Comparison of a New Serum Topiramate Immunoassay to Fluorescence Polarization Immunoassay

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Abstract: Topiramate is a newer anticonvulsant used to treat epilepsy, migraines, bipolar disorder, posttraumatic stress, and other conditions. Serum topiramate concentrations are measured to determine optimal levels, address therapeutic failure or drug–drug interactions, and assess compliance. Two high-throughput assays for serum topiramate measurement were compared: the Seradyn fluorescence polarization immunoassay (FPIA) on an Abbott TDx/FLx instrument and a new immunoassay from ARK Diagnostics performed on an Olympus AU680 automated analyzer. Precision, linearity, limit of quantitation, carryover, spike recovery, and endogenous interferences were found to be acceptable for the ARK assay. These studies were complemented by comparison of 120 patient samples analyzed using both methods. The ARK immunoassay performed comparably to FPIA with minimal difference in serum topiramate concentrations within the therapeutic range (2.0–20 μg/mL). A slight systematic discordance was observed at higher concentrations (greater than 30 μg/mL) with ARK immunoassay results being on average 6% higher than FPIA. Thus, the ARK immunoassay appears to provide acceptable analytical performance and comparability to FPIA; furthermore, the assay is compatible with high-throughput autoanalyzers.

Key Words: topiramate, FPIA, immunoassay, anticonvulsant

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INTRODUCTION

Topiramate is a second-generation anticonvulsant useful for treatment of epilepsy and migraine headaches as well as various off-label applications such as bipolar disorder and posttraumatic stress.[1] Its pharmacokinetic profile is favorable, showing greater than 80% oral bioavailability and low potential for drug interactions; side effects are mild with weight loss and somnolence commonly reported.[1,2]

Despite the relative absence of serious adverse effects and pharmacokinetic-related problems that plague use of older antiepileptic drugs,[3] topiramate monitoring is still helpful for optimizing individual therapy, managing comedications capable of altering topiramate concentrations, addressing therapeutic failure, and assessing compliance.[2] The recommended therapeutic range for topiramate is wide, 2 to 20 μg/mL (6–60 μmol/L).[4]

Unlike many of the newer anticonvulsants, topiramate monitoring has been facilitated by the availability of commercially produced immunoassays. One of the major platforms for this has been fluorescence polarization immunoassay (FPIA), which is in widespread use and has been validated against a liquid chromatography–tandem mass spectrometry assay for topiramate.[5] Using FPIA as the reference method, we evaluated the performance of a new immunoassay (ARK Diagnostics, Sunnyvale, CA). This evaluation was a collaborative study in conjunction with ARK Diagnostics as part of their application to the Food and Drug Administration for approval of the topiramate assay.

MATERIALS AND METHODS

Topiramate Measurement

The topiramate Innofluor FPIA (Seradyn Products, Indianapolis, IN) was performed on an Abbott TDx (Abbott Laboratories, Abbott Park, IL) instrument. Samples were transported frozen and brought to room temperature before analysis. The FPIA assay was performed according to the manufacturer's instructions. The assay is calibrated from 2.0 to 32.0 μg/mL with three levels of quality controls (3.0, 10.0, and 24.5 μg/mL) run immediately before patient samples to verify calibration accuracy. Samples with topiramate concentrations greater than 32 μg/mL were diluted with the manufacturer's zero calibrator before analysis.

The ARK Topiramate Assay (ARK Diagnostics) was performed on an Olympus AU680 (Olympus, Center Valley, PA) automated analyzer. All assays followed the manufacturer's recommendations and each run included assessment of quality controls (in duplicate) to confirm accurate calibration. Briefly, 150 μL of Reagent 1 (including the antitopiramate antibody, glucose-6-phosphate substrate, and nicotinamide adenine dinucleotide [NAD] cofactor) and 75 μL of Reagent 2 (including the enzyme glucose-6-phosphate dehydrogenase labeled with topiramate) are mixed with 3 μL of each serum sample, calibrator, or control. Reaction of the enzyme with substrate and cofactor converts NAD to NADH, which is assessed by measuring absorbance at 310 nm. The assay

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is calibrated against standards at 2.0, 4.0, 8.0, 24.0, and 60.0 μg/mL.

Sample Selection

Patient serum samples sent to the laboratory for routine topiramate measurements were analyzed by both FPIA and the ARK immunoassay. Patient samples were selected according to their FPIA topiramate concentration to evenly distribute the number tested throughout the analytical range of the ARK immunoassay. Very few patient samples exceeded 20 μg/mL; thus, additional samples were spiked with topiramate to evaluate the higher portion of the analytical range. Of these spiked samples, 20 were made using drug-free serum and 31 were made using topiramate-containing patient sera. Use of patient samples was approved by the Mayo Clinic Institutional Review Board.

RESULTS

The ARK immunoassay was developed for Hitachi automated analyzers but was adapted to the Olympus AU680 platform for this study (for parameters, see "Materials and Methods"). Performance of the ARK immunoassay was evaluated, including studies of within- and between-day precision, assay linearity, limit of quantitation, carryover, spike recovery, and susceptibility to endogenous interferences such as hemolysis and lipemia. After ascertaining these analytical characteristics, a method comparison including 100 patient samples and 20 spiked-matrix samples was performed using FPIA as the reference method.

The ARK immunoassay is calibrated from 2.0 to 60.0 μg/mL topiramate with the suggested analytical range between 1.5 and 54.0 μg/mL. Precision within the analytical range was tested at three concentrations (2.5, 10.0, and 40.0 μg/mL) using the quality control material (QCM) supplied with the assay kit. Intraday precision studies consisted of 20 measurements of each QCM split into two runs of 10 measurements each. Intraday coefficients of variation (CVs) were between 2.64% and 2.94% for the three samples (Table 1). Individual measurements ranged from 92% to 110% of the nominal value of each QCM; means of all 20 replicates were within ±4% of nominal values.

Interday precision was assessed by measuring each QCM eight times within a single day (split into two runs of four replicates each) over the course of 5 days for a total of 40 measurements for each sample. CVs were between 3.01% and 3.75%; individual measurements ranged from 88% to 110% of nominal with mean values within ± 2.5% of the nominal value (Table 1). Throughout the assessment of the ARK immunoassay, QCM values were analyzed in duplicate with each run of samples. Therefore, to determine interday precision over a longer period of time, all available QCM values were compiled for a total of 60 measurements made during the course of 3 months. Precision remained consistent with the results of the interday study with CVs between 2.94% and 3.47%, individual measurements ranging from 90.3% to 108.3% of nominal, and all mean values within ± 2.5% of nominal.

TABLE 1. Precision Studies for the ARK Immunoassay

	Topiramate (μg/mL)		
Study	**2.5**	**10.0**	**40.0**
Intraday			
Mean	2.4	10.3	40.4
SD	0.067	0.302	1.068
CV (%)	2.78	2.94	2.64
Percent of nominal (mean)	96.6	102.6	101.0
Percent of nominal (range)	92.0–100.0	99.0–110.0	98.0–107.0
Interday			
Mean	2.4	10.2	40.5
SD	0.082	0.309	1.520
CV (%)	3.33	3.01	3.75
Percent of nominal (mean)	97.9	102.4	101.2
Percent of nominal (range)	88.0–108.0	96.0–109.0	95.3–110.0
All quality control for study			
Mean	2.45	10.23	39.86
SD	0.077	0.301	1.385
CV (%)	3.14	2.94	3.47
Percent of nominal (mean)	97.9	102.3	99.6
Percent of nominal (range)	92.0–104.0	96.0–108.0	90.3–108.3

SD, standard deviation; CV, coefficient of variation.

Carryover and interference studies were all acceptable. There was no detectible carryover in low-concentration (2.0 μg/mL) samples after injection of high-concentration (80 μg/mL) material. For interference studies, two concentrations of topiramate (targets 5.0 and 20.0 μg/mL) were spiked into samples containing high levels of potentially interfering substances and then compared with spiked normal sera as controls. There were negligible effects in reported topiramate concentrations compared with controls in the presence of endogenous interfering substances, including gross lipemia, hemoglobin (1.0 g/dL), bilirubin (unconjugated or conjugated, 70 mg/dL), uric acid (30 mg/dL), or serum proteins (albumin or γ-globulin, 12 g/dL).

The analytical range is listed as 1.5 to 54.0 μg/mL with calibrators from 2.0 to 60.0 μg/mL. The assay showed good linearity throughout the majority of the analytical range; 25 samples spiked at concentrations between 2.4 and 60.0 μg/mL were analyzed in six replicates (two runs of three replicates each). All samples with concentrations above the low calibrator had mean values within 5% of the nominal value (mean, 101.2%; range, 96.1%–104.7%) with CVs less than 6.5% (mean, 3.69%; range, 1.31%–6.45%; Supplemental Table 1).

Samples at concentrations below the lowest calibrator (2.0 μg/mL) did not perform as well, even within the analytical range (Table 2); CVs were increased as were differences between reported concentrations and nominal values. For example, CVs at 1.8 μg/mL and 1.5 μg/mL were 8.2% and 8.4%, respectively, with mean values at 89.8% and 95.6% of the nominal concentration. However, all values within the analytical range (i.e., greater than 1.5 μg/mL) met the acceptability criteria stated in the package insert, namely CVs less than 20% and recovery within 15% of the nominal value. Of

TABLE 2. Low-End Precision for the ARK Immunoassay

Nominal concentration (μg/mL)	0.5	0.6	1	1.2	1.5	1.8	2.4	2.5	3.0
Mean	0.36	0.38	0.93	1.00	1.43	1.62	2.33	2.42	3.10
SD	0.05	0.04	0.06	0.09	0.12	0.13	0.15	0.12	0.14
Percent coefficient of variation	13.5	10.6	6.1	8.9	8.4	8.2	6.5	4.8	4.6
Percent nominal	72.5	63.9	93.3	83.3	95.6	89.8	97.2	96.7	103.3

SD, standard deviation.

note, these analyses were performed within a single day; based on the precision studies described, it is expected that imprecision would increase slightly between measurements on different days.

To complete the assessment, 120 samples were analyzed by both the ARK immunoassay and FPIA. To obtain even distribution of sample concentrations throughout the analytical range, patient sera (n = 69) were selected according to their FPIA topiramate concentration. Very few patients had topiramate greater than 20 μg/mL; thus, additional samples were spiked with topiramate to create high-concentration comparison material. These spiked samples were made in either drug-free commercial serum (n = 20) or in topiramate-containing patient serum (n = 31) and were supplemented with additional drug.

The comparison between the ARK immunoassay and the FPIA method is shown in Figure 1. Overall agreement is good with a correlation coefficient $R^2 = 0.982$ and a linear regression slope of 1.075. However, eight samples showed greater than 20% discordance (range, –22.2% to –31.8%) between the two methods; all were patient sera that had not been spiked with additional topiramate (Figure 1, middle panels). Repeat testing of greater than 20% discordant samples by both methods improved agreement considerably (range, 0.0% to –13.8%) with the majority of change occurring in the FPIA concentrations. Repeated FPIA values differed from initial measurements by a mean of –26.7% (range, –19.4% to –32.5%) in contrast to ARK immunoassay results, which differed from original values by an average of –2.4% (range, –7.4% to +7.6%). Inclusion of the repeat measurements improved overall correlation ($R^2 = 0.993$) with minimal effect on the linear regression slope (Figure 2, left panels). No explanation for the greater than 20% discordant samples was apparent; QCM precision on the FPIA assay was less than 5% for all concentrations tested.

Analysis of the spiked sera revealed that samples with high (greater than 30 μg/mL) topiramate concentrations show consistently higher results when analyzed by ARK immunoassay compared with FPIA measurements (Figure 1, right panels). Given that the FPIA assay is only calibrated to 32.0 μg/mL, it cannot be determined whether this phenomenon is a true bias between the two methods or whether it stems from the dilution necessary for FPIA analysis on such high-concentration samples. Agreement between the two methods

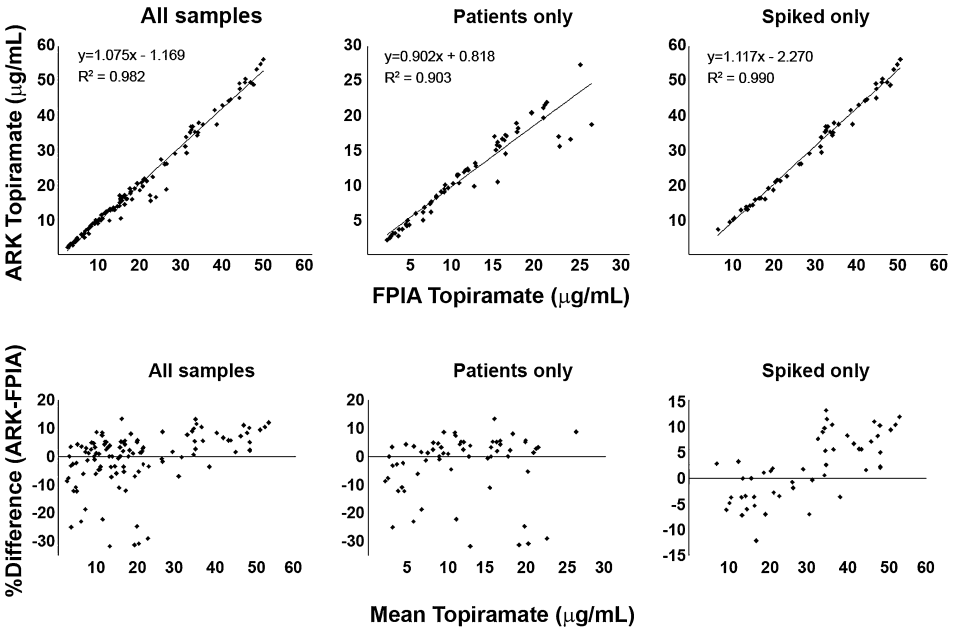

FIGURE 1. Initial method comparison between fluorescence polarization immunoassay (FPIA) and the ARK immunoassay. *Top panels* depict the linear regression correlations between FPIA (reference method, x-axes) and the ARK immunoassay (y-axes). *Bottom panels* show the corresponding Bland-Altman plots comparing the difference between methods (as ARK concentration–FPIA concentration) against the mean value for each sample. Samples shown include all sera tested (*left panels*), nonspiked patient samples only (*middle panels*), and all spiked samples (*right panels*).

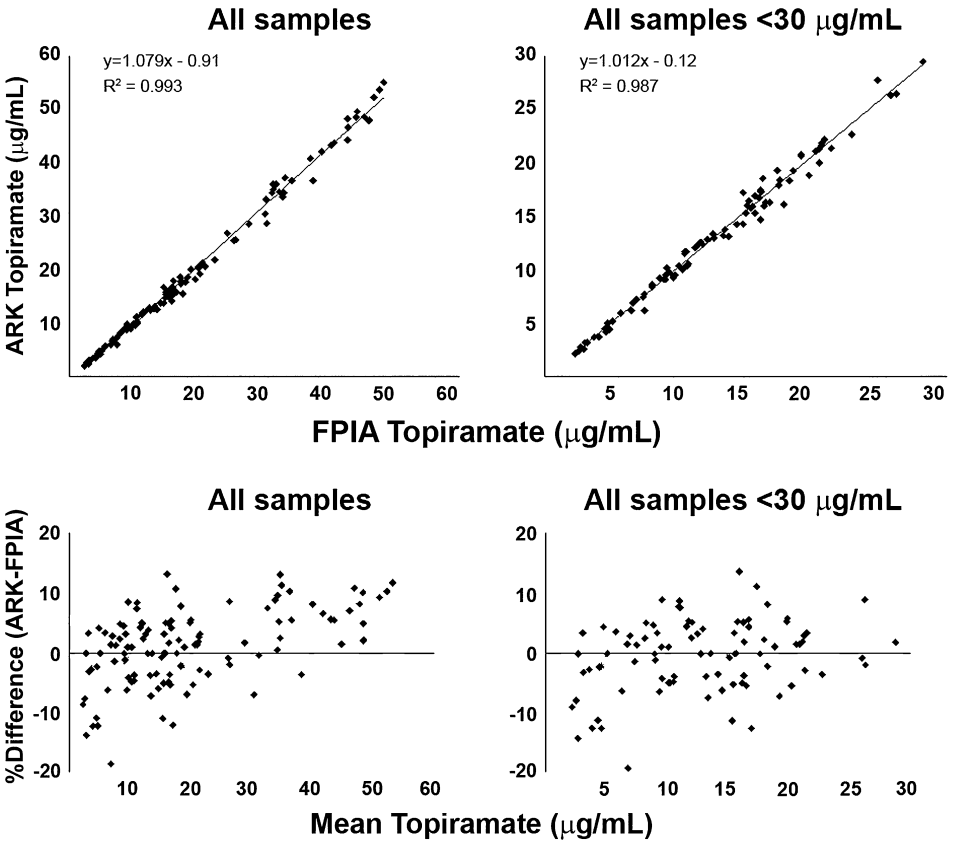

FIGURE 2. Method comparison after repeat testing. Samples showing greater than 20% discordance between methods (see Figure 1) were retested by both topiramate assays. The initial values were replaced with the repeated measurements in this figure. *Top panels* depict the linear regression correlations between fluorescence polarization immunoassay (FPIA) (reference method, x-axes) and the ARK immunoassay (y-axes). *Bottom panels* show the corresponding Bland-Altman plots comparing the difference between methods (as ARK concentration – FPIA concentration) against the mean value for each sample. Samples shown include all sera tested (*left panels*) or all samples with mean topiramate concentrations less than 30 μg/mL (*right panels*).

is excellent for all samples less than 30 μg/mL whether native patient samples or spiked (Figure 2, right panels); this encompasses the entire therapeutic range (2–20 μg/mL).

DISCUSSION

Assessment of the ARK topiramate immunoassay suggests that this new commercial assay is sufficiently robust for a clinical laboratory. Precision is good with CVs below 6.5% throughout the majority of the analytical range, although slightly higher at low topiramate concentrations. The assay is consistent over time as evidenced by CVs of approximately 3% for QCMs tested throughout the duration of the study. There was negligible effect from endogenous interferences such as hemoglobin and lipemia; exogenous interferences such as potentially crossreacting therapeutic agents were not assessed in these studies but are addressed in the package insert. Although only serum samples were analyzed in this study, the package insert states that performance is also acceptable with sodium or lithium heparin or potassium EDTA as anticoagulants.

The analytical range for the ARK immunoassay is stated as 1.5 to 54.0 μg /mL, which is considerably wider than the analytical range for the comparator FPIA assay (2.0–32.0 μg/mL). However, the clinical importance of extending this range is debatable; very few (nonspiked) patient samples were seen above the upper limit of the therapeutic range (20 μg/mL) with none above 30 μg/mL. Agreement between the two methods was poorer above 30 μg/mL, although whether this reflects a true bias or dilution-related error cannot be determined. Finally, the ARK immunoassay performs less well at concentrations below the lowest calibrator (2.0 μg/mL), even within the stated analytical range. Despite the concerns at high and very low topiramate concentrations, the overall performance of the ARK immunoassay is quite good with excellent correlation to the comparator method throughout the therapeutic range.

CONCLUSIONS

The ARK topiramate immunoassay provides acceptable performance for routine use in clinical laboratories. The correlation to the existing FPIA method is good throughout the therapeutic range with a small high bias apparent at higher concentrations. The immunoassay is amenable to high-throughput analyzers and may therefore allow laboratories without FPIA instrumentation to include topiramate in their test menus.

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APPENDIX TABLE. Linearity of the ARK Topiramate Immunoassay

Nominal concentration (μg/mL)	**3.6**	**4.0**	**4.2**	**4.8**	**5.0**	**5.4**	**6.0**	**6.0**	**10.0**	**12.0**	**15.0**
Mean	3.65	4.05	4.25	5.02	4.95	5.60	6.28	6.17	10.03	12.37	14.80
SD	0.138	0.259	0.187	0.117	0.226	0.155	0.147	0.163	0.437	0.476	0.429
Percent CV	3.78	6.39	4.40	2.33	4.56	2.77	2.34	2.65	4.35	3.85	2.90
Percent nominal	101.4	101.3	101.2	104.5	99.0	103.7	104.7	102.8	100.3	103.1	98.7
Nominal concentration (μg/mL)	**18.0**	**24.0**	**30.0**	**30.0**	**36.0**	**42.0**	**45.0**	**48.0**	**54.0**	**55.0**	**60.0**
Mean	18.80	24.45	30.80	28.83	36.63	42.02	43.92	49.95	53.98	56.10	60.90
SD	0.780	0.740	1.241	0.698	1.172	0.549	1.706	1.355	1.669	3.249	1.448
Percent CV	4.15	3.02	4.03	2.42	3.20	1.31	3.88	2.71	3.09	5.79	2.38
Percent nominal	104.4	101.9	102.7	96.1	101.8	100.0	97.6	104.1	100.0	102.0	101.5

SD, standard deviation; CV, coefficient of variation.