ARK™ Homogeneous Enzyme Immunoassays for the Newer Antiepileptic Drugs

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Introduction

ABSTRACT

Therapeutic monitoring of the current new generation of antiepileptic drugs (AEDs) is benefitted by the advent of immunoassays for their quantitative measurement in serum or plasma. These relatively new AEDs are used for treatment control of seizures and pain management. Homogeneous enzyme immunoassays for the measurement of topiramate, zonisamide and levetiracetam were developed by ARK Diagnostics, Inc. The performance of three new assays on the Roche/Hitachi 917 automated clinical chemistry analyzer is described. Six-level calibration for topiramate (0 to 60 µg/mL), zonisamide (0 to 80 µg/mL) and levetiracetam (0 to 100 µg/mL) and tri-level controls for each analyte were used to establish precision and other performance. Within-laboratory precision was generally below 5% CV for all assays. Analytical recovery and endogenous interference studies demonstrated performance within 10% of expected levels. Lower limits of quantitation for topiramate (1.5 µg/mL), zonisamide (2.0 µg/mL), and levetiracetam (2.0 µg/mL) were based on accuracy within 15% and precision within 20% CV. Using Passing Bablok regression analysis for method comparison: ARK Topiramate = 0.99 FPIA - 0.17 (r2 = 0.99, n=113, range 1.5 to 53.4 µg/mL); ARK Topiramate = 0.99 GC - 1.19 (r2 = 0.97, n=28, range 5.3 to 53.0 µg/mL); ARK Levetiracetam = 1.02 LCMS/MS + 0.91 (r2 = 0.97, n=98, range 2.1 to 86.4 µg/mL); ARK Zonisamide = 1.13 HPLC + 0.26 (r2 = 0.96, n=110, range 5.1 to 46.1 µg/mL). ARK assays measured AEDs in serum or plasma as demonstrated on the Roche/Hitachi 917, and show good correlation with comparative methods. All reagents, calibrators, and controls are supplied as stable liquids, ready-to-use, and are well-suited for routine TDM.

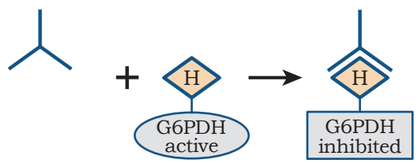
Quantitative measurements by reference methodologies were performed at clinical laboratories, and frozen specimens were shipped to ARK Diagnostics, Inc. for analyses by the ARK immunoassays. ARK Diagnostics gratefully acknowledges the following laboratories:

GC (topiramate) – William Beaumont Hospital, Royal Oak, MI
LC/MS/MS (levetiracetam) and HPLC (zonisamide) – MedTox Laboratories, New Brighton, MN

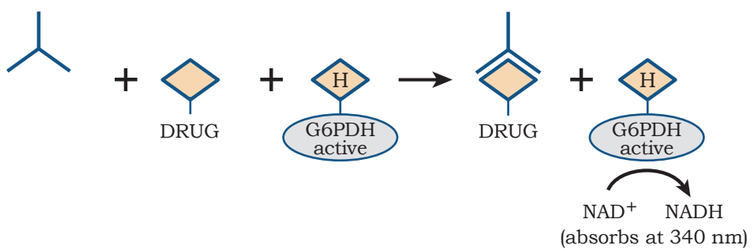
METHODS

ARK Assays employ homogeneous enzyme immunoassay for quantifying topiramate, levetiracetam, and zonisamide in human serum or plasma. Assays were evaluated on the Roche/Hitachi 917, using a six-point calibration curve. ARK tri-level quality controls were run.
The assay principle is shown in the following figure. Increasing reaction rate correlates to increasing drug concentration.

A) Absence of drug



B) Presence of drug



Precision

Precision was determined for ARK Assays as described in CLSI/NCCLS Protocol EP5-A2. ARK Tri-level controls containing drug were assayed in quadruplicate twice a day for 20 days. Grand mean, standard deviation (SD) for within-run, between-day, and total coefficients of variation (% CVs) were calculated.

			WITHIN RUN	BETWEEN DAY	TOTAL
Sample	**N**	**Mean (µg/mL)**	**CV (%)**	**CV (%)**	**CV (%)**
Topiramate Assay					
2.5 µg/mL	160	2.4	3.5	2.0	4.3
10.0 µg/mL	160	10.2	2.4	1.4	2.7
40.0 µg/mL	160	40.2	2.9	1.6	3.2
Levetiracetam Assay					
7.5 µg/mL	160	7.5	3.4	3.2	4.5
30.0 µg/mL	160	29.4	2.9	2.8	3.7
75.0 µg/mL	160	73.4	2.9	2.8	4.2
Zonisamide Assay					
5.0 µg/mL	160	5.0	4.1	3.2	5.1
25.0 µg/mL	160	24.4	3.8	2.3	4.5
50.0 µg/mL	160	50.6	3.9	2.6	5.3

Lower Limit of Quantitation

Limit of quantitation was evaluated according to CLSI/NCCLS EP17-A. Pooled human serum was supplemented with known amounts of each drug and assayed 40 times. The LLOQ of the ARK Assay is defined as the lowest concentration for which acceptable inter-assay precision (≤20% CV) and recovery (±15%) is observed. Data shown were obtained on Roche/Hitachi 917.

Sample	N	Mean (µg/mL)	RMSS	CV (%)	Recovery (%)
Topiramate Assay					
1.0 µg/mL	40	1.0	0.05	5.3	96.0
Levetiracetam Assay					
2.0 µg/mL	40	2.0	0.20	10.0	100.0
Zonisamide Assay					
2.0 µg/mL	40	1.9	0.07	4.0	93.4

Drug Metabolites

Metabolite	Level Tested (µg/mL)	Parent Drug (µg/mL)	(%) Crossreactivity	Parent Drug Level (µg/mL)	(%) Crossreactivity
Topiramate Assay 9-Hydroxy-topiramate	40	5	1.2	20	1.6
Levetiracetam Assay ucb L057: 2-pyrrolidone-*N*-butyric acid	250	15	-0.2	50	1.3
Zonisamide Assay NAZ (N-Acetyl Zonisamide)	50	15	1.7	45	5.5
SMAP (2-Sulfamoylacetyl phenol)	50	15	18.2	45	19.5

Specificity

Measurements of topiramate (5.0 and 20.0 µg/mL), levetiracetam (15.0 and 50.0 µg/mL), and zonisamide (15.0 and 45.0 µg/mL) were evaluated in the presence of other anti-epileptic drugs and potentially co-administered compounds, and assayed along with a serum control. The following compounds did not interfere (≤10% error) at the levels tested.

Topiramate: Summary of Specificity

Compound	Conc. (µg/mL)	Compound	Conc. (µg/mL)	Compound	Conc. (µg/mL)	Compound	Conc. (µg/mL)
Acetominophen	50	Diazepam	50	Lamotrigine	100	Phenytoin	50
Acetazolamide	50	Dichlorphenamide	40	Levetiracetam	200	Primidone	100
Alprazolam	20	Ethosuximide	500	Methysergide	100	Protriptyline	20
Amitriptyline	10	Famotidine	50	Metoprolol	100	Salicylic Acid	750
Acetylsaliyclic Acid	100	Felbamate	500	Nadolol	150	Sulfanilamide	2000
Atenolol	50	Flurazepam	20	Naproxen	600	Tiagabine	200
Caffeine	100	Furosemide	10	Nimodipine	100	Tolbutamide	750
Carbamazepine	100	Gapapentin	100	Notriptyline	10	Valproic Acid	200
Chlorthalidone	100	Hydrochlorthiazide	60	Oxcarbazepine	50	Verapamil	100
Clonazepam	50	Ibuprofen	500	Phenelzine	15	Viagabatrin	150
Clorazepate	20	9 OH Topiramate	40	Phenobarbitol	40	Zonisamide	200

Levetiracetam: Summary of Specificity – Other Anti-epileptic or Coadministered Drugs

Compound	Conc. (µg/mL)	Compound	Conc. (µg/mL)	Compound	Conc. (µg/mL)	Compound	Conc. (µg/mL)
Acetominophen	200	Erythromycin	200	Nortriptyline	20	Theophylline	250
Acetylsalicylic acid	1000	Ethosuximide	250	Oxcarbazepine	50	Tiagabine	200
Amitriptyline	20	Felbamate	250	Phenobarbital	200	Topiramate	250
Caffeine	100	Gabapentin	100	Phenytoin	200	Trimethoprim	20
Carbamazepine	120	Heparin	200	Primidone	100	Valproic Acid	250
Clonazepam	50	Hydrochlorothiazide	20	Probenecid	250	Verapamil	100
Cyclosporin A	40	Ibuprofen	500	Salicylic Acid	500	Vigabatrin	150
Diazepam	50	Lamotrigine	250	Sulfamethoxazole	400	Warfarin	250
Digoxin	40	Naproxen	500	Sulfisoxazole	250	Zonisamide	250

Zonisamide: Summary of Specificity – Other Anti-epileptic or Coadministered Drugs

Compound	Conc. (µg/mL)	Compound	Conc. (µg/mL)	Compound	Conc. (µg/mL)	Compound	Conc. (µg/mL)
2-Ethyl-2-phenylamalonamide	1000	Erythromycin	200	L-Tryptophan	50	Theophylline	250
Acetaminophen	200	Ethosuximide	1000	Oxcarbazepine	50	Tiagabine	200
Caffeine	100	Felbamate	1000	Phenobarbital	400	Topiramate	250
Carbamazepine-10, 11-epoxide	120	Gabapentin	100	Phenytoin	200	Trimethoprim	20
Carbamazepine	120	Heparin	200	Primidone	100	Valproic Acid	1000
Clonazepam	50	Ibuprofen	500	Salicylic Acid	500		
Cyclosporin A	40	Lamotrigine	300	Sulfamethoxazole	400		
Diazepam	20	Levetiracetam	400	Sulfisoxazole	1000		

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Analytical Recovery

Accuracy (analytical recovery) was performed by adding concentrated each drug into human serum negative for each drug. Test sample concentrations were presented the table below. Two analytical runs of three replicates of each sample were assayed. The results of the six replicates were averaged and compared to the theoretical target concentration and the percentage recovery was calculated.

Topiramate Analytical Recovery

Target (µg/mL)	Mean (µg/mL)	SD	CV (%)	Recovery (%)
1.5	1.4	0.10	7.2	95.6
2.5	2.7	0.05	1.9	106.7
5.0	5.3	0.22	4.1	106.0
10.0	10.4	0.35	3.4	103.8
15.0	15.5	0.28	1.8	103.4
30.0	30.8	0.89	2.9	102.6
45.0	47.3	1.22	2.6	105.0
55.0	58.9	1.77	3.0	107.1

Levetiracetam Analytical Recovery

Target (µg/mL)	Mean (µg/mL)	SD	CV (%)	Recovery (%)
2.0	1.9	0.25	13.0	95.8
4.0	3.8	0.21	5.6	94.6
10.0	10.0	0.32	3.2	100.0
20.0	19.2	0.67	3.5	95.9
45.0	44.1	1.44	3.3	98.0
80.0	79.3	2.44	3.1	99.1
100.0	105.3	4.72	4.5	105.3

Zonisamide Analytical Recovery

Target (µg/mL)	Mean (µg/mL)	SD	CV (%)	Recovery (%)
2.0	2.0	0.15	7.7	98.3
4.0	4.2	0.23	5.6	104.2
8.0	8.3	0.29	3.5	103.1
16.0	16.1	0.44	2.7	100.6
30.0	27.8	0.88	3.2	92.7
70.0	66.2	5.37	8.1	94.6
80.0	85.1	5.50	6.5	106.3

Endogenous Interference

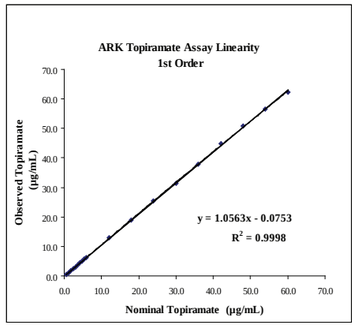
Interference studies were conducted using CLSI/NCCLS Protocol EP7-A2 as a guideline. Clinically high concentrations of the following potentially interfering substances in serum with known levels of topiramate (5.0 and 20.0 µg/mL), levetiracetam (15.0 and 50.0 µg/mL), and zonisamide (15.0 and 45.0 µg/mL) were evaluated. Each sample was assayed along with a serum control of each drug. Measurement of each drug resulted in ≤10% error in the presence of interfering substances at the concentrations tested.

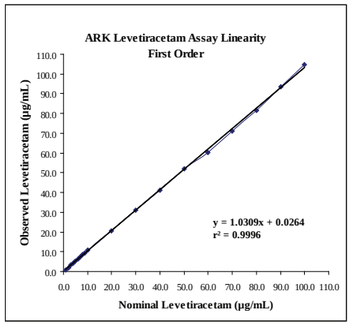
Substance	Topiramate Assay Interferent Concentration	Levetiracetam Interferent Concentration	Zonisamide Assay Interferent Concentration
Albumin	12 g/dL	12 g/dL	12 g/dL
Bilirubin	60 mg/dL	70 mg/dL	70 mg/dL
Cholesterol	301 mg/dL	535 mg/dL	651 mg/dL
Gamma-Globulin	10 g/dL	12 g/dL	12 g/dL
Hemoglobin	1000 mg/dL	1000 mg/dL	1000 mg/dL
Intralipid®	1500 mg/dL	1500 mg/dL	1500 mg/dL
Rheumatoid Factor	1000 IU/mL	1100 IU/mL	1100 IU/mL
Triglycerides	1105 mg/dL	1033 mg/dL	1204 mg/dL
Uric Acid	30 mg/dL	30 mg/dL	30 mg/dL

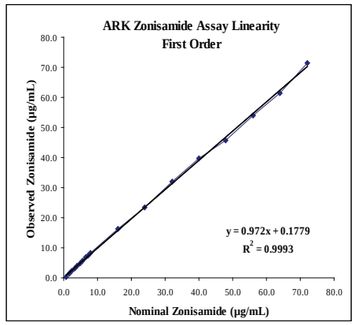
Linearity and Assay Range

Linearity studies were performed as suggested in CLSI/NCCLS Protocol EP6-A; the percentage difference between the predicted 1st order and 2nd order polynomial was within 10%. Negative pooled human serum was supplemented with each topiramate, levetiracetam, or zonisamide and then diluted proportionally. Regression plots of observed versus expected concentrations are shown for the entire linear range determined on the Roche/Hitachi 917.

	Topiramate (µg/mL)	Levetiracetam (µg/mL)	Zonisamide (µg/mL)
LLOQ	1.5	2.0	2.0
ULOQ	54.0	100.0	72.0
Linearity	1.2 to 54.0	3.0 to 100.0	0.8 to 72.0
Calibration Range	0.0 to 60.0	0.0 to 100.0	0.0 to 80.0

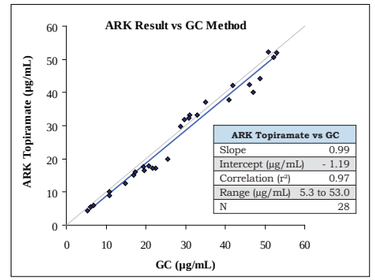


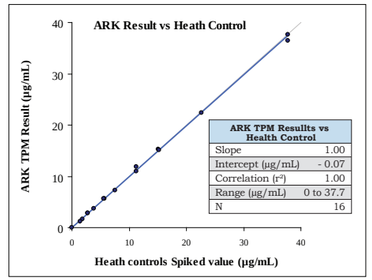


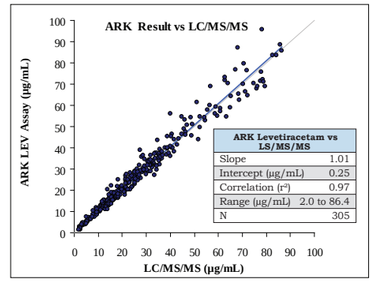


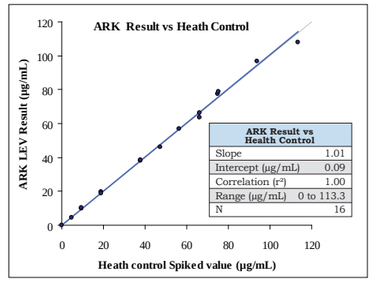
Method Comparison & Proficiency Samples

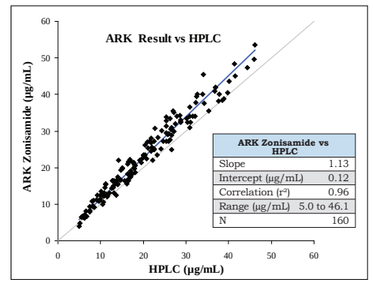
Clinical specimens from patients treated with each drug were analyzed. Heath Controls (UKNEQAS) were tested. Passing-Bablock regression of the comparison is shown in the figures below.

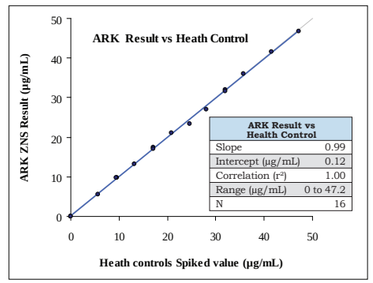












Conclusions

Performance was demonstrated on the Roche/Hitachi 917 System. Performance of the assay showed good precision, accuracy, specificity and linearity with excellent correlation to reference methodology. ARK reagents, calibrators and controls are provided as separate kits in liquid form ready-to-use.

INTENDED USE - GENERIC

ARK AED Assays are homogeneous enzyme immunoassays intended for the quantitative determination of anti-epileptic drugs in human serum or plasma on automated clinical chemistry analyzers. The results obtained are used in the diagnosis and treatment of AED overdose and in monitoring levels of AED to help ensure appropriate therapy.

ARK™ Topiramate Assay - FDA-Cleared, Licensed in Canada, and CE Marked

Assays under regulatory review – Performance characteristics have not been established

ARK™ Levetiracetam Assay – 510(k) pending ARK™ Zonisamide Assay – 510(k) pending