

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

A. 510(k) Number:

k070183

B. Purpose for Submission:

New device

C. Measurand:

Insulin autoantibodies (IAA)

D. Type of Test:

Semi-quantitative, radioimmunoassay (RIA)

E. Applicant:

KRONUS Market Development Associates, Inc.

F. Proprietary and Established Names:

KRONUS IAA 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:

OCN, Insulin autoantibodies (IAA)

4. Panel:

Immunology 82

H. Intended Use:

1. Intended use(s):

The KRONUS IAA RIA Assay Kit is for the semi-quantitative determination of antibodies to insulin in human serum. The KRONUS IAA RIA Assay is useful as an aid in the diagnosis of Type I diabetes mellitus (autoimmune mediated diabetes) in patients who have not received insulin therapy.

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 set for I¹²⁵ and a refrigerated centrifuge capable of 1500 x g

I. Device Description:

The KRONUS IAA RIA Assay Kit consists of:

1. Lyophilized I¹²⁵ Insulin Tracer (human recombinant).

2. Assay buffer

3. Insulin autoantibody calibrators: lyophilized, 0.0, 0.4, 1.0, 10, and 50 U/mL

4. Goat anti-human IgG (contains precipitation enhancer)

5. Control sera: Control I (low) and Control II (moderate)

J. Substantial Equivalence Information:

1. Predicate device name(s):
KRONUS Glutamic Acid Decarboxylase Antibody (GADAb) RIA Assay
2. Predicate 510(k) number(s):
k051061
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	Insulin Autoantibody (IAA) RIA Assay Kit	Glutamic Acid Decarboxylase Antibody (GADAb) RIA Assay Kit
Intended Use	Semi-quantitative detection of autoantibodies	Same
Indications for Use	Aid in the diagnosis of Type I diabetes mellitus (autoimmune mediated diabetes)	Aid in the diagnosis of Type I diabetes mellitus (autoimmune mediated diabetes) in patients who have not received insulin therapy
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 the reaction is measured	Same
Detection instrument	Gamma counter	Same
Antigen label	Iodine 125 (I^{125})	Same
Controls	2 levels	Same

Differences		
Item	Device	Predicate
	KRONUS Insulin Autoantibody (IAA) RIA Assay Kit	KRONUS Glutamic Acid Decarboxylase Antibody (GADAb) RIA Assay Kit
Analyte	Insulin autoantibodies	Glutamic acid decarboxylase antibodies
Precipitating reagent	Goat anti-human IgG with precipitation enhancer	Protein A

Differences		
Item	Device	Predicate
Calibrators	5 calibrators: 0.0, 0.4, 1.0, 10, and 50 U/mL	7 calibrators: 0.0, 1.0, 3.0, 10, 30, 120, and 300 U/mL
Initial incubation time	Overnight (16-24 hours)	2 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)”

L. Test Principle:

Calibrators, controls and patient samples are incubated overnight with human recombinant I¹²⁵ insulin. During this incubation, antibody binds to the tracer. Anti-human IgG is added and the tubes are incubated for one hour during which time the antibodies present are bound by anti-human IgG and removed from solution. Assay buffer is then added and the tubes are centrifuged. After centrifugation, the supernatants are decanted or aspirated. The resulting pellets are then washed and centrifuged an additional time followed by decanting or aspiration of the supernatants. The amount of radioactivity in the pellets is directly proportional to the amount of insulin autoantibody 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

Four samples (A-D) ranging from 1.29 to 35.53 U/mL of IAA were assayed in 20-21 replicates each. The mean, SD and coefficient of variation (%CV) were determined. For sample A, the %CV was 7.91%; for B, the %CV was 2.56%; the %CV was 5.05% for Control C; and for Control D, the %CV was 7.76%. In addition, data were submitted to demonstrate precision of the assay closer to the cut-off (0.4 U/mL) by running 27 replicates of 4 samples (E-H) with values of 0.47, 0.58, 0.69 and 0.67 U/mL. Percent CVs ranged from 8.97 to 14.04%.

Intra-assay								
Sample	A	B	C	D	E	F	G	H
N	20	20	21	21	27	27	27	27
Mean	1.29	35.53	18.05	6.48	0.47	0.58	0.69	0.67
SD	0.10	0.91	0.91	0.5	0.04	0.07	0.09	0.09
%CV	7.91	2.56	5.05	7.76	8.97	12.31	12.66	14.04

Inter-assay

Ten samples ranging from 0.45 to 17.46 U/mL of IAA were assayed in 10-14 replicates each. Several of the samples had values close to the assay cut-off. The

mean, SD and coefficient of variation (%CV) were determined. Percent CVs ranged from 9.09 to 16.45%.

Inter-assay										
Sample	1	2	3	4	5	6	7	8	9	10
N	14	14	14	14	14	14	10	10	10	10
Mean	17.46	0.97	6.41	0.50	0.48	0.45	0.58	0.58	0.68	0.66
SD	1.71	0.16	0.99	0.06	0.04	0.06	0.09	0.07	0.07	0.09
%CV	9.81	16.45	15.4	12.8	9.09	12.42	15.26	12.27	10.0	13.8

b. Linearity/assay reportable range:

Linearity

The measuring range for the assay is from 0.40 to 50 U/mL. As each patient sample will have a different dilution curve due to the nature of autoantibody affinities and avidities, linearity is variable. KRONUS makes no linearity claims and dilutions are not advised for patient samples with insulin autoantibody concentrations above 50 U/mL. The highest calibrator (50 U/mL) 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 >50 U/mL.

Recovery

Five serum samples of varying insulin autoantibody levels were diluted and measured in the assay. The recoveries ranged from 84.7% to 127% with a mean recovery across all samples of 108%. The studies showed varying results for low, moderate and high sample dilutions across all five samples. KRONUS included a description of problems relating to dilution of patient samples in the Dilution Recovery section of the labeling: Each patient sample will have a unique dilution curve as most patient samples positive for autoantibodies have a polyclonal mixture of antibodies with varying affinities and avidities.

Hook effect

Two serum samples with IAA values >50 U/mL were diluted in kit zero calibrator. No hook effect was observed.

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

There is no reference standard available for insulin autoantibodies and no claim was made for traceability.

Stability

Stability was established via both real-time at 2-8°C and accelerated stability studies at 37°C on various kit components as well as whole kit studies. Based on these studies a shelf-life of 8 weeks was established for the assay.

d. Detection limit:

Limit of Blank

The limit of blank for the assay was determined by sequentially testing a negative control 21 times. A calibration curve of %B/T (binding of tracer) versus concentration was constructed. The mean and standard deviation were calculated and the mean + 3 SD were interpreted from the curve. The limit of blank was computed to be 0.054 U/ml. The limit of detection was not

determined.

Functional sensitivity

The functional sensitivity (defined as the lowest level yielding an inter-assay CV not greater than 20%) was determined to be 0.4 U/mL. KRONUS recommends that results below the functional sensitivity of the assay be reported as less than 0.4 U/mL.

e. Analytical specificity:

Hemoglobin

Samples from patients positive for insulin autoantibodies and normal healthy blood donors were spiked with hemoglobin at a level of 500 mg/dL and then analyzed. Percent differences ranged from 0-14.3% for all positive samples.

Bilirubin

Samples from insulin autoantibody positive patients and healthy blood donors were spiked with 20 mg/dL of bilirubin and analyzed. The % differences for all positive samples ranged from 0-25%.

Lipids

Samples positive for insulin autoantibodies as well as from healthy blood donors were spiked with lipid at approximately 3000 mg/dL and 1000 mg/dL. Percent differences ranged from 0.4-16.7% and 0.0-33.3% respectively.

The user is instructed to avoid the use of grossly hemolyzed and lipemic samples.

Additional data were submitted for the sample closest to the assay cut-off. The low sample had a predetermined range of 0.7-1.6 U/mL and 3.2-4.1 %B/T. The %B/T for the 0.4 and 1.0 U/mL calibrators were included in the table below for comparison.

	Neat sample		Spiked sample		Calibrator %Bound	
	%Bound	U/mL	%Bound	U/mL	0.4 U/mL	1.0 U/mL
Hemoglobin 500 mg/dL	3.4	1.4	3.5	1.6	2.2	3.2
Bilirubin 20 mg/dL	4.1	1.0	3.8	0.8	2.6	4.1
Lipid 3000 mg/dL	3.4	0.8	3.4	0.7	2.3	3.6
Lipid 1000 mg/dL	3.3	0.8	3.2	0.6	2.3	3.6

f. Assay cut-off:

Fifty normal healthy controls with no family history of diabetes mellitus were assayed. There were 33 females and 17 males ranging in age from 20-61 years. All samples (100%) contained less than 0.4 U/mL. In addition, an assay of sera from 100 healthy blood donors showed 97 samples contained less than 0.4 U/mL. Of the 3 samples above 0.4 U/mL, 2 contained <0.6 U/mL and one gave a result of 6.0 U/mL. Given these results, values less than

or equal to 0.4 U/mL are considered negative for insulin autoantibodies and values greater than 0.4 U/mL are considered positive.

2. Comparison studies:

a. *Method comparison with predicate device:*

Samples from 150 healthy blood donors and 80 Type 1 diabetic patients were tested on both assays. The positive percent agreement (PPA) was 48%; the negative percent agreement (NPA) was 89%; and the overall agreement (OA) was 80%.

	KRONUS GAD RIA		
KRONUS IAA	+	-	Total
+	24	19	43
-	26	161	187
Total	50	180	230

PPA: $24/50 = 48\%$; NPA: $161/180 = 89\%$; and OA: $185/230 = 80\%$

There is a lower frequency of IAA in patients diagnosed with Type 1 diabetes in adulthood. When results for the disease positive patients for which age data are available (n=60) are ranked by age, the proportion of samples positive for GAD antibodies remains approximately equivalent (73% positive ages 7-18; 61 positive ages 19-46 years). The proportion of IAA positive samples shows a marked variation (82% positive ages 7-18; 33% positive 19-46 years). This is in agreement with literature furnished by KRONUS and may explain the 26 “false negative” results in the comparison.

b. *Matrix comparison:*

Not applicable because both assays use serum as matrix.

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. The samples were collected within 6 months of diagnosis. Using a cut-off of >0.4 U/mL, 25 (42%) were found to contain autoantibodies to insulin. In another study, KRONUS tested samples from an additional 20 confirmed Type I diabetes patients. Of the sera tested, 15 (75%) were positive for insulin autoantibodies. Combined this shows a clinical sensitivity of 50% (40/80). Published literature included in the submission showed clinical sensitivity for insulin autoantibodies in the target population ranging from 27 to 66%.

b. *Clinical specificity:*

KRONUS submitted data for 67 patients with non-diabetic diseases: 17 Graves' disease, 20 Hashimoto's thyroiditis, 10 SLE, 10 celiac disease, and 10 rheumatoid arthritis patients. All patient sera were negative for IAA.

Combining results from all groups the following results were obtained:

IAA assay result	Diabetes		Total
	+	-	
+	40	3	43
-	40	214	254
Total	80	217	297

Clinical sensitivity: 50% (40/80) (95% CI 39-61)

Clinical specificity: 98.6% (214/217) (95% CI 96-100)

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 <0.4 U/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 a substantial equivalence decision.