ARK™ Immunoassays for Antiepileptic Drugs: Gabapentin, Lamotrigine, Levetiracetam, Topiramate, Zonisamide

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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 on automated clinical chemistry analyzers available in the central laboratory. Homogeneous enzyme immunoassays for the measurement of gabapentin, lamotrigine, levetiracetam, topiramate, and zonisamide were developed by ARK Diagnostics, Inc. The performance of these assays on the Roche/Hitachi 917 automated clinical chemistry analyzer is described. Six-level calibration for gabapentin (0 to 40 µg/mL), lamotrigine (0 to 40 µg/mL), levetiracetam (0 to 100 µg/mL), topiramate (0 to 60 µg/mL), zonisamide (0 to 80 µg/mL) and tri-level controls for each analyte were used to establish precision and other performance. Total (within-laboratory) precision of tri-level controls ranged respectively for gabapentin (3.6 to 5.6%), lamotrigine (4.1 to 6.1%), levetiracetam (3.7 to 4.5%), topiramate (2.7 to 4.3%) and zonisamide (4.5 to 5.3%). Analytical recovery and endogenous interference studies demonstrated performance within 10% of expected levels. Lower limits of quantitation for gabapentin (0.5 µg/mL), lamotrigine (0.85 µg/mL), levetiracetam (2.0 µg/mL), topiramate (1.5 µg/mL), and zonisamide (2.0 µg/mL) were based on accuracy within 15% and precision within 20% CV. Using Passing Bablok regression analysis for method comparison: ARK Gabapentin = 0.96 LC/MS/MS – 0.06 (r^2 = 0.96, n=183, range 1.0 to 39.0 µg/mL); ARK Lamotrigine = 1.01 HPLC + 0.37 (r^2 = 0.97, n=193, range 1.00 to 36.7 µg/mL); ARK Levetiracetam = 1.02 LC/MS/MS + 0.91 (r^2 = 0.97, n=98, range 2.1 to 86.4 µg/mL); ARK Topiramate = 0.99 FPIA - 0.17 (r^2 = 0.99, n=113, range 1.5 to 53.4 µg/mL); ARK Zonisamide = 1.13 HPLC + 0.26 (r^2 = 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.

PRODUCT DESCRIPTIONS

The advent of immunoassay technology to the testing of the newer antiepileptic drugs (AED) facilitates rapid turnaround of reportable assay results from the central laboratory at local hospitals and moderate complexity clinical laboratories.

ARK Diagnostics, Inc. designs, develops and manufactures in vitro diagnostic (IVD) medical devices for the quantitative measurement of therapeutic drugs. ARK holds a quality management system certificate for ISO 13485:2003 CMDCAS. For each target drug analyte, a set of test kit products comprise assay reagents (R1 – Antibody/Substrate and R2 – Enzyme), Calibrators (6-level set) and Controls (3-level set). More specific information can be obtained at the company website www.ark-tdm.com.

ASSAY PRINCIPLE

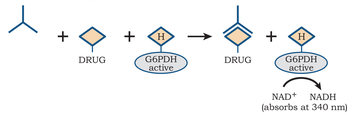
ARK AED Assay is a homogeneous immunoassay based on competition between drug in the specimen and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. As the latter binds antibody, enzyme activity decreases. In the presence of drug from the specimen, 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.

METHODS

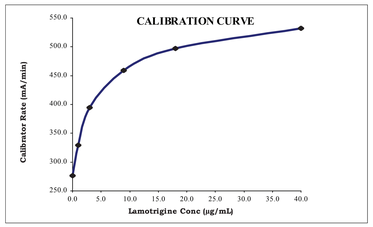
ARK assays employ homogeneous enzyme immunoassay for quantifying therapeutic drugs in human serum or plasma. Assays were evaluated on the Roche/Hitachi 917, using a six-point calibration curve. ARK tri-level quality controls were run. Quantitative measurements by reference methodologies were performed at clinical laboratories, and frozen specimens were shipped to ARK Diagnostics, Inc. for analyses by the ARK immunoassays. 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



Calibration curve example for the ARK Lamotrigine Assay for illustration



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.

Sample	N	Mean (µg/mL)	Within Run CV (%)	Between Day CV (%)	Total CV (%)
Gabapentin Assay					
2.5 µg/mL	160	2.5	3.3	3.9	5.6
8.0 µg/mL	160	7.9	2.6	3.3	4.4
25.0 µg/mL	160	24.6	1.9	2.7	3.6
Lamotrigine Assay					
2.00 µg/mL	160	2.08	3.4	2.5	4.1
12.00 µg/mL	160	11.70	3.6	2.4	4.2
25.00 µg/mL	160	24.23	4.1	4.4	6.1
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
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
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)	RMSSD	CV (%)	Recovery (%)
Gabapentin Assay					
0.5 µg/mL	40	0.45	0.04	9.3	89.5
Lamotrigine Assay					
0.85 µg/mL	40	0.77	0.02	2.9	90.1
Levetiracetam Assay					
2.0 µg/mL	40	2.0	0.20	10.0	100.0
Topiramate Assay					
1.0 µg/mL	40	1.0	0.05	5.3	96.0
Zonisamide Assay					
2.0 µg/mL	40	1.9	0.07	4.0	93.4

Analytical Recovery

Accuracy (analytical recovery) was performed by spiking concentrated drug into human serum negative for each drug. The results of the six replicates were averaged and compared to the theoretical target concentration and the percentage recovery was calculated. Acceptance criteria: Recovery (±10%).

Assay	Target Range (µg/mL)	Average Recovery (%)
Gabapentin	1.0 – 40.0	100.9 ± 1.8
Lamotrigine	0.85 – 40.0	99.2 ± 2.7
Levetiracetam	2.0 – 100.0	98.4 ± 3.6
Topiramate	1.5 – 55.0	103.8 ± 3.7
Zonisamide	2.0 – 80.0	100.0 ± 5.0

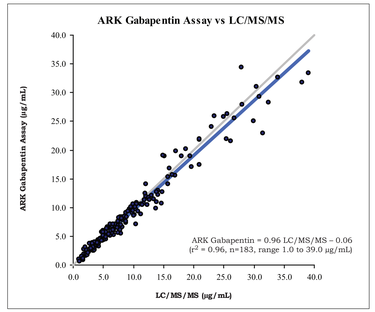
Linearity and Assay Range

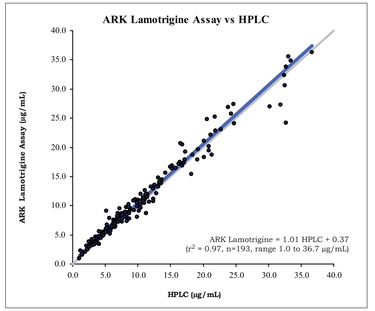
Linearity studies were performed as suggested in CLSI/NCCLS Protocol EP6-A. Negative pooled human serum was supplemented with the target drug analyte and then diluted proportionally. The lower limit of quantitation (LLOQ), upper limit of quantitation (ULOQ), linearity range and calibration range were tabulated for the respective ARK AED assays.

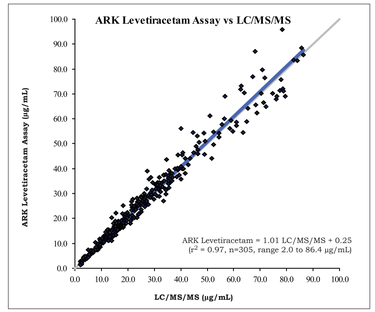
	Gabapentin (µg/mL)	Lamotrigine (µg/mL)	Levetiracetam (µg/mL)	Topiramate (µg/mL)	Zonisamide (µg/mL)
LLOQ	0.75	0.85	2.0	1.5	2.0
ULOQ	40.0	40.0	100.0	54.0	72.0
Linearity Range	0.75 to 40.0	1.0 to 40.0	2.0 to 100.0	1.2 to 54.0	0.8 to 72.0
Calibration Range	0.0 to 40.0	0.0 to 40.0	0.0 to 100.0	0.0 to 60.0	0.0 to 80.0

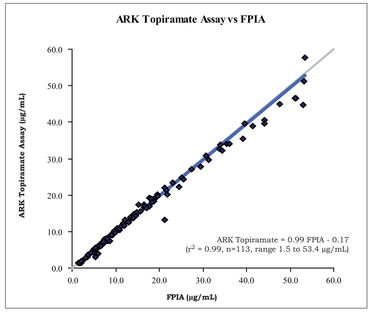
Method Comparison

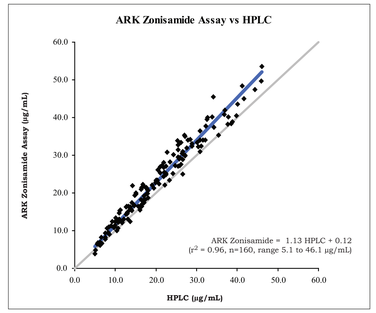
Correlation studies were performed using CLSI/NCCLS Protocol EP9A-2 as a guideline. Clinical specimens from patients treated with each drug were analyzed. Results from ARK AED Assays were evaluated in comparison to results from clinical predicate devices (FPIA, fluorescence polarization immunoassay) or reference methodology (HPLC or LC/MS/MS). Passing-Bablock regression of the comparisons are shown in the figures below.

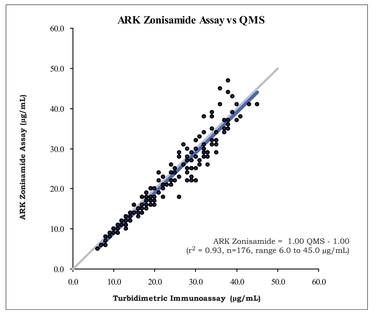












Specificity

ARK AED Assays were not interfered (≤10% error) by commonly co-administerd drugs. Specificity studies were conducted using CLSI/NCCLS Protocol EP7-A2 as a guideline. Consult package insert labeling for more specific information.

DRUG/METABOLITES

Metabolites of the parent drugs (gabapentin, lamotrigine, levetiracetam, topiramate, zonisamide) were tested at high concentrations in the presence of their parent at either low or high parent drug levels. Due to structural similarity with the target parent drug as analyte, pregabalin and trimethoprim if coadministered and at high concentration may interfere with the gabapentin and lamotrigine assay respectively.

Cross-Reactivity was calculated as follows:

- "TEST" sample contains parent drug plus metabolite
- "CONTROL" sample contains only parent drug

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

Drug/Metabolite	Level Tested (µg/mL)	Parent Drug (µg/mL)	% Crossreactivity	Parent Drug (µg/mL)	% Crossreactivity
Gabapentin Assay					
Pregabalin	100	2	1.1	20	2.0
Lamotrigine Assay					
Lamotrigine-2-N-glucuronide	50	3	2.4	15	1.9
Lamotrigine-2-N-methyl	400		0.04		0.2
Lamotrigine-2-N-oxide	80		3.7		3.6
Trimethoprim	40		4.4		3.0
Levetiracetam Assay					
ucb L057: 2-pyrrolidone-N-butyric acid	250	15	- 0.2	50	1.3
Topiramate Assay					
9-Hydroxy-topiramate	40	5	1.2	20	1.6
Zonisamide Assay					
NAZ (N-Acetyl Zonisamide)	50	15	1.7	45	5.5
SMAP (2-Sulfamoyltacetyl phenol)	50		18.2		19.5

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 gabapentin (2 and 20 µg/mL), lamotrigine (3 and 15 µg/mL), levetiracetam (15 and 50 µg/mL), topiramate (5 and 20 µg/mL), and zonisamide (15 and 45 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.

Assay Substance	Gabapentin	Lamotrigine	Levetiracetam	Topiramate	Zonisamide
	Interferent Concentration				
Albumin	12 g/dL	12 g/dL	12 g/dL	12 g/dL	12 g/dL
Bilirubin	70 mg/dL	70 mg/dL	70 mg/dL	60 mg/dL	70 mg/dL
Cholesterol	623 mg/dL	623 mg/dL	535 mg/dL	301 mg/dL	651 mg/dL
Gamma-Globulin	12 g/dL	12 g/dL	12 g/dL	10 g/dL	12 g/dL
Hemoglobin	1000 mg/dL	1000 mg/dL	1000 mg/dL	1000 mg/dL	1000 mg/dL
Intralipid®	1500 mg/dL	1000 mg/dL	1500 mg/dL	1500 mg/dL	1500 mg/dL
Rheumatoid Factor	1100 IU/mL	1100 IU/mL	1100 IU/mL	1000 IU/mL	1100 IU/mL
Triglycerides	1220 mg/dL	618 mg/dL	1033 mg/dL	1105 mg/dL	1204 mg/dL
Uric Acid	30 mg/dL	30 mg/dL	30 mg/dL	30 mg/dL	30 mg/dL

Conclusions

Performances of ARK AED Assays were demonstrated on the Roche/Hitachi 917 System. Assays showed good precision, accuracy, specificity and linearity with excellent correlation to reference methodology.

ADVANTAGES

- ARK reagents, calibrators and controls are provided as separate kits in liquid form, ready-to-use.
- Assays may be performed on automated clinical chemistry analyzers in the local central laboratory.
- Quick turnaround for reporting assay results, same day is possible, benefitting patient management.

INTENDED USE - GENERIC

ARK's 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. AED concentrations can be used as an aid in management of patients treated with the respective AED.

AVAILABILITY

ARK AED Assays are available in the USA, Canada and Europe.
Check the website for documentation and other current information, www.ark-tdm.com.