

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

A. 510(k) Number:

k051061

B. Purpose for Submission:

New device

C. Measurand:

Glutamic acid decarboxylase (GAD) autoantibodies

D. Type of Test:

Semi-quantitative, radioimmunoassay (RIA)

E. Applicant:

KRONUS Market Development Associates, Inc.

F. Proprietary and Established Names:

KRONUS Glutamic Acid Decarboxylase Antibody (GADAb) RIA Assay Kit

G. Regulatory Information:

1. Regulation section:

21 CFR § 866.5660, Multiple Autoantibodies Immunological Test System

2. Classification:

Class II

3. Product code:

NWG, Autoantibodies, glutamic acid decarboxylase (GAD), used for the semi-quantitative determination of glutamic acid decarboxylase (GAD) antibodies in human serum as an aid in the diagnosis of Type I diabetes mellitus (autoimmune mediated diabetes).

4. Panel:

Immunology 82

H. Intended Use:

1. Intended use(s):

The KRONUS GADAb RIA Assay Kit is for the semi-quantitative determination of glutamic acid decarboxylase antibody in human serum. The KRONUS GADAb RIA Assay is useful as an aid in the diagnosis of Type I diabetes mellitus (autoimmune mediated diabetes).

2. Indication(s) for use:

Same as the 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 GADAb RIA Assay Kit consists of:

1. Lyophilized I¹²⁵ GAD₆₅ Tracer (human recombinant).
2. Assay buffer
3. GAD antibody calibrators – ready to use
4. Protein A precipitating reagent

5. Two control sera: A. close to the cut-off, and B. moderate to high

J. Substantial Equivalence Information:

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

Similarities		
Item	Device	Predicate
	KRONUS Glutamic Acid Decarboxylase Antibody (GADAb) RIA Assay Kit	KRONUS Acetylcholine Receptor Antibody (AChRAb) Assay Kit
Intended Use	Semi-quantitative detection of autoantibodies	Same
Matrix	Serum	Same
Test principle	Radioimmunoassay	Same
Test platform	Antibodies bind to labeled antigen in liquid phase (test tube), the antigen-antibody complexes are precipitated and radioactivity measured.	Same
Detection instrument	Gamma counter	Same
Differences		
Item	Device	Predicate
Analyte	Anti-glutamic acid decarboxylase antibodies	Anti-acetylcholine receptor
Indications for Use	Aid in the diagnosis of Type I diabetes mellitus (autoimmune mediated diabetes)	Aid in the differential diagnosis of Myasthenia Gravis
Precipitating reagent	Protein A	Anti-human IgG

K. Standard/Guidance Document 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)”

L. Test Principle:

Calibrators, controls and patient samples are incubated for two hours with human recombinant I¹²⁵ GAD₆₅. During this incubation, antibody binds to the tracer. Protein A is added and the tubes are incubated for one hour during which time the antibodies present are bound by Protein A and removed from solution. Assay buffer is then

added and the tubes are centrifuged. After centrifugation, the supernatants are decanted or aspirated. The amount of radioactivity in the pellets is directly proportional to the amount of antibody contained in the samples. Calibrator concentrations are plotted on semi-log graph paper and the concentration of antibody in the unknowns is interpolated from the curve.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
 - a. *Precision/Reproducibility:*

Intra-assay

Two controls containing low and high levels of GAD antibodies were assayed in 25 replicates each. The mean, SD and coefficient of variation (%CV) were determined. For the low sample the mean was 6.4 U/mL and the %CV was 3.58%. For the high sample the mean was 42.66 U/mL and the CV % was 3.73%. In addition, data were submitted to demonstrate precision of the assay closer to the 1.0 U/mL cut-off by running 18 replicates of 2 samples with values of 2.6 and 1.2 U/mL.

Intra-Assay				
Sample	1	2	3	4
N	25	25	18	18
Mean	6.4	42.6	2.6	1.2
SD	0.23	1.59	0.1	0.1
%CV	3.6	3.7	3.7	6.7

Inter-assay

Two controls containing low and high levels of GAD antibodies were assayed in 25 independent runs. The mean, SD and coefficient of variation (%CV) were determined. For the low sample (mean = 6.1 U/mL) the %CV was 4.9% and for the high sample (mean = 42.9 U/mL) the %CV was 6.99%. KRONUS submitted data for 5 samples closer to the cut-off with values ranging from 1.2 to 37.5 U/mL.

Inter-Assay							
Sample	A	B	C	D	E	F	G
N	18	18	18	18	18	25	25
Mean	2.3	37.5	11.4	2.8	1.2	2.3	4.5
SD	0.2	3.5	1.1	0.2	0.1	0.17	0.21
%CV	10.8	9.7	9.4	6.2	8.1	7.4	4.7

- b. *Linearity/assay reportable range:*

Linearity

The measuring range for the assay is from 0 to 300 U/mL. Because each patient sample will have a different dilution curve due to the nature of autoantibody affinities and avidities, linearity is variable. Patient dilutions are not advised for samples with GAD antibody concentrations above 300 U/mL. The 300 U/mL calibrator represents the approximate maximum binding of the

tracer in the assay and allows for most samples to be read off the curve without need of dilution. Samples with results above the highest calibrator are reported as “>300 U/mL”.

Recovery

The calibrators were spiked with three serum samples of varying GAD antibody levels (5.2, 7.3 and 9.6 U/mL). Recoveries ranged from 83.6 to 117.6%.

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
There is no reference standard available and no claim was made for traceability.

- d. *Detection limit:*

Lower detection limit

The lower detection limit of the assay was determined by sequentially testing the zero calibrator 20 times. A calibration curve of %B/T (binding of tracer) versus concentration was constructed. The mean and standard deviation were calculated and the mean + 2 standard deviations (SD) and the mean + 3 SD were interpreted from the curve. The lower detection limit was computed to be 0.11 U/ml which is the mean of 20 determinations plus 2 SD. The cut-off for the assay is set at >1 U/mL for positive.

Functional sensitivity

The functional sensitivity (defined as the lowest level yielding an average inter-assay CV not greater than 20%) was determined to be 0.85 U/mL. KRONUS recommends that results below the functional sensitivity of the assay be reported as less than 0.85 U/mL.

- e. *Analytical specificity:*

Hemoglobin

Samples from patients positive for antibodies to GAD and normal healthy blood donors were spiked with hemoglobin levels up to 490 mg/dL and analyzed. Percent differences ranged from 0-12% for all positive samples.

Bilirubin

Samples from GAD antibody positive patients and healthy blood donors were spiked with 2 to 20 mg/dL of bilirubin and analyzed. With the exception of one sample (difference 24%), the % difference for all positive samples ranged from 0-17%. Other samples with similar results to the outlier showed % differences ranging from 2-8%.

Lipids

Samples positive for GAD antibodies as well as from healthy blood donors were spiked with lipid at approximately 3000 mg/dL and 1000 mg/dL.

Percent differences ranged from 0-17%

- f. *Assay cut-off:*

Fifty normal healthy controls with no family history of diabetes mellitus (DM) were assayed. There were 33 females and 17 males ranging in age from 20-61 years. Forty-eight samples (96%) contained less than 0.35 U/mL and all samples contained 0.5 U/mL of GAD antibodies or less. Given these results, values less than or equal to 1 U/mL are considered negative for GAD antibodies and values greater than 1 U/mL are considered positive.

In an additional study to validate the appropriateness of the assay cut-off, 100 human blood donor serum specimens were tested. Of the 100 tested, 91 (91%) yielded results indistinguishable from 0 U/mL, and 95% contained less than 0.35 U/mL GAD antibodies. Three sera gave values of 1.0 U/mL or greater and all three were reduced by incubation with unlabeled GAD suggesting they contained specific GAD antibodies.

2. Comparison studies:

a. *Method comparison with predicate device:*

Not applicable as there was no comparison to a predicate device. The new device claims were supported by clinical studies and published literature.

b. *Matrix comparison:*

Not applicable.

3. Clinical studies:

a. *Clinical Sensitivity:*

Sixty Type I diabetes serum samples (35 males aged 7 to 46 years and 25 females aged 10 to 35 years) were assayed. Using a cut-off of >1 U/mL, 40 (67%) were found to contain antibodies to GAD. In another study, KRONUS tested samples from an additional 93 confirmed Type I diabetes patients. Of the sera tested, 66 (71%) were positive for GAD antibodies

b. *Clinical specificity:*

KRONUS submitted data for 98 patients with non-diabetic diseases: 27 Graves' disease, 12 Hashimoto's thyroiditis, 20 Addison's disease, 19 myasthenia gravis, 10 SLE, and 10 rheumatoid arthritis patients. Combining results from these groups with 153 serum results from Type I diabetes subjects and 150 normal subjects, the following table was submitted:

	Target = Diabetes present	Non-target = Diabetes absent = Other autoimmunity	Normals
POSITIVE:			
	Study 1: 40/60 (67%) Study 2: 66/93 (71%)	Graves': 1/27 (4%) Hashimoto's: 0/12 (0%) Addison's: 0/20 (0%) Myasthenia Gravis: 0/19 (0%) SLE: 1/10 (10%) RA: 0/10 (0%)	Study A: 0/50 (0%) Study B: 2/100 (2%)
Total positive	106/153 (69%)	2/98 (2%)	2/150 (1%)
Combined	106/153 (69%)		4/248 (2%)
Negative:			
	Study 1: 20/60 (33%) Study 2: 27/93 (29%)	Graves': 26/27 (96%) Hashimoto's: 12/12 (100%) Addison's: 20/20 (100%) Myasthenia Gravis: 19/19 (100%) SLE: 9/10 (90%) RA: 10/10 (100%)	Study A: 50/50 (100%) Study B: 98/100 (98%)

	Target = Diabetes present	Non-target = Diabetes absent = Other autoimmunity	Normals
Total negative	47/153 (31%)	96/98 (98%)	148/150 (99%)
Combined	47/153 (31%)		244/248 (98%)

GADAb assay result	Diabetes present/absent		Total
	(+)	(-)	
(+)	106	4	110
(-)	47	244	291
Total	153	248	401

Clinical sensitivity: 69.3% (106/153) (95% CI; 62, 76)

Clinical specificity: 98.4% (244/248) (95% CI; 96, 100)

Overall agreement: 87.3% (350/401)

Published literature included in the submission showed a clinical sensitivity for GAD antibodies ranging from 64 to 83% and a clinical specificity ranging from 98-100%.

- c. Other clinical supportive data (when a. and b. are not applicable):*
Not applicable.
4. **Clinical cut-off:**
See assay cut-off.
 5. **Expected values/Reference range:**
The expected value for the normal population is ≤ 1 U/mL. However, 2-3% of the general population are positive for GAD antibodies.

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.