

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

A. 510(k) Number:

k073590

B. Purpose for Submission:

New device

C. Measurand:

Autoantibodies to IA-2 (insulinoma-associated protein 2), a tyrosine phosphatase-like protein

D. Type of Test:

Semi-quantitative, radioimmunoassay (RIA)

E. Applicant:

KRONUS Market Development Associates, Inc.

F. Proprietary and Established Names:

KRONUS IA-2 Autoantibody RIA Assay Kit

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
OIF: Tyrosine phosphatase (IA-2) autoantibody assay	Class II	21 CFR 866.5660 Multiple autoantibody immunological test system	Immunology (IM82)

H. Intended Use:

1. Intended use(s):

The KRONUS IA-2 Autoantibody RIA Assay Kit is for the semi-quantitative determination of antibodies to tyrosine phosphatase (IA-2) in human serum. The KRONUS IA-2 Autoantibody 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 radiation counter set for I¹²⁵ and a refrigerated centrifuge capable of 1500 x g

I. Device Description:

The KRONUS IA-2Ab RIA Assay Kit consists of:

1. Lyophilized I¹²⁵ IA-2 Tracer (human recombinant).
2. Assay buffer
3. IA-2 Autoantibody calibrators: lyophilized, 0.0, 0.75, 2.0, 10, and 50 U/mL
4. Protein A reagent
5. Control sera: Control A (low) and Control B (high)

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
	Tyrosine Phosphatase Autoantibody (IA-2Ab) RIA	Glutamic Acid Decarboxylase Autoantibody (GADAb) RIA
Intended Use	Semi-quantitative detection of autoantibodies	Same
Indications for Use	Aid in the diagnosis of Type I diabetes mellitus (autoimmune mediated diabetes)	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 the reaction is measured	Same
Detection instrument	Gamma counter	Same
Antigen label	Iodine 125 (I ¹²⁵)	Same
Calibrators	5 calibrators: 0.0, 0.75, 2.0, 10, and 50 U/mL	5 calibrators: 0.0, 0.4, 1.0, 10, and 50 U/mL
Precipitation reagent	Protein A	Same
Controls	2 levels	Same

Differences		
Item	Device	Predicate
	Tyrosine Phosphatase Autoantibody (IA-2Ab) RIA	KRONUS Glutamic Acid Decarboxylase Autoantibody (GADAb) RIA
Analyte	Tyrosine phosphatase autoantibodies	Glutamic acid decarboxylase autoantibodies

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

FDA guidance: *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 IA-2. 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 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 IA-2 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

Eight samples with values ranging from 0.98 to 27.6 U/mL of IA-2 autoantibody were assayed in 20 or 25 independent runs. Four of the samples had values close to the assay cut-off of 1.0 U/mL. Percent CVs ranged from 2.1 to 15.2%.

Intra-Assay								
Sample	A	B	C	D	E	F	G	H
N	25	25	25	25	25	25	20	20
Mean (U/mL)	0.98	1.72	2.61	6.4	15.1	27.6	1.91	1.33
SD	0.15	0.11	0.05	0.20	0.40	0.70	0.10	0.12
%CV	15.2	6.2	2.1	2.5	2.8	2.5	5.0	8.7

Inter-assay

Eight samples with values ranging from 0.50 to 25.83 U/mL of IA-2 autoantibody were assayed in 11-25 replicates each. Several of the samples had values close to the assay cut-off. The %CVs ranged from 3.30 to 25.28%.

Inter-Assay								
Sample	1	2	3	4	5	6	7	8
N	25	25	25	25	25	25	11	12
Mean (U/mL)	0.50	1.21	3.74	6.10	15.0	25.83	1.47	1.55
SD	0.13	0.26	0.15	0.20	0.80	1.34	0.22	0.16
%CV	25.28	21.63	3.94	3.30	5.30	5.18	15.2	10.1

Lot-to-Lot

Two control samples, one low and one high levels of IA-2Ab were assayed in 11 kit lots. The %CV for the samples ranged from 0.02 to 0.03%.

Lab-to-Lab

One-hundred fifty samples (suspected Type 1 diabetics) were assayed at two different laboratories. The correlation between laboratories was $r=0.991$. The overall agreement of positive and negative was 99.3% (149/150).

b. *Linearity/assay reportable range:*

Linearity

The measuring range for the assay is from 0.6 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 patient dilutions are not advised. 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 should be reported as >50 U/mL. Samples below 0.6 (Limit of Quantitation) should be reported as <0.6 U/mL.

Recovery

The IA-2 RIA calibrators were spiked with 3 serum samples of varying IA-2 autoantibody levels, were diluted and measured in the assay. The recoveries ranged from 110% to 157% with a mean recovery across all samples of 134%. 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: “As the dynamics of antibody-antigen interactions are affected by both antibody concentration and affinity each patient sample will have a different dilution profile and KRONUS does NOT recommend that patient samples are diluted for use in this assay.”

Hook effect

Four serum samples ranging from 7.5 to >50 U/mL were diluted in kit zero calibrator. No hook effect was observed.

Hook Effect							
1	U/mL	2	U/mL	3	U/mL	4	U/mL
Neat	7.5	Neat	22.8	Neat	36.6	Neat	>50
1:2	6.2	1:2	14.4	1:2	29.2	1:2	>50
1:4	4.9	1:4	8.5	1:4	22.5	1:4	29.3
1:8	3.9	1:8	5.1	1:8	15.9	1:8	13.2
1:16	2.9	1:16	3.2	1:16	10.6	1:16	7.0
1:32	2.1	1:32	2.1	1:32	7.3	1:32	3.9
1:64	1.4	1:64	1.3	1:64	5.3	1:64	2.2
1:128	0.8	1:128	0.7	1:128	3.7	1:128	1.0
1:256	0.6	1:256	0.5	1:256	2.5	1:256	0.5

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

There is no reference standard available for IA-2 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.

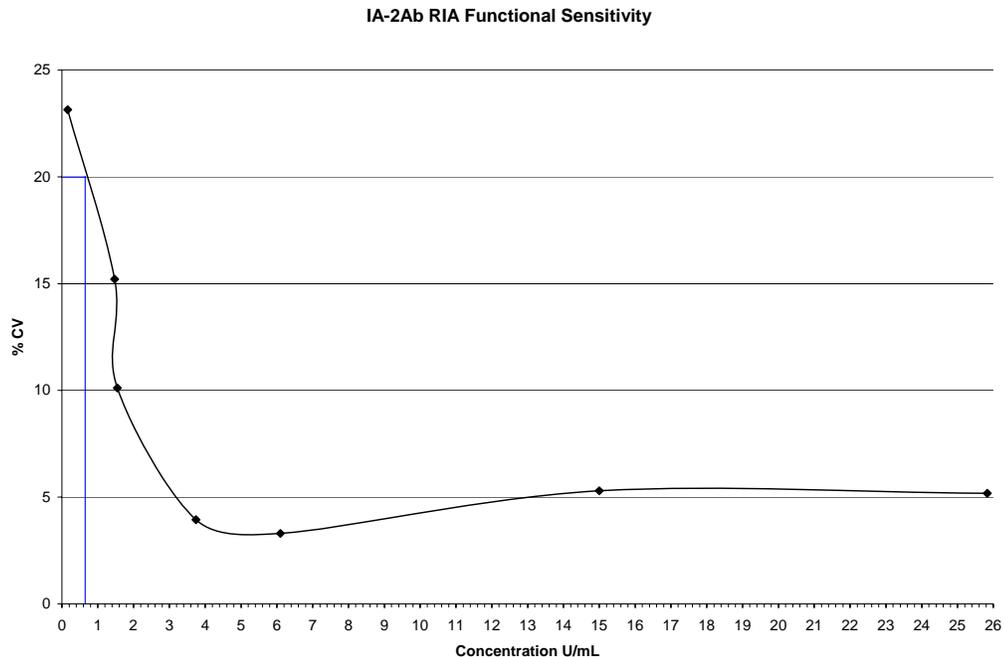
d. *Detection limit:*

Limit of Blank (LoB)

The LoD for 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 SD were interpreted from the curve. The limit of blank was computed to be 0.19 U/ml.

Limit of Quantitation (LoQ)

The LoQ (defined as the lowest level yielding an inter-assay %CV not greater than 20% using inter-assay precision) was established by plotting a graph of mean concentration versus %CV. The 7 samples tested ranged from 0.16-25.83 U/mL. The LoQ was determined to be 0.6 U/mL and KRONUS recommends that results below this value be reported as less than 0.6 U/mL.



e. *Analytical specificity:*

Fifteen samples were tested before and after the addition of the potentially interfering substance. This included 9 IA-2 autoantibody positive samples (1.7-20.9 U/mL) and 6 negative samples (≤ 0.53 U/mL).

Hemoglobin

Samples were spiked with hemoglobin at a level of 500 mg/dL and then analyzed. Percent differences ranged from -11.8 to 4.2% for all positive samples.

Bilirubin

Samples were spiked with 20 mg/dL of bilirubin and analyzed. The % differences for all positive samples ranged from -3.5 to 5.9%.

Lipids

Samples were spiked with lipid at approximately 3000 mg/dL and 1000

mg/dL. Percent differences for all positive samples ranged from -4.2 to 0.0% and -5.8 to 1.5%, respectively.

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

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 1.0 U/mL. In addition, sera from 113 healthy blood donors showed 112 samples contained less than 1.0 U/mL. One sample gave a result of 1.0 U/mL. Given these results, values less than or equal to 1.0 U/mL are considered negative for IA-2 autoantibodies and values greater than 1.0 U/mL are considered positive.

2. Comparison studies:

a. *Method comparison with predicate device:*

Samples from 50 healthy blood donors and 60 Type 1 diabetic patients were tested on both assays.

KRONUS IA-2Ab RIA	KRONUS GADAb RIA		
	+	-	Total
+	29	4	33
-	9	68	77
Total	38	72	110

Positive Percent Agreement: 76% (29/38) (95% CI = 62-88)

Negative Percent Agreement: 94% (68/72) (95% CI = 88-98)

Overall Agreement: 88% (97/110) (95% CI = 81-93)

b. *Matrix comparison:*

Not applicable because both assays use serum as matrix.

3. Clinical studies:

a. *Clinical Sensitivity and Specificity:*

The clinical studies included 795 subjects: Type 1 diabetes (n=277); Type 2 diabetes (n=204); healthy blood donors (n=163); and 151 patients with other types of autoimmune diseases.

Clinical Status	Number of Patients positive for IA-2Ab	% Positive
Graves' Disease	1/60	2%
Hashimoto's Thyroiditis	0/47	0%
Systemic Lupus Erythematosus	0/10	0%
Myasthenia Gravis	0/34	0%
healthy blood donors	1/163	1%
Type 2 Diabetes	5/204	2%
Type 1 Diabetes	136/277	49%

Clinical Sensitivity, Specificity and Agreement

IA-2Ab Assay	Type 1 Diabetes		Total
	+	-	
+	136	7	143
-	141	511	652
Total	277	518	795

Clinical sensitivity: 49% (136/277) (95% CI 43-55)

Clinical specificity: 98.6% (511/518) (95% CI 97-99)

Published literature included in the submission showed clinical sensitivity for IA-2 autoantibodies in the target population ranging from 25 to 89%.

- 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:
The expected value for the normal population is <1.0 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.