ARK Diagnostics, Inc.

ARK™ *Topiramate Assay*

This ARK Diagnostics, Inc. package insert for the ARK Topiramate 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.

Customer Service

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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 5015-0001-00

1 Name

ARK™ ***Topiramate Assay***

2 Intended 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.

3 Summary and Explanation of the Test

Topiramate (2,3:4,5-Di-*O*-isopropylidene-ß-D-fructopyranose sulfamate) is an anti-convulsant drug approved for use in the treatment of epilepsy and is often prescribed as monotherapy or as one component of a multiple anti-epileptic drug therapy.[1]

4 Principles of the Procedure

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

5 Reagents

REF	Product Description	Quantity/Volume
5015-0001-00	**ARK Topiramate Assay** **Reagent R1 – Antibody/Substrate** rabbit polyclonal antibodies* to topiramate, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, preservatives, and stabilizers	1 X 28 mL
	Reagent R2 – Enzyme topiramate epitope labeled with bacterial G6PDH, buffer, bovine serum albumin, preservatives, and stabilizers	1 X 14 mL

*Antibodies are produced selectively to an epitopic moiety of topiramate.

Reagent Handling and Storage

ARK Topiramate 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,** laboratory professional use.
- For prescription use only. *Caution: U.S. Federal Law restricts this device to sale by or on the order of a licensed practitioner.*
- 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 topiramate. 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
- 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.[2]
- 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 erroneously low results.

- Each laboratory should consult available literature and internal data regarding specimen stability.
- Based on studies performed by ARK Diagnostics, 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 Topiramate Assay – **REF** 5015-0001-00

Materials Required – Provided Separately
ARK Topiramate Calibrator – **REF** 5015-0002-00
Quality Controls – ARK Topiramate Control – **REF** 5015-0003-00

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 Topiramate 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 Topiramate Calibrators A, B, C, D, E, and F; test 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. Calibrator A is the calibration blank.

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 Topiramate 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 54 µg/mL, manually dilute the specimen with zero calibrator (CAL A). 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 topiramate to µmol/L topiramate, multiply µg/mL by 2.95. The topiramate value from this assay should be used in conjunction with other clinical information. 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 sections **Specimen Collection and Preparation for Analysis** and **Specific Performance Characteristics**.

11 Expected Values

A therapeutic range for topiramate has not been well established. An inconsistent correlation exists between levels of circulating topiramate and toxicity, adverse effects or clinical efficacy.[3] Therefore, monitoring topiramate concentration in patients is warranted.

Reference ranges for seizure control have been proposed variously, which include trough sample concentrations from 5 to 20 µg/mL (15 to 60

μmol/L)[3-6] and 2 to 10 μg/mL[7] in neuropsychopharmacotherapy. Monitoring of topiramate is recommended during and after pregnancy.[8]

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.

12 Specific Performance Characteristics

Data are representative of performance on automated clinical chemistry analyzers. 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 Topiramate 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 (often considered ≤20% CV with ±15% recovery). The LOQ was determined to be 1.5 μg/mL, and may depend on analyzer-specific performance.

Assay Range

The range of the assay is 1.5 to 54.0 μg/mL. Report results below this range as <1.5 μg/mL or below the analyzer-specific lower LOQ established in your laboratory. Report results above this range as >54.0 μg/mL or above the analyzer-specific upper LOQ established in your laboratory.

Accuracy

Accuracy (analytical recovery) was performed by adding concentrated topiramate drug into human serum negative for topiramate. A stock concentrate of highly pure topiramate was added volumetrically to human serum negative for topiramate, representing drug concentrations across the assay range. Six replicates of each sample were assayed on an automated clinical chemistry analyzer. The results were averaged and compared to the target concentration and percent recovery calculated. Results are shown below.

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

Theoretical Concentration (µg/mL)	Mean Recovered Concentration (µg/mL)	Percent Recovery
1.5	1.4	95.6
2.5	2.7	106.7
4.0	4.2	104.2
5.0	5.3	106.0
6.0	6.4	106.7
10.0	10.4	103.8
15.0	15.5	103.4
30.0	30.8	102.6
45.0	47.3	105.0
55.0	58.9	107.1

Linearity

Linearity studies were performed as suggested in CLSI Protocol EP6-Ed2. A 60.0 µg/mL serum sample was prepared and dilutions were made proportionally with human serum negative for topiramate. Topiramate concentrations ranged from 1.8 to 54 µg/mL. Linearity was demonstrated between 1.8 and 54.0 µg/mL with no more than 12% deviation from linearity. Results are tabulated below.

Nominal Value (µg/mL)	Mean (µg/mL) (N=6)	Deviation from Linearity (µg/mL)	% Deviation from Linearity
1.8	1.7	-0.21	-11.3%
2.4	2.4	-0.11	-4.6%
3.0	3.0	-0.14	-4.4%
3.6	3.7	-0.04	-1.0%
4.2	4.4	0.07	1.7%
4.8	4.9	-0.03	-0.6%
5.4	5.8	0.20	3.6%
6.0	6.3	0.05	0.7%
18.0	18.9	0.25	1.4%
30.0	31.4	0.35	1.1%
42.0	44.8	1.32	3.0%
54.0	56.5	0.65	1.2%

Method Comparison

Correlation studies were performed using CLSI Protocol EP9-A2. Results from the ARK Topiramate assay were compared with results from a

commercially available FPIA Immunoassay. The topiramate concentrations ranged from 1.5 µg/mL to 53.4 µg/mL. Results of the Passing-Bablok[9] regression analysis for the study are shown below.

Slope	0.99
y-intercept	- 0.17
Correlation Coefficient (r^2)	0.99
Number of Samples	113

Precision

Precision was determined as described in CLSI Protocol EP5-A2. 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. The within run, between day, total SD, and percent CVs were calculated. Results are shown below. Acceptance criteria: <10% total CV.

			Within Run		Between Day		Within-Lab	
Sample	**N**	**Mean (µg/mL)**	**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

Interfering Substances

Interference studies were conducted using CLSI Protocol EP7-A2 as a guideline. Clinically high concentrations of the following 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. Measurement of topiramate resulted in ≤10% error in the presence of interfering substances at the levels tested.

Interfering Substance	Interferent 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
Rheumatoid Factor	1000 IU/mL

Triglycerides	1105 mg/dL
Uric Acid	25 mg/dL

Specificity

Cross-reactivity was tested for a known metabolite of topiramate. Other medications routinely administered with topiramate and anti-epileptic drugs were also tested to determine whether these compounds affect the quantitation of topiramate concentrations using the ARK Topiramate Assay. High levels of these compounds were spiked into serum pools containing low (5 µg/mL) and high (20 µg/mL) therapeutic levels of topiramate. The samples were analyzed and the topiramate concentrations of samples containing interferent were compared to the control serum.

Metabolites

Metabolites of topiramate are found primarily in urine of patients being administered topiramate therapy.[10] ARK Topiramate Assay serum and plasma results are unlikely to be affected by metabolism of topiramate drug, since plasma levels of metabolites are usually not clinically significant. The following metabolite was tested for cross-reactivity.

Metabolite	Metabolite Conc. (µg/mL)	Percent Cross-Reactivity		Percent Interference	
		Low Topiramate	High Topiramate	Low Topiramate	High Topiramate
9-Hydroxy-topiramate	40.0	1.2	1.6	8.6	3.2

Drug Interference

Topiramate-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 topiramate (approximately 5 and 20 µg/mL) and assayed along with a serum control of topiramate. Measurement of topiramate resulted in ≤10% error in the presence of drug compounds at the levels tested.

Compound	Concentration (µg/mL)	Compound	Concentration (µg/mL)
Acetaminophen	50	Levetiracetam	200
Acetozolamide	50	Methysergide	100
Alprazolam	20	Metoprolol	100
Amitriptyline	10	Nadolol	150
Acetylsalicylic acid	100	Naproxen	600

Atenolol	50	Nimodipine	100
Caffeine	100	Nortriptyline	10
Carbamazepine	100	Oxcarbazepine	50
Chlorthalidone	100	Phenelzine	15
Clonazepam	50	Phenobarbital	40
Clorazepate	20	Phenytoin	50
Diazepam	50	Primidone	100
Dichlorphenamide	40	Protriptyline	20
Ethosuximide	500	Salicylic Acid	750
Famotidine	50	Sulfanilamide	2000
Felbamate	500	Tiagabine	200
Flurazepam	20	Tolbutamide	750
Furosemide	10	Valproic Acid	200
Gabapentin	100	Verapamil	100
Hydrochlorothiazide	60	Vigabatrin	150
Ibuprofen	500	Zonisamide	200
Lamotrigine	100		

13 References

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14 Trademarks

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