

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k080523

B. Purpose for Submission:

New device

C. Measurand:

Acetylcholine Receptor Blocking Auto-antibody

D. Type of Test:

Semi-quantitative, radioimmunoassay (RIA)

E. Applicant:

KRONUS® Market Development Associates, Inc.

F. Proprietary and Established Names:

KRONUS I¹²⁵ Acetylcholine Receptor Blocking Antibody Kit

G. Regulatory Information:

1. Regulation section:
866.5660 Multiple Autoantibodies Immunological Test System
2. Classification:
Class II
3. Product code:
NST, Autoantibodies, Acetylcholine Receptor, Acetylcholine Blocking and Non-Blocking.
4. Panel:
Immunology (82)

H. Intended Use:

1. Intended use(s):
The KRONUS Blocking AChRAb RIA Assay Kit is for the semi-quantitative determination of blocking antibodies to the acetylcholine receptor in human serum. The KRONUS Blocking AChRAb Assay is useful as an aid in the diagnosis of myasthenia gravis (MG).
2. Indication(s) for use:
Same as intended use
3. Special conditions for use statement(s):
The device is for prescription use only
4. Special instrument requirements:
Gamma counter for I¹²⁵

I. Device Description:

The KRONUS Blocking AChRAb RIA Assay Kit consists of the following components:

1. Blocking AChRAb Receptor, lyophilized.
2. Blocking AChRAb I¹²⁵ toxin, lyophilized.
3. Reconstitution buffer, ready-to-use.
4. Positive and Negative References, ready-to-use.
5. Non-specific binding (NSB) Reagent.
6. Concanavalin-A-Sepharose, ready-to-use.

7. Normal human serum, ready-to-use.
8. Wash buffer, ready-to-use.

J. Substantial Equivalence Information:

1. Predicate device name(s):
KRONUS® I¹²⁵ Acetylcholine Receptor Antibody (AChRAb) Assay Kit.
2. Predicate 510(k) number(s):
k042248
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Reference Classification Regulation	21 CFR 866.5660 – Multiple autoantibodies immunological test system	Same
Indication for Use	Aid in the diagnosis of Myasthenia gravis	Same
Test System Principle	Radioimmunoassay	Same
Test Matrix	Serum	Same
Detection Equipment	Gamma Counter	Same

Differences		
Item	Device	Predicate
Analyte	Blocking antibodies to the acetylcholine receptor	Binding antibodies to the acetylcholine receptor
Test Platform	Blocking AChR autoantibodies bind to acetylcholine receptors, inhibit the binding of radiolabeled α -bungarotoxin, are precipitated with Con A-sepharose and are detected as an inhibition of ¹²⁵ I α -bungarotoxin	Autoantibodies to AChR react with ¹²⁵ I-labeled acetylcholine receptors, are precipitated with anti-human IgG and read off a calibration curve.
Measuring range	0.5 to 1000 ng/mL	0.4 to 350 ng/mL
Sample size	2 μ L	58 μ L
Precipitation Reagent	Con A sepharose	Anti-human IgG
Stability: Open	7 days	112 cumulative hours

K. Standard/Guidance Documents referenced (if applicable):

Review Criteria for In Vitro Diagnostic Devices for the Assessment of Thyroid Autoantibodies Using Direct Immunofluorescence Assay (IFA), Indirect Hemagglutination (IHA), Radioimmunoassay (RIA) and Enzyme Linked Immunosorbent Assay ELISA) where appropriate.

L. Test Principle:

The test methodology is first of a kind to detect blocking auto-antibodies to acetylcholine receptors. Patient specimens and references are incubated for one hour at room temperature with detergent-solubilized fetal and adult human acetylcholine receptors (AChR). After that hour, I^{125} labeled α -bungarotoxin is added to the tubes, and any acetylcholine blocking antibodies in the sample compete with the I^{125} - α -bungarotoxin for a limited number of binding sites on the receptors. The resulting ACHR/blocking Ab or bungarotoxin complexes are then precipitated with Con-A sepharose. After centrifugation, the supernatant is aspirated and the pellet containing ACHR/blocking Ab or bungarotoxin complexes is counted in a gamma counter. Counts are inversely proportional to the amount of autoantibody present.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Intra-assay precision. Five serum samples containing a range of levels of blocking auto-antibodies to AChR were assayed between 9 and 25 times in the same run. The means, standard deviations and coefficients of variation were determined and are presented in the table below. Imprecision was less than 14.4 % CV and is acceptable for this type of assay.

Intra-Assay					
Sample	A	B	C	D	E
N	25	20	9	9	9
Mean (ng/mL)	53.9	27.8	26.2	17.3	14.9
SD (ng/mL)	2.1	4.01	1.32	0.86	1.15
% CV	3.9	14.4	5.03	4.96	7.72

Inter-assay precision. Five serum samples containing a range of levels of blocking auto-antibodies to AChR were assayed in 10-20 independent runs. The means, standard deviations and coefficients of variation were determined and are presented in the table below. Imprecision was less than 12.2 % CV and is acceptable for this type of assay.

Inter-Assay				
Sample	1	2	3	4
n	20	20	20	16
Mean (ng/mL)	62.2	56.0	15.8	25.0
SD (ng/mL)	1.6	1.7	1.6	3.1
% CV	2.5	3.0	9.8	12.2

Lab-to-Lab Reproducibility. Ten serum samples from diagnosed myasthenia gravis patients containing a range of levels of blocking auto-antibodies to AChR including 0 at two different laboratories using the KRONUS Blocking AChRAb RIA assay kit. The correlation between laboratories using these specimens was $r = 0.97$ indicating excellent between laboratory reproducibility.

Lot-to-Lot Reproducibility. Four dilutions of the positive reference sample provided in the KRONUS kit (70% inhibition to 9.5% inhibition) one positive serum sample from a diagnosed myasthenia gravis patient and two healthy blood donors were assayed using three kit lots. The undiluted positive reference was assayed using a further 16 kit lots. The %CVs for each sample ranged from 0.79 to 15.5% and was deemed by the company to be acceptable for this product.

b. *Linearity/assay reportable range:*

The sponsor states in the Limitations section of the package insert: “The relationship between acetylcholine receptor 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, some investigators 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 data from within the linear range. Normal human serum is provided in the kit so that this type of analysis can be made if required.” This was seen graphically in the high dose hook effect study. In the package insert the sponsor recommends using a cutoff of 20% inhibition to indicate positive results for blocking auto-antibodies, thus, the assay is actually qualitative and a linear range is not applicable to this assay. A reportable range is not declared in the package insert. The high dose hook effect study showed that the assay was not linear above approximately 55% inhibition for either sample studied. 100% inhibition would mean a patient sample blocked all acetylcholine receptors in the assay, which would mean the assay contains inadequate reagent to detect auto-antibodies in all patient samples. Since the indication for use is to aid in the diagnosis of myasthenia gravis, there is no clinical significance to the quantitative value of the test result.

High dose hook effect. Two serum samples from myasthenia gravis patients with high levels of blocking antibody were diluted in normal human serum to determine if there was any hook effect. The hook effect study showed that the assay was not linear above approximately 55% inhibition for either sample; but that there is no hook effect to give false results when the recommended cutoff is used to determine that the patient samples contain blocking antibody.

Recovery Study: A sample (A) positive for blocking antibodies to the acetylcholine receptor was diluted with 2 further samples (B & C) with varying levels of blocking acetylcholine receptor antibodies and measured in

the KRONUS Blocking AChRAb RIA Assay. Below is a table showing the expected (calculated) vs. the observed (measured) recovery for each of the dilutions. The recoveries ranged from 89% to 119% with a mean recovery across samples of 106%. Recovery is acceptable for a test of this type.

Samples	Observed % Inhibition	Expected % Inhibition	% Recovery
100% Sample A	53.21	-	-
20% Sample A + 80% Sample B	64.73	54.25	119
40% Sample A + 60% Sample B	65.77	55.29	119
60% Sample A + 40% Sample B	65.61	56.32	116
80% Sample A + 20% Sample B	63.69	57.36	111
100% Sample B	58.40	-	-

Samples	Observed % Inhibition	Expected % Inhibition	% Recovery
100% Sample A	49.8	-	-
20% Sample A + 80% Sample C	38.5	40.8	94
40% Sample A + 60% Sample C	29.0	31.8	91
60% Sample A + 40% Sample C	24.5	22.8	107
80% Sample A + 20% Sample C	12.3	13.8	89
100% Sample C	4.8	-	-

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

No reference material available.

d. *Detection limits:*

The Limit of Blank: The Limit of Blank (LOB) was 2.92% calculated as the mean of 19 determinations of the negative reference plus three standard deviations. The LOB, calculated with 70 normal samples, was 8.7%.

The Limit of Quantitation (defined as functional sensitivity): The Limit of Quantitation (LOQ), defined as the lowest level yielding an inter-assay CV not greater than 20%, of the KRONUS Blocking AChRAb RIA was determined from the inter-assay reproducibility study by plotting the %CV vs. %inhibition and was determined to be 13%. It is recommended that results below the LOQ of the assay be reported as 'less than 13%'.

e. *Analytical specificity:*

Interference Studies:

- i. *Hemoglobin:* Seven serum samples from patients with AChR blocking autoantibodies (11 – 75% Inhibition) and 3 serum samples from normal healthy blood donors (0% inhibition) spiked with 500 mg/dL hemoglobin were analyzed in the KRONUS Assay. The samples spiked with

hemoglobin were compared to the same sample without spiking. Acceptance criterion was $\pm 7\%$. Results showed no significant interference at the concentration tested. However, the Specimen Collection and Handling section of the package insert states that samples that are hemolyzed may interfere with assay performance.

- ii. *Bilirubin*: Seven serum samples from patients with AChR blocking autoantibodies (14.5 – 74% Inhibition) and 3 serum samples from normal healthy blood donors (0% inhibition) spiked with 20 mg/dL bilirubin were analyzed in the KRONUS Assay. The samples spiked with bilirubin were compared to the same sample without spiking. Percent recovery was calculated. Acceptance criterion was $\pm 23\%$. Results showed significant interference for two of the samples with low levels of blocking antibody present. The Specimen Collection and Handling section of the package insert states that samples that contain bilirubin may interfere with assay performance.
- iii. *Lipids*: Seven serum samples from patients with AChR blocking autoantibodies (18 – 78% Inhibition) and 3 serum samples from normal healthy blood donors spiked with 20% Intralipid, an oil-in-water emulsion manufactured by Fresenius Kabi (3000 mg/dL and 1000 mg/dL) were analyzed in the KRONUS Assay. The samples spiked with lipid were compared to the same sample without spiking. Acceptance criterion was $\pm 23\%$. Results with 3000 mg/dL showed significant interference for one of the samples with low levels of blocking antibody present. Results at 1000 mg/dL showed no significant interference. The package insert claims no interference seen at 1000 mg/dL, however, the Specimen Collection and Handling section of the package insert states that samples that contain lipid may interfere with assay performance.

f. *Assay cut-off*:

The package insert recommends that the cutoff for positive blocking antibodies be 20% inhibition. There does not seem to be any justification for this choice. No literature references were cited or discussed. Serum samples from 70 normal healthy blood donors gave a range of % inhibition of 0 – 14.6% inhibition. Samples from one person with diagnosed Myasthenia Gravis had 14% inhibition with low levels of binding antibodies. The choice of cutoff appears to be best guess between diagnosed patients and normal healthy blood donors.

2. Comparison studies:

- a. *Method comparison with predicate device*: A split sample method comparison study was performed using serum samples from 70 healthy blood donors and 52 patients with Myasthenia Gravis. The Binding AChRAb RIA (predicate) and Blocking AChRAb (new device) had a positive percent agreement (PPA) of 71%, a negative percent agreement (NPA) of 100% and an overall percent agreement of (OPA) 91%. The relatively low PPA is attributed to the fact that the new device detects a subpopulation of acetylcholine receptor antibodies.

		Predicate Device - Binding AChRAb RIA		
		+	-	Total
New Device-Blocking AChRAb RIA	+	27	0	27
	-	11	84	95
	Total	38	84	122

PPA: $27/38 = 71\%$ (95% C.I. = 56 to 84)

NPA: $84/84 = 100\%$ (95% C.I. = 97 to 100)

OPA: $111/122 = 91\%$ (95% C.I. = 85 to 95)

b. Matrix comparison:

Not applicable. Serum is the only recommended matrix.

a. Clinical studies:

- a. Clinical Sensitivity and Specificity.* To determine clinical sensitivity, serum specimens from fifty two (52) diagnosed myasthenia gravis patients were assayed in the KRONUS Blocking AChRAb RIA assay. Utilizing the cutoff of >20%, 27 (52%) were found to contain blocking antibodies to the acetylcholine receptor. Utilizing the cutoff of 0.5 nmol/L or greater, 38 samples were positive in the predicate KRONUS Binding AChRAb assay.

To determine clinical specificity, 70 healthy blood donors and 50 patients that had other autoimmune diseases (as listed in EXPECTED VALUES) were assayed in the KRONUS Blocking AChRAb RIA and the predicate KRONUS Binding AChRAb assay.

Results demonstrated that The KRONUS Blocking AChRAb has a percent clinical sensitivity, specificity and agreement of 52%, 100% & 85% respectively. The predicate KRONUS Binding AChRAb has a clinical sensitivity, specificity and agreement of 73%, 91% & 84%. The 95% confidence intervals were calculated using the binomial normal approximation interval method.

Blocking AChRAb				Binding AChRAb (predicate)			
RIA Result	Myasthenia Gravis			RIA Result	Myasthenia Gravis		
	Present	Absent	Total		Present	Absent	Total
Positive	27	0	27	Positive	38	6	44
Negative	25	120	145	Negative	14	64	78
Total	52	120	172	Total	52	70	122

Blocking AChRAb % Sensitivity: 52 (95% C.I. = 39 to 65)
 % Specificity: 100 (95% C.I. = 98 to 100)

Binding	% Sensitivity: 73	(95% C.I. = 60 to 84)
AChRab	% Specificity: 91	(95% C.I. = 83 to 97)

Since the new device detects only a subpopulation of acetylcholine receptor autoantibodies, it is expected that it will have different clinical sensitivity and specificity from the predicate device.

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

4. Clinical cut-off:

See assay cut-off.

5. Expected values/Reference range:

Sera from 70 individual healthy blood donors were assayed in the KRONUS Blocking AChRab RIA assay kit and gave a mean % inhibition of $1.1\% \pm 2.9\%$ (absolute range 0-14.6%). Individuals with other autoimmune diseases were assayed also and gave the results provided below reported as % inhibition.

Patient Group	Patients Positive for Blocking AChRab	
	Number	%
Myasthenia Gravis	27/52	52
Graves Disease	0/10	0
Hashimoto's Thyroiditis	0/5	0
Rheumatoid Arthritis	0/10	0
Type 1 Diabetes	0/13	0
Systemic Lupus Erythematosus	0/5	0
Addison's Disease	0/5	0
Lambert-Eaton Myasthenic Syndrome	0/2	0

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.