

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

A. 510(k) Number:

k072135

B. Purpose for Submission:

New device

C. Measurand:

Glutamic acid decarboxylase autoantibodies (GADA)

D. Type of Test:

Quantitative enzyme-linked immunosorbent assay (ELISA)

E. Applicant:

KRONUS Market Development Associates, Inc.

F. Proprietary and Established Names:

KRONUS Glutamic Acid Decarboxylase Antibody (GADAb) ELISA Assay Kit

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
NWG, Autoantibodies, glutamic acid decarboxylase (GADA)	Class II	21 CFR 866.5660 Multiple autoantibodies immunological test system	Immunology 82

H. Intended Use:

1. Intended use(s):

The KRONUS GADAb ELISA Assay Kit is for the semi-quantitative determination of glutamic acid decarboxylase antibody in human serum. The 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):

Prescription use only

4. Special instrument requirements:

ELISA plate reader, plate shaker or rotator

I. Device Description:

The device consists of: ELISA strip wells coated with GAD₆₅, GAD₆₅ biotin, streptavidin-peroxidase, positive and negative control materials, 6 levels of calibrators, GAD-biotin reconstitution buffer, streptavidin-peroxidase reconstitution buffer, wash solution, peroxidase substrate (TMB), and stop solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):

KRONUS Glutamic Acid Decarboxylase Antibody (GADAb) RIA

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

k051061

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	KRONUS Glutamic Acid Decarboxylase Antibody (GADAb) ELISA Assay	KRONUS Glutamic Acid Decarboxylase Antibody (GADAb) RIA Assay
Intended Use	Semi-quantitative detection of GAD autoantibodies	Semi-quantitative detection of GAD autoantibodies
Indications for Use	Aid in the diagnosis of Type I diabetes mellitus (autoimmune mediated diabetes)	Same
Matrix	Serum only	Same
Controls	Positive and negative	Same

Differences		
Item	Device	Predicate
Test principle	Enzyme-linked immunosorbent assay	Radioimmunoassay
Autoantibody capture	GADAb bind to GAD ₆₅ coated on solid phase	GADAb react with ¹²⁵ I-labeled GAD ₆₅ in liquid phase
Solid phase	Microtiter plate	Not applicable
Precipitating reagent	Not applicable	Protein A
Second capture antigen	GAD ₆₅ biotin	Not applicable
Enzyme/substrate	Streptavidin peroxidase/TMB	Not applicable
Detection instrument	ELISA plate reader (spectrophotometer)	Gamma counter
Calibrators	5 levels: 5, 18, 35, 120, 250 IU/mL	7 levels: 0, 1, 3, 10, 30, 120, 300 U/mL
Standardization	GAD Standard NIBSC 97/550	Not standardized against a reference material

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:

The new device assay depends on the ability of GAD autoantibodies to act divalently and form a bridge between GAD coated on ELISA plate wells and liquid phase GAD-biotin. The GAD-biotin bound is then quantitated by addition of streptavidin

peroxidase and a chromogenic substrate (TMB) with reading of final absorbance at 450nm. The absorbance of each well is directly proportional to the amount of antibody present. Calibrator values are plotted on semi-log graph paper and the antibody concentrations of the controls and patient samples are interpolated from the curve.

M. Performance Characteristics (if/when applicable):

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

Intra-assay

To assess intra-assay precision, 6 samples containing a range of GADAb levels were assayed in 21-26 replicates.

	Samples (IU/mL)					
	1	2	3	4	5	6
N	25	25	26	26	21	21
Mean	27.23	19.98	7.01	7.49	4.24	4.81
SD	7.12	1.70	0.25	0.23	0.69	0.19
%CV	7.32	8.49	3.57	3.12	16.2	4.0

Inter-assay

To assess inter-assay precision, 8 samples containing a range of GADAb levels were assayed in 15-20 runs.

	Samples (IU/mL)							
	1	2	3	4	5	6	7	8
N	20	20	15	20	20	20	20	20
Mean	96.9	21.0	7.9	8.1	17.4	8.8	3.41	5.74
SD	5.5	1.1	0.9	0.8	1.1	1.2	0.77	0.37
%CV	5.7	5.2	11.5	10.1	6.2	13.2	22.7	6.5

Lab-to-lab reproducibility

One hundred fifty samples (suspected Type I diabetics) were assayed at 2 different laboratories. The correlation between laboratories was $r = 0.970$ and the overall agreement for positive/negative was 98.7% (148/150).

- b. *Linearity/assay reportable range:*

Linearity

The measuring range for the assay is from 5 to 250 IU/mL. Each patient sample will have a different dilution curve due to the nature of autoantibodies, affinities and avidities. Linearity is variable and sample dilutions are not advisable. Three high positive samples were serially diluted and assayed. All diluted in a linear fashion. Results above the highest calibrator should be reported as >250 IU/mL.

Hook-effect

Three high samples were serially diluted in kit negative control. No hook effect was observed for samples up to 253.3 IU/mL (calculated result).

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
The GADAb ELISA calibrators are standardized against the international reference preparation NIBSC Islet Cell Antibody 97/550.

Stability data demonstrated the assay has a shelf-life of 9 months.

- d. *Detection limit:*

The lower detection limit of the assay was determined by sequentially testing the kit negative control 20 times. A calibration curve of absorbance (450nm) versus concentration was constructed. The mean and SD were calculated and the mean +2SD and +3SD were read off the calibration curve to give an IU/mL value. The lower detection limit was computed to be 0.06 IU/mL which is the mean of 20 replicates +2SD.

Functional sensitivity/Limit of Quantitation (LoQ)

The functional sensitivity (defined as the lowest level yielding an average inter-assay CV not greater than 20%) was determined. The means of the samples ran to establish inter-assay reproducibility were plotted against their %CVs and functional sensitivity was determined to be 4.0 IU/mL. KRONUS recommends that results below the functional sensitivity or LoQ of the assay be reported as less than 4.0 IU/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 500 mg/dL and analyzed. Percent differences ranged from 0.7-19.1% for all positive samples.

Bilirubin

Samples from GAD antibody positive patients and healthy blood donors were spiked with 20 mg/dL of bilirubin and analyzed. The % difference for all positive samples ranged from 0.9-11.1%.

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 0.2-16.1%

The package insert states that lipemic or grossly hemolyzed serum samples are not to be used.

- f. *Assay cut-off:*

Twenty samples from healthy blood donors were assayed. All samples contained 1.5 IU/mL or less of GAD autoantibodies. Given these results, values less than or equal to 5 IU/mL are considered negative for GAD antibodies and values greater than 5 IU/mL are considered positive. In an additional study to validate the appropriateness of the assay cut-off, 100 healthy male blood donor serum specimens were tested. Of the 100 tested, 98 (98%) yielded results less than or equal to 5 IU/mL.

2. Comparison studies:

a. *Method comparison with predicate device:*

Three hundred sixty seven samples were tested. This group included 99 Type I diabetics (60 newly diagnosed (35 males aged 7 to 46 years and 25 females aged 10 to 35 years) and 39 confirmed Type I diabetes patients); 68 patients suspected of having Type I diabetes mellitus; 40 Type II diabetics; 40 other autoimmune disease patients (20 Graves' disease, 10 Hashimoto's thyroiditis, and 10 rheumatoid arthritis); and 120 samples from healthy blood donors.

The comparison showed the following:

		Predicate device – GADAb RIA		
		+	-	Total
New device – GADAb ELISA	+	101	24	125
	-	3	239	242
	Total	104	263	367

Positive Percent Agreement: $(101/104) = 97.1\%$ (95% CI = 93-99)
 Negative Percent Agreement: $(239/263) = 90.9\%$ (95% CI = 87-94)
 Overall Agreement: $(340/367) = 92.6\%$ (95% CI = 90-95)

b. *Matrix comparison:*

Both assays use serum as the recommended matrix.

3. Clinical studies:

a. *Clinical Sensitivity and Specificity:*

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 >5 IU/mL, 44 (73%) were positive. In another study, KRONUS tested samples from an additional 39 confirmed Type I diabetes patients. Of the sera tested, all 39 (100%) were positive for GAD autoantibodies.

KRONUS also submitted data for 40 patients with non-diabetic diseases: 20 Graves' disease, 10 Hashimoto's thyroiditis, and 10 rheumatoid arthritis patients. Combining results from these groups with 99 serum results from Type I diabetes subjects and 120 normal subjects, the following table was submitted:

	Target = Diabetes present	Non-target = Diabetes absent = Other autoimmunity	Normals
POSITIVE:			
	Study 1: 44/60 (73%) Study 2: 39/39 (100%)	Graves': 0/20 (0%) Hashimoto's: 0/10 (0%) RA: 0/10 (0%)	Study A: 0/20 (0%) Study B: 1/100 (1%)
Total positive	83/99 (83.8%)	0/40 (0%)	1/120 (<1%)
Combined	83/99 (83.8%)		1/160 (<1%)

	Target = Diabetes present	Non-target = Diabetes absent = Other autoimmunity	Normals
NEGATIVE:			
:	Study 1: 16/60 (27%) Study 2: 0/39 (0%)	Graves': 20/20 (96%) Hashimoto's: 10/10 (100%) RA: 10/10 (100%)	Study A: 20/20 (100%) Study B: 99/100 (99%)
Total negative	16/99 (16%)	40/40 (100%)	119/120 (99%)
Combined	16/99 (16%)		159/160 (99%)

Calculating the clinical sensitivity and specificity showed the following:

		Type I Diabetes		Total
		Present	Absent	
GADAb assay result	+	83	1	84
	-	16	159	175
	Total	99	160	259

Clinical sensitivity: 83.8% (83/99) (95% CI: 76-90)

Clinical specificity: 99.4% (159/160) (95% CI: 97-100)

Overall agreement: 93.4% (242/259) (95% CI: 90-96)

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

b. 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 ≤ 5 IU/mL. However, some patients with other autoimmune diseases and 2-3% of the general population can be positive for GAD autoantibodies.

N. Proposed Labeling:

The labeling is sufficient and 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.