Application Of Levetiracetam (Keppra) Monitoring To Standard Laboratory Analyzers

G. J. Abraham, H. Gang, N. Qureshi
Analytical Diagnostic Lab, Brooklyn, NY.

Abstract

Background and Methods
Levetiracetam (Keppra) is an antiepileptic approved for use as adjunctive therapy in the treatment of epilepsy. It is currently measured by labor intensive LC-MS methods. To simplify and standardize the monitoring of LTA for diagnostic laboratories, a homogeneous EIA method has been developed. We tested this EIA assay on our current analyzers (Roche/Hitachi 917, Roche/Cobas c501, Hitachi 747). Levetiracetam calibration standards were prepared by ARK Diagnostics, Inc. in pooled human serum and were assayed in duplicate on the Roche/Hitachi 917 and Roche/Cobas c501 chemistry analyzers. As the latter binds antibody, the enzyme activity decreases. In the presence of drug, free enzyme activity increases and this increase is directly proportional to drug concentration. The homogeneous EIA LTA assay had been previously tested on the Roche/Hitachi 917 analyzer by ARK Diagnostics, Inc. and assayed 6 times. The LLOQ of the ARK Levetiracetam Assay is defined as the lowest concentration for which acceptable assay precision (±20% CV) and recovery (>80%) is observed. The calibration curve was verified with 6-level controls. A typical calibration curve is shown in the Figure below.

Method Comparison 1
Twenty-four archived patient specimens from patients treated with levetiracetam were analyzed using the ARK Levetiracetam Assay on the Roche/Cobas c501 chemistry analyzer and LC-MS/MS. Comparison by Passing-Bablock regression of the results is shown in the Figure below.

Method Comparison 2
Twenty four archived patient specimens from patients treated with levetiracetam were analyzed using the ARK Levetiracetam Assay on the Roche/Cobas c501 chemistry analyzer and Roche/Hitachi 917. Comparison by Passing-Bablock regression of the results is shown in the Figure below.

Discussion and Conclusions
The ARK Levetiracetam Assay was calibrated using a full calibration (6-point) procedure. To perform a full calibration, the ARK Levetiracetam Calibrators 0.0, 5.0, 12.5, 25.0, 50.0, and 100.0 µg/mL were assayed in duplicate. Calibration curve was verifid with three-level controls. A typical calibration curve is shown in the Figure below.

Precision
Precision was determined by assaying the same replicate on eight replicates of each level in control containing levetiracetam. Mean, present recovery from target, standard deviation (SD), and coefficients of variation (%CV) were calculated for each level. The precision ranged from 1.7 to 6.9%.

Conclusions
The ARK Levetiracetam Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of levetiracetam in human serum on automated clinical chemistry analyzers. Assay performance on the Roche/Cobas c501 automated clinical chemistry analyzers showed good precision and accuracy with excellent patient sample correlation to LC-MS/MS. The lower limit of quantification (LLOQ) was 2.0 µg/mL. In conclusion, analytical utility of the ARK Levetiracetam Assay for routine quantification of levetiracetam in serum or plasma on the Roche/Cobas c501 was demonstrated.

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Analytical Recovery
Analytical recovery was performed by adding concentrated levetiracetam (LLOQ) into human serum negative for levetiracetam. Test sample concentrations were 10, 100, 300, 500, 800, and 1000 µg/mL. Three replicates of each sample were assayed. The results were assayed and compared to the theoretical target concentration and the percentage recovery was calculated. The amount of levetiracetam recovered from serum ranged from 110% to 122%.

Mean percent recovery: 112.4