510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k111956

B. Purpose for Submission:

New Device

C. Measurand:

Voltage Gated Calcium Channel (VGCC) Antibody

D. Type of Test:

Semi-quantitative RIA Assay

E. Applicant:

KRONUS Market Development Associates, INC.

F. Proprietary and Established Names:

KRONUS Voltage Gated Calcium Channel (VGCC) Antibody RIA Assay Kit

G. Regulatory Information:

1. Regulation section:

   21 CFR § 866.5660, Multiple autoantibody immunological test system

2. Classification:

   Class II

3. Product code:

   PAF, Voltage gated calcium channel (VGCC) antibody assay

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use(s):

   The KRONUS Volgate Gated Calcium Channel (VGCC) Antibody RIA Assay Kit is for the semi-quantitative determination of antibodies to voltage gated calcium channels in human serum. The VGCC Antibody may be present in patients diagnosed with Lambert-Eaton Myasthenic Syndrome (LEMS). The assay result is not to be used alone and is to be used in conjunction with other clinical, electrodiagnostic and laboratory findings.

2. Indication(s) for use:

   Same as Intended use.

3. Special conditions for use statement(s):

   For prescription only.

4. Special instrument requirements:

   RIA Gamma Counter set for ^{125}I

I. Device Description:

The KRONUS VGCC Antibody RIA Assay is a radioimmunoassay in which VGCC autoantibodies, if present in a patient's serum, are allowed to interact with P/Q subtype voltage gated calcium channels labeled with ^{125}I- ω-conotoxin MVIIC. The VGCC and autoantibody complexes are precipitated with anti-human IgG and VGCC autoantibody levels are directly proportional to the radioactive counts. The assay kit is available in two formats: 12 tubes or 24 tube kits. The 12 tube kit includes 1 lyophilized vial each of VGCC labeled with ^{125}I-ω-conotoxin MVIIC; Total Binding; and Non-Specific Binding (lyophilized to be reconstituted with 0.7 mL distilled water). It also includes the following ready to use: individual vials: Positive control (150 uL), Negative control (200uL), Anti-human IgG (4 mL), incubation buffer (10 mL) and wash solution (120mL). The 24 tube kit includes 2 vials each of kit components.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   KRONUS AChRAB RIA Assay Kit

2. Predicate 510(k) number(s):
   k042248

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Trade name	Kronus Voltage Gated Calcium Channel (VGCC) Antibody RIA Assay Kit	Kronus I^{125} Acetylcholine Receptor Antibody Assay Kit
Method/Principle	RadioImmunoAssay (RIA)	Same
Sample Matrix	Human serum	Same
Controls	Positive and negative	Same
Assay Format	Semi-quantitative	Same
Solid phase	Tubes	Same
Precipitation Reagent	Anti-human IgG	Same
Shaker	Vortex mixer	Same
Signal	Radioactivity	Same
Detection equipment	Gamma counter	Same

Differences		
Item	Device	Predicate
Indication for Use	VGCC Ab may be present in patients diagnosed with LEMS. Assay result is not to be used alone and is to be used in conjunction with other clinical, electrodiagnostic and laboratory findings.	Aid in differential diagnosis of Myasthenia gravis
Intended Use	Measurement of VGCC antibody	Measurement of AchRAb antibody
Analyte	VGCC antibodies	AchRAb antibody
Test Platform	Autoantibodies to VGCC react with VGCC labeled with ^{125}I-ω-conotoxin MVIIC, are precipitated with anti-human IgG and concentration calculated from the radioactive counts.	Autoantibodies to AChR react with ^{125}I-labeled acetylcholine receptors, are precipitated with anti-human IgG and read off a calibration curve.
Incubation periods	Two 1-hour incubation	Two 2-hours incubation
Units of measure	pmol/L	nmol/L
Analytical sensitivity	2.86 pmol/L	0.26 nmol/L
Cut-off	Positive: >30 pmol/L Negative: ≤ 30 pmol/L	Positive: ≥0.5 nmol/L Equivocal: 0.21-0.49

Differences		
Item	Device	Predicate
		nmol/L Negative: ≤0.2 nmol/L

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

None

L. Test Principle:

Patient specimens and controls are incubated for one hour at room temperature with P/Q subtype voltage-gated calcium channel (VGCC) labeled with ^{125}I-ω-conotoxin MVIIC. The resulting bound complexes of labeled VGCC and autoantibody are then immunoprecipitated with anti-human IgG. Non-specific binding in the assay is determined using a preparation of VGCC which have been labeled with ^{125}I-ω-conotoxin in the presence of an excess of unlabeled conotoxin. After centrifugation, the supernatant is aspirated and the pellet containing labeled VGCC/autoantibody-bound complex is counted in a gamma counter. Counts are directly proportional to the amount of autoantibody present.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Intra-assay

The intra-assay precision was determined by testing four serum specimens 20-25 times. The serum specimens consisted of two high anti-VGCC specimen (144.6 and 62.1 pmol/L), additional one negative (25.5 pmol/L) and one close to the cut-off (31.1 pmol/L). Results showed %CV ranged from 6.9-15.5% (see table below).

Intra-assay Performance of VGCC Ab RIA Assay

Specimen	A	B	C	D
n	25	25	20	20
Mean (pmol/L)	144.6	62.1	25.5	31.0
SD	9.91	9.63	2.84	4.63
CV %	6.9	15.5	11.11	14.92

Inter-assay

The inter-assay precision was determined by testing four serum specimens in triplicate, once a day for ten days. Two additional specimens (5-6) were also tested in triplicate, once a day for eight days and then twice a day for two days. The serum specimens consisted of 4 specimens with high anti-VGCC concentrations (60.8-383.5 pmol/L), 2 specimens close to the cut-off (31.3 and 28.0 pmol/L), and the %CVs ranged from 9.3%-16.9% (see table below).

Inter-assay Performance of VGCC Ab RIA Assay

Specimen	1	2	3	4	5	6
N	20	20	20	20	10	10
Mean	142.4	60.8	383.5	66.4	31.3	28.0
SD	20.85	8.72	55.79	9.99	5.29	2.61
CV %	14.6	14.3	14.5	15.0	16.9	9.3

Lot to lot reproducibility:

Ten kit lots were tested using three specimens over a period of 80 weeks. The values of the three specimens were comparable between the ten lots and demonstrated reproducibility with %CV ranging from 9.6-14.6% CV.

b. Linearity/assay reportable range:

Assay reportable range:
The assay reportable range was established using the method described in "Verifying the reportable range of an analytical method in clinical chemistry" as follows: (P Marquis, Service de biochemie, Centre Hospitalier,Metz, France, http://www.multiqc.com/ReportableRange)

A LEMS patient serum (Pat 13) was diluted with dilution buffer to give 5 evenly spaced concentrations and assayed in the KRONUS VGCC Ab RIA. The kit positive control had a value of 150 pmol/L and in addition to the zero point provided 2 fixed points for the calculation. The kit positive control was given a tolerance interval of 33.3% (100 – 200 pmol/L). The tolerance polygon shows the reportable range to be 0 – 405 pmol/L.

Assay Linearity

The serum specimen with an initial concentration of 133 pmol/L, was diluted with kit negative control to give dilutions of 1/5, 1/7.5, 1/10, 1/15, and 1/20. The neat and diluted specimens were assayed using the VGCC Ab RIA kit protocol, including the kit negative and positive controls. The dilution data is provided below:

VGCC Ab RIA Linearity Data

Dilution	Concentration pmol/L
Neat	133
1/5	95
1/7.5	63
1/10	51
1/15	39
1/20	26

The Limitation section of the package insert includes the following Linearity statement: "The relationship between VGCC antibody concentration and CPM bound in the assay is only linear over a limited range. Furthermore, the linear range is different in different sera. In order to overcome this problem, it is possible to dilute antibody positive sera in normal human sera and assay several dilutions. This enables the linear range to be established for each individual patient serum. Antibody concentrations are then calculated using binding data from within the linear range.

Hook-effect

Three high serum specimens (from 322-445 pmol/L) were serially diluted and no hook effect was observed.

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

The VGCC control production is done according to approved standard operating procedures (purchased from approved supplier).

Shelf life

Data from 4 lots demonstrated the assay has a shelf life of 11 weeks. Testings were performed at 0, 5, 10, 11 and 12 week intervals.

d. *Detection limit:*

The limit of blank was computed to be 2.86 pmol/L from sequentially testing the negative control 20 times.

The limit of detection of 7.8 pmol/L was calculated from 25 determinations each from four healthy blood donor specimens (n = 100 total).

The Limit of Quantitation (LoQ), defined as the lowest level yielding an inter-assay CV not greater than 20%, is 19.5 pmol/L.

e. Analytical specificity:

Interference by endogenous substances: No interference was observed in 5 specimens (at 0.8, 9.2, 60, 147 and 318 pmol/L concentrations) spiked with hemoglobin up to 5 mg/dL, bilirubin up to 20 mg/dL; lipids at 1000 and 3000 mg/dL and ten Rheumatoid arthritis specimens (with concentration levels from 44-863 IU/mL.)

Crossreactivity with other autoantibodies: The KRONUS VGCC Ab RIA Assay Kit was tested with 36 sera on other auto immune diseases and conditions for LEMS differential diagnosis: 46 Graves Disease; 11 Hashimoto's Thyroiditis; 10 Rheumatoid arthritis; 10 Type 1 Diabetes 10 Systemic Lupus Erythematosus; 11 Addison's Disease; 32 Myasthenia gravis; 5 Polymyalgia rheumatica; 10 Chronic fatigue syndrome; 5 Neuropathy; 3 Polymyositis; 3 SCLC and 1 Guillain-Barré Syndrome. All specimens were negative with the KRONUS VGCC Ab RIA Assay Kit. The Limitations section of the package insert includes the LEMS differential diseases/conditions tested and not tested with VGCC Ab Kit.

f. Assay cut-off:

The assay cut-off for the KRONUS VGCC Ab RIA Assay Kit was determined empirically by testing specimens from 160 individual healthy blood donors. As healthy blood donors are expected to be negative for VGCC antibodies, the cutoff was set at 30 pmol/L, which was the highest concentration observed.

2. Comparison studies:

a. Method comparison with predicate device:

Refer to Clinical studies.

b. Matrix comparison:

Not applicable.

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

The clinical studies were evaluated on 207 clinically defined samples from patients. The 207 clinically defined samples had the following diagnosis: 50 patients with LEMS; 154 with other autoimmune diseases; 3 with Small Cell Lung Carcinoma. The 154 other autoimmune disease samples were from: 32 myasthenia gravis; 46 Graves Disease; 11 Hashimoto's Thyroiditis; 10 Rheumatoid arthritis; 10 Type 1 Diabetes 10 Systemic Lupus Erythematosus;

11 Addison's Disease; 5 Polymyalgia rheumatica; 10 Chronic fatigue syndrome; 5 Neuropathy; 3 Polymyositis; 1 Guillain-Barré Syndrome. The VGCC Ab RIA Assay Kit sensitivity and specificity were 100% (50/50) and 100% (157/157) respectively (refer to table below).

		Diagnosis		
		Positive (Target Disease: LEMS*)	Negative (Non-Target Diseases as listed above)	Totals
VGCC Ab RIA Assay Kit	Positive	50	0	50
	Negative	0	157	157
	Total	50	157	207

*LEMS is a rare disorder with a prevalence of 4 per million and approximately 400 known cases in the USA (per National Organization for Rare Disorders).

b. Other clinical supportive data (when a. is not applicable):

Not applicable.

4. Clinical cut-off**:**

Refer to assay cut-off.

5. Expected values/Reference range:

The expected value from 160 healthy individual blood donors was ≤30 pmol/L.

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 a substantial equivalence decision.