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Validation of a New Immunoassay for Quantification of Topiramate

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Topiramate (Topamax®) is an important anticonvulsant drug for which therapeutic drug monitoring (TDM) can be clinically indicated and would be improved through commercialization of additional automated assays performed using common open channel analyzers.

OBJECTIVE: Immunoassay reagents developed by ARK Diagnostics, Inc. (Sunnyvale, CA) for quantification of topiramate were evaluated and results compared to those generated by FPIA.

METHODS: The ARK™ Topiramate Assay was performed with an Olympus AU400 (Olympus Diagnostics, Center Valley, PA). The INNOFLUOR® FPIA Assay System for topiramate (Seradyn Diagnostics/Thermo Scientific, Indianapolis, IN) was performed using an Abbott TDx® Analyzer. Both assays were performed according to manufacturer instructions and all measurements were performed in triplicate (or more). The ARK assay was evaluated for accuracy (recovery) by analyzing two batches of samples spiked at ten concentrations between 1.5 and 55 µg/mL. Linearity (assay range) was determined by analyzing two batches of spiked samples over two concentration ranges (0.6 to 6.0 µg/mL, and 6.0 to 60.0 µg/mL) that were prepared by serial dilution. Precision was determined by analyzing quadruplicate control samples at three concentrations for five days. Carryover was evaluated by a series of samples containing 80.0 µg/mL, followed by a sample containing 2.0 µg/mL, in five replicates. Analytical interference was evaluated with residual clinical samples containing various concentrations of potentially interfering substances with known concentrations of topiramate, along with a topiramate control. Three replicates of each sample and their respective controls were analyzed. The ARK assay was compared to the FPIA assay with residual patient specimens. All residual patient samples were de-identified in compliance with an IRB-approved protocol. Twenty specimens were fortified with topiramate to achieve a concentration of 30 to 60 µg/mL to evaluate the upper range of the ARK assay, because the FPIA assay had a reportable range of 0.6 to 32.0 µg/mL. These 20 specimens were diluted with zero calibrator for analysis by FPIA. Nine discordant specimens whose ARK results differed by more than 20% from the FPIA results were retested twice by both methods.

RESULTS: The LLOQ for the ARK assay was 1.0 µg/mL. The ULOQ was 60.0 µg/mL. Percent recovery ranged from 94.5 to 107.5%. A first order regression fit of the linearity data was y = 1.1620x - 0.1111 for sample concentrations 0.6 to 6.0 µg/mL and y = 1.0156x + 1.2407 for sample concentrations 6.0 to 60 µg/mL. All precision data (within run, between day and total) demonstrated ≤6.8% CV. The linear regression equation for the patient specimens tested by both methods was y = 0.93x - 0.06, r2 = 0.98 (n=116, range 1.0 to 59.1 µg/mL).

CONCLUSION: Good correlation was observed between patient specimens analyzed using the ARK assay and the FPIA assay. The ARK topiramate assay performed comparably to the FPIA assay but offers a wider reportable range (1.0 to 60.0 µg/mL vs 1.0 to 32.0 µg/mL) while maintaining excellent precision. Commercialization of the ARK reagents may improve accessibility of topiramate TDM.