

Thermo Scientific QMS[®] Lamotrigine Immunoassay on HITACHI[®] 917 System

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Abstract

Background: Lamotrigine, an antiepileptic drug (AED) approved by the FDA in 1994, is now available in more than 90 countries worldwide. It has a broad spectrum of clinical efficacy in the treatment of epilepsy and is often prescribed as monotherapy and polytherapy. Concomitant administration of lamotrigine with other AEDs makes it subject to various pharmacokinetic interactions, which can strongly inhibit or increase the metabolism of lamotrigine. Concentrations in patients on lamotrigine monotherapy have also shown significant inter-patient variability. These characteristics of lamotrigine emphasize the clinical need to closely manage each patient's medication regimen. Maintaining optimal serum/plasma concentrations within the suggested therapeutic range, 3 to 15 µg/mL, is achieved by therapeutic drug monitoring (TDM). TDM can be both effective and efficient in the optimization of individual patient therapy when used as a means to define serum concentrations which best control seizures as well as those which are associated with AED-specific side effects.

Objective: This study evaluates the performance of the Thermo Fisher Scientific QMS Lamotrigine Immunoassay for use on automated clinical chemistry analyzers.

Methods: The Thermo Scientific QMS Lamotrigine Immunoassay is a homogenous turbidimetric immunoassay that utilizes the QMS technology for analyzing lamotrigine in human serum or plasma in a non-linear, competitive binding format. The results were generated on the HITACHI 917 platform. The immunoassay range was determined to be 2 to 40 µg/mL. The accuracy of the immunoassay was confirmed by performing method correlations (NCCLS protocol EP6-A2) with HPLC on lamotrigine patient samples. Studies at three different sites were performed with patient samples consisting of plasma or serum and distributed across the immunoassay range. QMS results were correlated to the HPLC value via Passing-Bablok regression, and results are tabulated below. In addition, precision (NCCLS EP5-A), LOQ, cross reactivity (metabolite, co-administered drugs, and endogenous substances) and on-board stability were measured and results are presented.

Method Correlation				
	Study 1	Study 2	Study 3	
Slope (95% confidence interval)	1.03	1.03	1.06	
y-intercept (µg/mL)	0.11	0.36	-0.14	
Correlation Coefficient (R)	0.96	0.96	0.94	
Standard Error of Estimates (µg/mL)	1.98	1.92	2.44	

Total Precision				
	Sample	SD	CV (%)	
Study 1	Low Patient Pool	0.08	2.8	
	Mid Patient Pool	0.21	2.0	
	High Patient Pool	0.58	2.4	
Study 2	Low Patient Pool	0.13	4.7	
	Mid Patient Pool	0.37	3.5	
	High Patient Pool	0.78	3.3	
Study 3	Low Patient Pool	0.16	5.9	
	Mid Patient Pool	0.38	3.6	
	High Patient Pool	0.75	3.2	

Introduction

Thermo Fisher Scientific has developed the first particle enhanced lamotrigine (anti-epileptic) immunoassay for the quantitative determination of lamotrigine levels in human serum or plasma. The FDA 510(k) cleared the product in January 2007. Monitoring lamotrigine (Figure 1a) serum or plasma concentrations allows physicians to aid in their patients' anticonvulsant drug therapy. The therapeutic range for lamotrigine has been reported in literature as 3 to 15 µg/mL (10 to 60 µmol/L).¹⁻³ Lamotrigine has an elimination half-life of 32 hours.^{4,5} Once a stable dose is reached, up to 7 days may be needed to reach a steady state concentration.⁴ There is no clear relationship between lamotrigine serum concentrations and clinical response across patients.¹ Due to individual patient differences and other co-administered medications, considerable overlap in lamotrigine serum concentrations has been observed between responders and non-responders as well as serum levels associated with seizure control and adverse effects.⁵ Lamotrigine drug concentrations should not be the only means of therapeutic drug management. Clinicians should carefully monitor patients during therapy initiation and dosage adjustments. It may be necessary to obtain multiple samples to determine expected variation of optimal concentrations for individual patients.

Methods & Materials

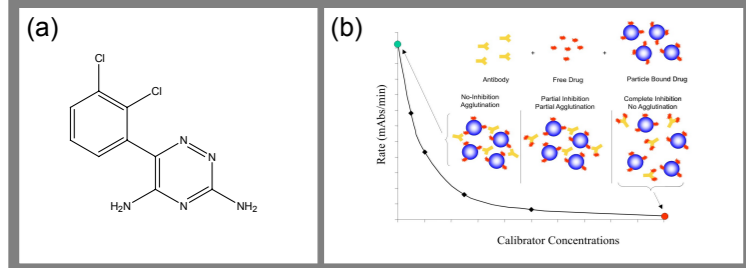
The immunoassay is based on competition between drug in the sample and drug coated onto a microparticle for antibody binding sites of the lamotrigine antibody reagent (Figure 1b). The lamotrigine-coated microparticle reagent is rapidly agglutinated in the presence of the anti-lamotrigine antibody reagent and in the absence of any competing drug in the sample. The rate of absorbance change is measured photometrically, and is directly proportional to the rate of agglutination of the particles. When a sample containing lamotrigine is added, the agglutination reaction is partially inhibited, slowing down the rate of absorbance change. A concentration-dependent classic agglutination inhibition curve can be obtained, with the highest rate of agglutination at the lowest lamotrigine concentration and the lowest agglutination rate at the highest lamotrigine concentration.

Reagents: R1 = Anti-Lamotrigine Polyclonal Antibody (Sheep), R2 = Lamotrigine-coated Microparticles

Calibrators: QMS Lamotrigine Calibrators A-F: A (1 x 2 mL) B-F (1 x 1 mL each)

Controls: QMS Lamotrigine Controls 1-3: 1 x 2.5 mL each

FIGURE 1. a) Structure of Lamotrigine
b) Representation of Calibration Curve via the QMS Technology



Results

Specificity: Cross-reactivity and interfering substance studies were conducted using NCCLS protocol EP7-A.⁶ Studies also were conducted to examine the cross-reactivity of the QMS Lamotrigine antiserum to the lamotrigine metabolites (lamotrigine N-2 glucuronide, lamotrigine N-2 methyl, and lamotrigine N-2 oxide) (Table 1), endogenous interferences (Table 2), and common and co-administered drugs (Table 3).

TABLE 1. Cross-Reactivity of Lamotrigine Metabolites

Metabolite	Concentration Tested (µg/mL)	Cross-Reactivity	Serum Levels
N-2 glucuronide	10 400	< 12%	10% of LTG level
N-2 methyl	10 400	< 5%	*ND
N-2 oxide	10 400	< 25%	*ND

TABLE 2. Endogenous Substances Tested

Substance	Concentration
Albumin	12 g/dL
Bilirubin	60 mg/dL
Cholesterol	500 mg/dL
Hemoglobin	1500 mg/dL
Gamma Globulin	10 g/dL
Rheumatoid Factor*	500 IU/mL
Triglycerides*	1500 mg/dL
Uric Acid*	20 mg/dL

*Prepared by diluting a natural patient sample with lamotrigine-spiked human serum pools.

TABLE 3. Cross-Reactivity with Common & Co-administered Drugs

Acetaminophen	Ethanol	Nortriptyline
Acetazolamide	Ethosuximide	Olanzapine
Acetylsalicylic Acid	Felbamate	Oxcarbazepine
Amikacin	Fluoxetine	Paroxetine
Amtripyline	Furosemide	Penicillin V
Amoxapine	Gabapentin	Perphenazine
Amphotericin B Soln	Gentamicin	Pherytoin
Ampicillin	Haloperidol	Phenobarbital
Ascorbic Acid	Heparin	Primidone
Bupropion	Ibuprofen	Procainamide
Caffeine	Imipramine	Prochlorperazine
Carbamazepine	Kanamycin Solution A	Ranitidine
Carbamazepine-10,11-Epoxy	Kanamycin Solution B	Rifampin
Carbenicillin	Levetiracetam	Risperidone
Chloramphenicol	Lidocaine	Sertraline
Chlorpromazine	Lincomycin	Spectinomycin
Citalopram	Mephenytoin	Sulfamethoxazole
Clobazam	Mesoridazine	Theophylline
Clozapepam	Methicillin	Thioridazine
Cyclosporin A	Methylprednisolone	Tobramycin
Desipramine	N-Acetylprocainamide	Topiramate
Diazepam	Nefazodone	Trazodone
Digloxin	Neomycin	Trimethoprim
Digoxin	Nacin	Valproic Acid
Doxepin	Nirvanol	Vancomycin
Ephedrine	Nitrazepam	Vigabatrin
Erythromycin	Nordoxepin	Zonisamide

Note: The common and co-administered drugs were tested at -3x the highest therapeutic range of the possible interferent with both low and high levels of lamotrigine.

Precision: Precision was determined as described in NCCLS protocol EP5-A2.⁷ A tri-level human serum based commercial control containing lamotrigine and patient sample pools representing low, medium and high therapeutic values were used in the study. Each level of control and patient pool was assayed in duplicate twice a day for twenty non-consecutive days. Each of the runs per day were separated by at least two hours. The study was performed at three different sites in order to verify lab to lab reproducibility. The results are shown in Table 4.

TABLE 4. Precision Summary

	N	STUDY 1			STUDY 2			STUDY 3		
		Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
Low Control	80	2.17	0.06	2.9%	2.09	0.04	1.9%	2.10	0.05	2.2%
Mid Control	80	15.51	0.29	1.9%	15.27	0.44	2.9%	15.24	0.26	1.7%
High Control	80	25.57	0.52	2.0%	25.09	0.69	2.8%	25.04	0.82	3.3%
Low Patient Pool	80	2.81	0.08	2.8%	2.74	0.04	1.5%	2.70	0.06	2.3%
Mid Patient Pool	80	10.79	0.21	2.0%	10.67	0.16	1.5%	10.42	0.23	2.2%
High Patient Pool	80	23.93	0.58	2.4%	23.68	0.56	2.4%	23.01	0.57	2.5%

Linearity & Accuracy: Linearity and accuracy were determined using NCCLS guideline EP6-A.⁸ A patient pool containing a high concentration of lamotrigine was diluted to create several concentrations spanning the assay range with a serum pool negative for lamotrigine. Accuracy samples were prepared by gravimetrically diluting a stock concentration of lamotrigine to several concentrations. For linearity, each sample was assayed at least n = 2 using the QMS Lamotrigine Immunoassay. The mean of the replicates was determined for each sample. The means were plotted using the calculated target on the x-axis and the observed mean on the y-axis. Regression equations of the 1st and 2nd order polynomial were used to calculate the predicted values (Figure 2). Linearity (Table 5) was considered acceptable if the percent deviation was less than 10% between the predicted 1st and 2nd order values. Accuracy (Table 6) was analyzed by comparing the observed mean to the theoretical gravimetric concentration. The QMS Lamotrigine Immunoassay range is 2.0 µg/mL to 40.0 µg/mL.

FIGURE 2. Regression Analysis for Linearity Determination

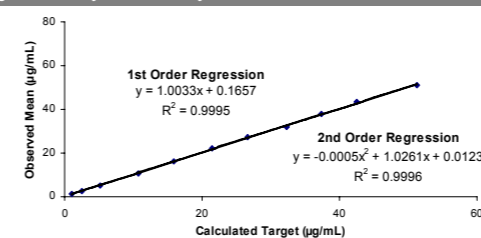


TABLE 5. Linearity

Calculated Target (x)	Observed Mean (µg/mL)	Predicted First Order	Predicted Second Order	Difference (µg/mL) 1st & 2nd Order	Deviation of 1st order from 2nd
51.20	51.20	50.87	50.94	0.07	0.14%
42.50	43.25	42.94	42.89	-0.05	-0.12%
37.38	37.67	37.38	37.28	-0.11	-0.29%
32.26	31.82	31.55	31.41	-0.14	-0.44%
26.62	27.34	27.08	26.94	-0.14	-0.54%
21.50	22.26	22.02	21.88	-0.13	-0.60%
15.87	16.15	15.93	15.84	-0.09	-0.57%
10.75	10.78	10.58	10.55	-0.03	-0.26%
5.12	5.19	5.01	5.07	0.06	1.21%
2.56	2.56	2.39	2.50	0.11	4.47%
1.02	1.23	1.06	1.20	0.14	11.66%

TABLE 6. Accuracy

Theoretical Conc. (µg/mL)	Mean Recovered Conc. (µg/mL)	SD	Percent CV	Percent Recovery
40.02	39.02	0.30	0.78	97.51
30.02	33.04	0.79	2.38	110.07
20.03	20.03	0.68	3.40	99.98
15.00	15.18	0.06	0.37	98.82
9.00	8.91	0.12	1.31	98.96
5.02	4.95	0.09	1.74	98.54
3.75	3.65	0.01	0.27	97.33
2.50	2.34	0.01	0.25	93.73
1.52	1.70	0.05	2.79	111.62

Sensitivity:

Least Detectable Dose (LDD): The LDD was determined to be 0.13 µg/mL.

The LDD of the QMS Lamotrigine Immunoassay is defined as the lowest drug concentration that can be distinguished from zero with 95% confidence. The LDD was determined by analyzing the lowest level calibrator n = 20.

Limit of Quantitation (LOQ): The LOQ is 2.0 µg/mL.

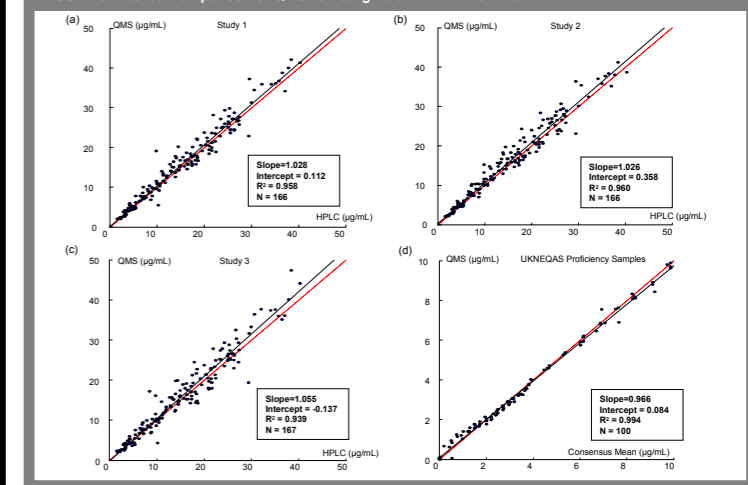
The LOQ (Table 7) of the QMS Lamotrigine Immunoassay is defined as the lowest drug concentration for which acceptable intra-assay precision is observed (often considered ≤ 10% CV) and recovery is between 85 to 115% of theoretical value. The LOQ was determined by analyzing diluted samples twice (n = 2) on 10 calibration curves over 5 days with the QMS Lamotrigine Immunoassay. The study was conducted on more than one HITACHI 917.

TABLE 7. Limit of Quantitation (LOQ)

Target Concentration (µg/mL)	0.35	0.72	1.74	2.75	3.78	4.88
AVG	0.51	0.98	1.82	2.68	3.64	4.88
n	20	20	20	20	20	20
SD	0.05	0.07	0.09	0.15	0.16	0.30
CV	9.80%	7.17%	4.95%	5.60%	4.40%	6.15%
Recovery	146%	136%	105%	97%	96%	100%

Method Comparison: Correlation studies were performed using NCCLS protocol EP9-A.⁹ Studies were performed internally and at two external clinical laboratory sites and were compared to an HPLC reference method.¹⁰ The results of the three studies are shown in Figures 3a through 3c. In addition, correlation studies were conducted with proficiency samples from Cardiff Heathconrol UKNEQAS TDM proficiency scheme (Figure 3d). QMS results were compared to the consensus mean of greater than 50 laboratories participating. In all cases, correlation of the QMS method to the reference method or consensus mean was excellent and resulted in Passing-Bablok¹¹ regression slopes of 0.96 to 1.6, negligible y-intercepts and R² correlation values greater than 0.93.

FIGURE 3. Method Comparison of QMS Lamotrigine on HITACHI 917 vs HPLC



Stability

- QMS Lamotrigine reagents display up to twelve months of real time stability at 2 to 8°C.
- Reagent stability on a HITACHI platform is greater than 50 days when uncapped and on-board.
- Calibration curve is stable for 38 days on HITACHI platforms when reagents are left uncapped and on-board.

Conclusions

The performance results of QMS Lamotrigine Immunoassay on the HITACHI 917 show that the assay provides precise and reliable lamotrigine concentrations within the therapeutic range to aid in optimal patient care. In addition, the QMS Lamotrigine reagents, calibrators, and controls are liquid stable and are easy to use. As a result, optimized patient care is made much more readily available.

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