazepam	50	100.0	99.1
Cyclosporin A	40	101.2	104.9
Diazepam	20	98.0	100.8
Erythromycin	200	101.4	103.9
Ethosuximide	1000	99.9	100.5
Felbamate	1000	94.3	102.4
Gabapentin	100	100.9	105.3
Heparin	200 units/mL	104.1	102.7
Ibuprofen	500	101.3	105.9
Lamotrigine	300	100.0	99.8
Levetiracetam	400	95.6	97.9
L-Tryptophan	50	102.9	104.7
Oxcarbazepine	50	99.1	105.2
Phenobarbital	400	98.6	101.9
Phenytoin	200	105.1	106.7
Primidone	100	98.3	98.8
Salicylic Acid	500	104.7	106.6
Sulfamethoxazole	400	102.0	105.2
Sulfisoxazole	1000	95.8	98.3
Theophilline	250	101.7	100.3
Tiagabine	200	102.2	103.5
Topiramate	250	101.7	105.0
Trimethoprim	40	101.1	96.3
Valproic Acid	1000	99.8	101.2

13 References

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14 Trademarks

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