Performance Charactersitcs of ARK™ Diagnostics Quantitative Immunoassay for Levetiracetam on The Beckman Random Access Unicel® Dxc System & Comparsion to LC-MS-MS Batch Analysis.

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Introduction

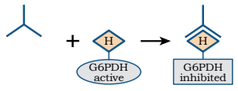
ABSTRACT

Background
Levetiracetam [(-)-(S)-α-ethyl-2-oxo-1-pyrrolidine acetamide] is a second generation anticonvulsant medication indicated as adjunctive therapy in the treatment of certain types of seizures in people with epilepsy. It is marketed under the trade name Keppra®. Levetiracetam is a single enantiomer and the precise mechanism(s) by which levetiracetam exerts its antiepileptic effect is unknown. However, high doses of levetiracetam can induce adverse effects, including dizziness, somnolence, asthenia, headache, behavioral problems, depression, and psychosis (Kanner et al., 2004). The therapeutic drug monitoring of levetiracetam concentrations plays an important role as an aid in management of patients treated with levetiracetam for toxicity issues.

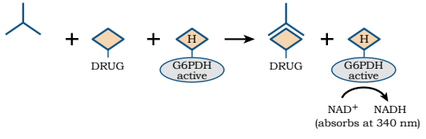
Objective
To evaluate the performance characteristics of the new ARK Diagnostics Levetiracetam Assay on the Beckman Random Access UniCel DxC system for routine clinical laboratory use.

Method
The ARK Levetiracetam Assay is a homogeneous immunoassay used in the quantitative determination of levetiracetam in human serum or plasma. When sample and reagents are mixed, drug in the sample competes with drug labeled by the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody so that the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. The NADH absorbance is directly proportional to drug concentration in the sample. Endogenous serum G6PDH does not interfere because the coenzyme functions only with the bacterial enzyme (from Leuconostoc mesenteroides) used in the assay. The ARK Levetiracetam Assay was evaluated using the Random Access UniCel DxC 600 analyzer. The assay was calibrated using a six point calibration curve (0 to 100 µg/mL). Performance of the assay was determined by assessing precision, limit of quantitation, linearity, endogenous interferences, Heath Controls (NEQAS, UK) proficiency samples performance and correlation studies using LC/MS/MS batch analysis.

A) Absence of drug



B) Presence of drug

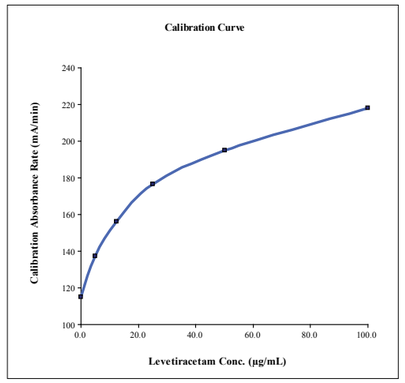


RESULTS
Precision on tri-level controls was 7.3% CV (7.9 µg/mL), 6.5% CV (29.5 µg/mL) and 7.8% CV (79.1 µg/mL). Limit of Quantitation (LOQ) was 2.0 µg/mL. Linearity was demonstrated from 2.0 to 100.0 µg/mL. No common endogenous substances (Hb, bilurubin, gamma globulin, uric acid, albumin, cholesterol and triglyceride) interference was observed with the measurement of levetiracetam at the levels tested. Recovery experiment using spiked samples at 1.0, 2.0, and 3.0 µg/mL showed acceptable recovery. Correlation studies were done using 50 patient samples with level ranging from 2.0 to 61.0 µg/mL were analyzed using reference the LC/MS/MS method showing acceptable statistical results (Passing Bablok regression analysis: y (Beckman DxC) = 1.01 (LC/MS/MS) - 0.03, r^2 = 0.95).

CONCLUSIONS
The ARK Levetiracetam Assay is suitable for the quantitative measurement of Levetiracetam in serum and plasma on the UniCel Random access DxC 600 System. This assay correlated with LC/MS/MS and is well-suited for routine TDM use on the UniCel DxC 600 Random Access Clinical System. Compared to LC/MS/MS method, which is time consuming and expensive and requires highly skilled technical staff, the ARK Levetiracetam Assay can be used in routine clinical chemistry laboratories and can generate results within 30 minutes.

ARK Levetiracetam Assay Calibration Curve on the Beckman Coulter UniCel DxC 600Random Access Clinical System

A 6-point calibration curve was generated by testing calibrators in duplicate. Calibration curve was verified with two levels of quality controls. A representative calibration curve is shown.



Intra-Assay Precision

Precision was determined using ARK tri-level quality control samples. The data are derived from 2 runs, 10 replicates per run to give a total of 20 replicates of each control level.

QC Sample Conc. (µg/mL)	QC Assayed (Mean ± SD)	N	CV (%)	Recovery (%)
7.5 µg/mL	7.9 ± 0.58	20	7.3	105.8
30.0 µg/mL	29.5 ± 1.91	20	6.5	98.4
75.0 µg/mL	79.1 ± 6.20	20	7.8	105.4

Lower Limit of Quantitation (LOQ)

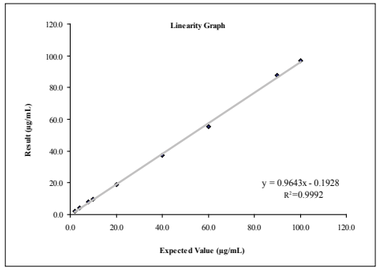
Three LOQ levels were tested below the lowest positive calibrator concentration (5.0 µg/mL). Pooled human serum representative of the patient specimen matrix was supplemented with levetiracetam to give concentrations of 1.0, 2.0 and 3.0 µg/mL. Five (5) replicates of each sample were tested.

Spiked Sample Conc. (µg/mL)	QC Assayed (Mean ± SD)	N	CV (%)	Recovery (%)
1.0 µg/mL	1.1 ± 0.30	5	26.8	112.3
2.0 µg/mL	2.3 ± 0.28	5	12.4	113.0
3.0 µg/mL	3.4 ± 0.49	5	14.3	114.7

Linearity and Accuracy

Linearity studies were performed by testing concentrations of levetiracetam across the assay range. Dilutions of this highest linearity level (100 µg/mL) were made proportionally with pooled human serum negative for levetiracetam. Four replicates of each sample were assayed.

Expected Value (µg/mL)	Result (µg/mL)	Recovery (%)	SD	CV (%)
2.0	2.1	103.8	0.17	8.2
4.0	4.1	101.9	0.39	9.5
8.0	8.1	100.9	0.59	7.3
10.0	9.9	98.5	0.57	5.8
20.0	19.0	94.9	1.49	7.9
40.0	37.2	93.1	2.81	7.6
60.0	55.4	92.3	4.70	8.5
90.0	87.5	97.3	7.65	8.7
100.0	97.1	97.1	8.67	8.9



Heath Control Proficiency Samples

Heath Controls (UK NEQAS:United Kingdom National External Quality Assessment Scheme, Cardiff Bioanalytical Services Ltd.16, MountStuat Stuart Square, Cardiff CF10 5DP U.K.) were evaluated. Five replicates of each sample were assayed. ARK Levetiracetam assay results were compared to Heath Control Spiked Values and all method Consensus Values.

Heath Spiked			
Sample	Heath AE0510 (4.8 µg/mL)	Heath AE0610 (66.3 µg/mL)	Heath AE0810 (18.9 µg/mL)
ARK Mean Value (µg/mL)	5.3	64.3	17.9
N	5	5	5
SD	0.59	0.46	0.56
CV (%)	11.1	0.7	3.1
Recovery (%)	109.6	97.0	94.9

Heath Consensus			
Sample	Heath AE0510 (4.8 µg/mL)	Heath AE0610 (64.4 µg/mL)	Heath AE0810 (18.7 µg/mL)
ARK Mean Value (µg/mL)	5.3	64.3	17.9
N	5	5	5
SD	0.59	0.46	0.56
CV (%)	11.1	0.7	3.1
Recovery (%)	109.6	99.8	95.9

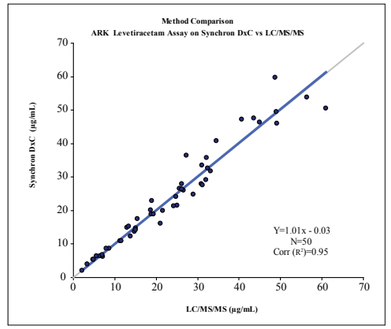
Endogenous Interference

Endogenous interfering specimens containing approximately 15 µg/mL of levetiracetam were tested in triplicate. The mean results of levetiracetam were calculated and the percentage recovery relative to the serum control result was determined.

Endogenous Substance	Endogenous Substance Conc.	Mean (µg/mL)	N	SD	CV (%)	Interference (%)
Cholesterol	406 mg/dL	16.0	3	1.07	6.7	-0.7
Triglyceride	1054 mg/dL	16.3	3	0.92	5.6	0.9
Bilirubin Conjugated	70 mg/dL	15.8	3	1.50	9.5	0.9
Bilirubin Unconj.	70 mg/dL	16.5	3	0.21	1.3	5.4
Hemoglobin	1000 mg/dL	17.4	3	1.21	7.0	7.5
Human γ-Globulin	12 g/dL	16.3	3	0.49	3.0	4.1
Human Albumin	10 g/dL	18.4	3	0.23	1.2	-4.5
Uric Acid	30 mg/dL	15.6	3	1.30	8.3	-0.1

Comparative Analysis

Patient samples dosed with Levetiracetam were analyzed using the ARK Levetiracetam Assay on the Beckman DxC chemistry analyzer and High Turbulence Liquid Chromatography-Tandem Mass Spectrometry (HTLC-MS/MS). Comparative analysis was performed using Passing-Bablock parameters (Bablok et al., 1998).



ARK Levetiracetam Assay vs LC/MS/MS	
Slope	1.01
Intercept (µg/mL)	0.03
Correlation Coefficient (r^2)	0.95
Number of Samples	50

Conclusions

The ARK Levetiracetam Assay is suitable for the quantitative measurement of levetiracetam in serum and plasma on the UniCel Random access DxC 600 System. The assay offers the following advantages to laboratories:

- **Small sample size**
- **No sample extraction or pretreatment**
- **Good precision and sensitivity**
- **No endogenous substance interference**
- **Excellent correlation to LC-MS/MS method**
- **Ready-to-use liquid reagents and calibrators**
- **Rapid turn-around time**