

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE**

A. 510(k) Number:

k062966

B. Purpose for Submission:

New assay and analyte.

C. Analyte:

lamotrigine

D. Type of Test:

turbidimetric immunoassay

E. Applicant:

Seradyn Corporation

F. Proprietary and Established Names:

QMS® Lamotrigine, calibrators and controls

G. Regulatory Information:

1. Regulation section: 21 CFR 862.3350, 862.3200, 862.3280
2. Classification: Class II
3. Product code: NWM, LAS, DLJ
4. Panel: 91

H. Intended Use:

1. Intended use(s):

See indications for use.

2. Indication(s) for use:

The QMS® Lamotrigine Assay is intended for the quantitative determination of lamotrigine in human serum or plasma on automated clinical chemistry analyzers. Lamotrigine concentrations can be used as an aid in management of patients treated with lamotrigine.

The QMS® Lamotrigine calibrator set is intended for calibration of the QMS® Lamotrigine Assay. The QMS® Lamotrigine Control set is intended for use in quality control of the QMS® Lamotrigine Assay.

3. Special conditions for use statement(s):

Lamotrigine drug values should not be the only means of therapeutic drug management. The assay should be used in conjunction with information available

from clinical evaluations and other diagnostic procedures.

It may be necessary to obtain multiple samples to determine the expected variation of optimal (steady-state) concentrations for individual patients.

See expected range below.

4. Special instrument requirements:

The assay has been validated by the manufacturer on the Hitachi 917 analyzer.

I. Device Description:

The QMS® Lamotrigine assay consists of separately packaged reagents (R1 and R2), calibrators (A through F), and controls (Levels 1, 2, 3).

The R1 Antibody Reagent includes

Lamotrigine Antibody Reagent: sheep antisera (polyclonal Ab) in a buffer as stabilizer and <0.1% sodium azide as preservative.

The R2 Microparticles include

Lamotrigine Microparticle Reagent: lamotrigine-coated microparticles and <0.1% sodium azide as preservative.

The human serum used for the control matrix is from pooled units drawn from donor patients. The serum is tested by FDA approved methods and confirmed to be nonreactive for Hepatitis B surface Ag (HBsAg), HIV Type 1 and 2 Antibodies (anti-HIV1/HIV-2), and Hepatitis C antibodies (anti-HCV).

See section M1c, below, for further information concerning controls and calibrators

J. Substantial Equivalence Information:

1. Predicate device name(s): QMS® Zonisamide Assay
2. Predicate 510(k) number(s): k051211
3. Comparison with predicate: The predicate device is used to quantitate zonisamide; this assay is used to quantitate lamotrigine. Both assays are for use in management of individuals treated with an anti-seizure drug. The assays use similar technology and are both for use on automated analyzers.

K. Standard/Guidance Document Referenced (if applicable):

CLSI documents: "Evaluation of Precision Performance of Clinical Chemistry Devices", EP5; "Evaluation of the Linearity of Quantitative Measurement", EP6; "Interference Testing in Clinical Chemistry", EP7; "Method Comparison and Bias Estimation Using Patient Samples", EP9.

L. Test Principle:

The assay is a homogeneous particle-enhanced turbidimetric assay, based on antibody-binding competition between drug in the sample and microparticle-bound drug.

Antibody binding causes microparticle agglutination. The rate of absorbance, measured spectrophotometrically, is proportional to the rate of agglutination.

M. Performance Characteristics (if/when applicable):

Performance was validated on a Hitachi 917 instrument.

1. Analytical performance:

a. Precision

Precision was evaluated for serum and plasma samples.

Serum:

Studies were performed at Seradyn and the two external laboratory sites, using the protocol described in CLSI EP5-A2. At each of the sites, human serum-based control samples (3 levels) and patient pools (3 levels) were run twice a day in duplicate for 20 non-consecutive days for a total of 80 replicates. The 3 human serum pools were prepared from samples obtained from patients on lamotrigine therapy. The high patient pool was supplemented with a lamotrigine serum drug stock in order to attain the desired concentration. Data from Seradyn was generated using one calibration curve. Data from external sites included multiple calibrations when needed. Each study site used the same lot of reagents, calibrators and controls. Results are summarized below:

Seradyn results

			Within Run (range over 3 sites)		Total (range over 3 sites)	
	N	mean	SD (ug/mL)	%CV	SD (ug/mL)	%CV
Low patient pool	80	2.17	0.04	1.6	0.06	2.9
Mid patient pool	80	15.51	0.18	1.1	0.29	1.9
High patient pool	80	25.57	0.39	1.5	0.52	2.0
Low control	80	2.81	0.05	1.6	0.08	2.8
Mid control	80	10.79	0.10	0.9	0.21	2.0
High control	80	23.93	0.40	1.7	0.58	2.4

Representative external site results

			Within Run (range over 3 sites)		Total (range over 3 sites)	
	N	mean	SD (ug/mL)	%CV	SD (ug/mL)	%CV
Low patient pool	80	2.10	0.05	2.2	0.09	4.4
Mid patient pool	80	15.24	0.26	1.7	0.39	2.6

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			Within Run (range over 3 sites)		Total (range over 3 sites)	
	N	mean	SD (ug/mL)	%CV	SD (ug/mL)	%CV
High patient pool	80	25.04	0.82	3.3	0.90	3.6
Low control	80	2.70	0.06	2.3	0.16	5.9
Mid control	80	10.42	0.23	2.2	0.38	3.6
High control	80	23.01	0.57	2.5	0.75	3.2

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Plasma:

The evaluation was performed at the manufacturer's site. Samples were prepared from patient plasma pools and were run in duplicate twice per day for 10 non-consecutive days, for a total of 40 replicates. There was a minimum of 2 hours between runs each day. Results are summarized below:

			Within Run		Total	
	N	mean	SD (ug/mL)	%CV	SD (ug/mL)	%CV
Low patient pool	40	3.20	0.04	1.1	0.07	2.3
Mid patient pool	40	11.90	0.14	1.1	0.36	3.0
High patient pool	40	26.61	0.38	1.4	0.86	3.2

Precision near upper limit of the assay range:

A 5-day evaluation was performed at the manufacturer's site to determine precision near the high end of the assay. Patient samples containing lamotrigine were supplemented with additional lamotrigine to attain concentrations near 40 ug/mL. Results are shown in the table below:

			Within Run		Total	
	N	Mean	SD (ug/mL)	%CV	SD (ug/mL)	%CV
High sample 1	20	40.86	0.67	1.7	1.2	2.8
High sample 2	20	39.67	0.43	1.1	0.9	2.1
High sample 3	20	40.67	0.61	1.5	0.9	2.2

b. Linearity/assay reportable range:

Linearity by dilution was evaluated based on the CLSI guideline EP6-A: "Evaluation of the Linearity of Quantitative Measurement". Serum and plasma samples were evaluated.

Serum:

The linearity evaluation for serum was performed by diluting a high patient pool to concentrations across the assay range. The high patient pool was supplemented with lamotrigine serum stock to achieve a final lamotrigine concentration of ~50 µg/mL. The concentration of this serum stock was determined gravimetrically. This high concentration pool was then diluted with QMS® Lamotrigine Calibrator A to 10 different concentrations according to the table below. Target concentrations at each level were determined, independently of the QMS® assay, by multiplying the high pool concentration by the dilution factor. Each level was analyzed n=2 on each of two instruments for a total of n=4. The average of all four replicates was used in the analysis. Results are summarized below.

Calculated Target (µg/mL)	Observed Concentration (µg/mL)
42.50	43.25
37.38	37.67
32.26	31.82
26.62	27.34
21.50	22.26
15.87	16.15
10.75	10.78
5.12	5.19
2.56	2.56
1.02	1.23 *

* 1.23 µg/mL is below the assay range.

Percents deviations, based on first and second order predicted values calculated as recommended in EP-6A, were below 1.2% for lamotrigine values between 5 and 40 µg/mL, and below 5% for 2.6 µg/mL.

Plasma:

For the linearity evaluation in plasma, a stock of lamotrigine was prepared to a concentration of 51.81 µg/mL (determined gravimetrically) in human plasma negative for lamotrigine. This stock was diluted with human plasma to the concentrations shown below. Each level was analyzed n=2. The table below demonstrates similar performance for plasma as for serum.

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Calculated Target (µg/mL)	observed concentration (µg/mL)
51.81	51.81
43.00	46.68
37.82	38.08
32.64	33.41
26.94	27.24
21.76	22.41
16.06	16.51
10.88	10.64
5.18	5.35
2.59	2.63

Spike/Recovery into Human Serum Negative for Lamotrigine:

An accuracy by recovery study was performed by spiking pharmaceutical grade lamotrigine drug into human serum negative for lamotrigine and preparing dilutions of this stock in human serum negative for lamotrigine. The samples prepared were at drug concentrations across the assay range. Each sample was assayed n=3 on two separate QMS® Lamotrigine calibration curves on two different Hitachi 917 analyzers. The results were averaged and compared to the target concentrations, determined gravimetrically, independently of the QMS® assay. Recoveries observed ranged from 95-109%.

These linearity data, together with the results on precision and recovery at 2 µg/mL (see detection limit, below) support the claimed assay range of 2-40 µg/mL.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

The calibrators consist of pharmaceutical grade lamotrigine, gravimetrically spiked into human serum containing preservatives. They are supplied in liquid form, ready for use, for storage at 2 to 8°C. The target calibrator concentrations are 0.0, 2.5, 5.0, 10.0, 20.0, and 40 µg/mL.

The calibrators are gravimetrically prepared (to 4 decimal places) to attain the target concentrations listed above. Additionally, the primary (reference) calibrator is analyzed by a validated HPLC methodology. The reference material used by that method is ≥99% purity. According to the manufacturer's criteria, the HPLC results must be within +/- 10% of the target values. Instruments, programmed using nominal values, and calibrated with primary calibrators are used to test secondary calibrators. Secondary calibrators must then recover within +/- 10% for the average of the replicates measured, according to the manufacturer's acceptance criteria. Calibrators are labeled with nominal values.

The controls are labeled with the gravimetric target concentrations of lamotrigine for each level (2, 15 and 25 µg/mL). According to manufacturer's acceptance criteria, each lot of controls will have concentrations verified within ±15% of target concentration. The package insert instructs each laboratory to establish its own ranges for each new lot of controls according to the laboratory's Standard Operating Procedures.

Calibrator and control stability:

The manufacturer's acceptance criteria for stability of calibrators and controls include change in recovery, as measured by HPLC, < 10% relative to time 0. Real-time stability studies are performed on all 6 levels calibrator material stored at 2-8 degrees C, and opened at test intervals (6, 12, and 18 months). A minimum of six replicates are tested (duplicates on three different curves) at each time point, and the average of the replicates is determined. Additional manufacturer's acceptance criteria include a stable calibrator signal over the claimed stability time period.

d. Detection limit:

The LOQ of the assay is the lowest drug concentration for which acceptable performance is observed. Criteria were defined by the manufacturer as $\leq 20\%$ CV, with recovery $\pm 15\%$ of target concentration. Two studies were performed to determine the LOQ of the QMS® Lamotrigine Assay in both serum and plasma.

Serum:

The samples evaluated were prepared by pooling patient samples that had concentrations at the low end of the therapeutic range and diluting the pool with QMS® Lamotrigine calibrator A (zero calibrator) to five different concentrations. The concentration of the 100% sample was measured using the QMS® assay, and the target concentration for the remaining samples was then calculated using the dilution factor. Each sample was run in 2 replicates per run on two different Hitachi analyzers. There were five runs per analyzer, over five non-consecutive days, for a total of 20 replicates per analyzer (n=40). Results are shown below:

Tested Target µg/mL	1.74	2.75	3.78	4.88
AVG	1.82	2.68	3.64	4.88
SD	0.09	0.15	0.16	0.30
% CV	4.74%	5.69%	4.30%	6.25%
Target-Recovery (ug/mL)	4.48%	-2.73%	-3.76%	0.00%

Plasma:

Samples were prepared by spiking a concentrated plasma stock of lamotrigine into plasma negative for lamotrigine and diluting with plasma to various concentrations. The following is a summary of the plasma data pooled from two analyzers.

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Tested Target µg/mL	2.00	2.74	3.54
AVG	2.21	2.83	3.54
SD	0.07	0.10	0.10
% CV	3.21%	3.38%	2.89%
Target-Recovery	10.69%	3.27%	0.00%

These data support the manufacturer's claimed LOQ of 2.0 ug/mL.

e. Analytical specificity:

Evaluations of assay specificity were performed according to CLSI EP7-A.

The assay was evaluated for potential interference from the N-2 glucuronide, N-2 methyl, and N-2 oxide metabolites of lamotrigine. Metabolites of >99% purity were spiked into serum at concentrations ranging from 5-400 ug/mL. Samples were also spiked with lamotrigine at concentrations of approximately 3 and 16 ug/mL. Control samples contained the same concentration of lamotrigine, with no metabolite. Replicate sample measurements (using 2 calibration curves) were obtained, with total n=6 observations. The mean of replicates was used in calculations of cross-reactivity.

Minimal or no cross-reactivity was observed for the N-2 glucuronide and N-2 methyl metabolites. Cross-reactivity with the N-2 oxide is shown below.

N-2 Oxide in Low Lamotrigine Sample (µg/mL)							
	Control	5	10	20	40	80	400
Avg	2.60	3.58	3.89	4.52	5.01	6.26	11.50
SD	0.03	0.04	0.07	0.01	0.11	0.02	0.04
Da-Dt	N/A	0.98	1.30	1.93	2.42	3.67	8.91
% X-rxty	N/A	19.7	13.0	9.6	6.0	4.6	2.2
N-2 Oxide in High Lamotrigine Sample (µg/mL)							
	Control	5	10	20	40	80	400
% X-rxty	ND	92.5	36.5	25.1	15.1	10.7	4.2
N-2 Oxide Control in Neg Human Serum (µg/mL)							
		5	10	20	40	80	400
Avg		0.33	0.48	0.91	1.61	2.37	7.82
SD		0.02	0.01	0.02	0.03	0.04	0.06

Potential endogenous interferents were spiked into human serum containing 3 and 16 ug/mL lamotrigine. Samples were assayed (n=4) and the average of replicates was determined. A summary of results is shown below.

Substance (concentration tested)	Percent recovery in presence of low concentration lamotrigine	Percent recovery in presence of high concentration lamotrigine
Albumin (12g/dL)	-8.9	-0.8
Bilirubin (60 mg/dL)	-2.5	-4.8
Cholesterol (500 mg/dL)	0.5	0.4
Hemoglobin (1500 mg/dL)	-7.0	3.9
Gamma globulin (10 g/dL)	2.6	-3.2
Rheumatoid factor (500 IU/mL)	6.3	-2.4
Triglyceride (1500 mg/dL)	10.0	-3.2
Uric acid (20 mg/dL)	9.5	6.0

Commonly prescribed and OTC drugs were tested for interference in the presence of lamotrigine. The drugs tested, and their concentrations, were based on the manufacturer's investigations of commonly co-prescribed drugs with lamotrigine, as well as CLSI EP7 guidelines. The list is included in the package insert. No significant interference was observed (<5% differences in lamotrigine concentrations recovered) under the testing conditions.

f. Assay cut-off:

Not applicable

2. Comparison studies: (outliers, repeat measurements, graphs)

a. Method comparison with predicate device:

Results obtained with the QMS® Lamotrigine Assay were compared to those obtained with a well-validated HPLC reference method in clinical use. One hundred and sixty eight banked samples were evaluated. Samples were evaluated at 3 sites, including the manufacturer's site. The samples represented approximately 138 individuals (patients treated with lamotrigine). No specific criteria (in terms of patient demographics) were applied in sample selection. Samples were obtained from patients in the US and Europe. Both serum and plasma samples were included. Ten individual patient samples were supplemented with additional lamotrigine in order that the samples would span the entire assay range.

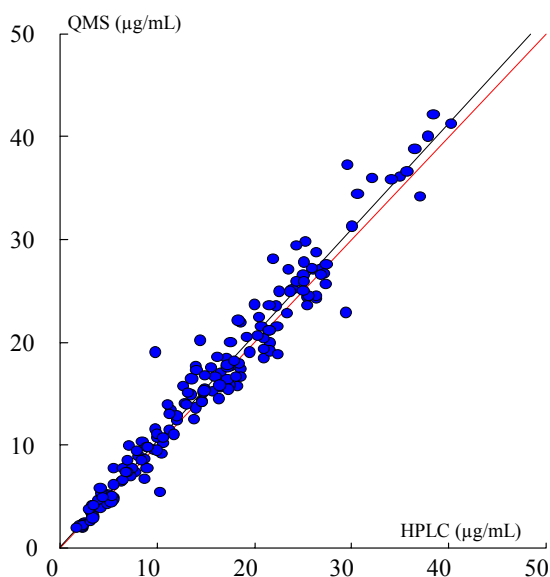
Samples were evaluated by the QMS® assay in accordance with the procedure described in the proposed package insert. The same reagent, calibrator, and control lots were used at all 3 sites. The table below summarizes results provided by the sponsor, based on Passing

Bablok analysis. Single measurements for each method were used in the analysis. The samples ranged in concentration on the Hitachi 917 from 2.02 µg/mL to 43.13 µg/mL, and by HPLC from 1.6 µg/mL to 40.3 µg/mL. No outliers were removed prior to analysis.

Study	Site performing QMS®	N	Slope (with 95% confidence intervals)	Y-Intercept (with 95% confidence intervals)	R	Standard error of estimate
1	Manufacturer	166	1.028 (95% confidence interval of 0.999 to 1.057)	0.112 (95% confidence interval of -0.212 to 0.508)	0.979	1.98
2	external site	166	1.026 (0.995 to 1.061)	0.358 (-0.028 to 0.545)	0.980	1.92
3	external site	167	1.055 (1.018 to 1.092)	-0.137 (-0.646 to 0.168)	0.969	2.44

An evaluation of the effect of the repeat measurements (from individuals) indicated that regression results were not significantly affected by the repeat measurements in this study.

The graph below illustrates representative results from one of the sites.



b. Matrix comparison:

Linearity, precision, and method comparison studies used both serum and plasma samples and similar performance was observed for both.

In addition, anticoagulant evaluations were conducted to determine sample recovery by the assay, as well as stability. Blood samples were drawn from normal donors in various collection tubes, including:

- Potassium EDTA - both K2 and K3
- Plain Serum – both glass and plastic
- Serum Separator (SST)
- Lithium Heparin
- Sodium Heparin
- Serum with Clot Activator
- Plasma Separator (PST)

Samples were spiked with lamotrigine and immediately analyzed as the Day 0 value. All samples up to day 14 refrigerated and day 42 frozen met the following manufacturer's criteria:

- >90% recovery when compared to Day 0
- ±10% recovery when compared to serum (baseline)

3. Clinical studies:

a. *Clinical Sensitivity:*

Not applicable. Not typically submitted for this type of assay.

b. *Clinical specificity:*

Not applicable. Not typical for this type of assay.

c. Other clinical supportive data (when a. and b. are not applicable):

The sponsor provided balanced and representative literature discussing measurement of lamotrigine for clinical use.

4. Clinical cut-off:

See expected values.

5. Expected values/Reference range:

A therapeutic range for lamotrigine has not been well established. Some reports in the literature suggest a target range for steady-state concentrations of 3 to 15 µg/mL. However, there is not a clear relationship between lamotrigine serum concentrations and clinical response and considerable overlap in lamotrigine concentrations has been observed between serum responders and non-responders as well as between serum levels associated with seizure control and adverse effects. In one study, the highest mean serum level (trough) reported was 8.8 µg/mL, and less than 15 % of patients reported an adverse event at serum concentrations less than 10 µg/mL. Mild to moderate adverse effects are more commonly associated with patients with lamotrigine concentrations above 13-15 µg/mL.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports substantial equivalence decision.