510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k171731

B. Purpose for Submission:

New device

C. Measurand:

Islet Antigen-2 (IA-2) Autoantibodies

D. Type of Test:

Quantitative enzyme-linked immunosorbent assay (ELISA)

E. Applicant:

KRONUS, Inc.

F. Proprietary and Established Names:

KRONUS IA-2 Autoantibody (IA-2Ab) ELISA Kit

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.5660 - Multiple Autoantibodies Immunological Test System

2. Classification:

   Class II

3. Product code:

   OIF - Tyrosine Phosphatase (IA-2) Autoantibody Assay

4. Panel:

   Immunology (IM82)

H. Intended Use:

1. Intended use(s):

   See Indications for use.

2. Indication(s) for use:

   The KRONUS IA-2 Autoantibody (IA-2Ab) ELISA Kit is for the quantitative determination of antibodies to Islet Antigen-2 (IA-2) in human serum. The KRONUS IA-2 Auto-antibody (IA-2Ab) ELISA Kit may be useful as an aid in the diagnosis of Type 1 diabetes mellitus (autoimmune mediated diabetes). The KRONUS IA-2 Autoantibody (IA-2Ab- ELISA Kit is not to be used alone and is to be used in conjunction with other clinical and laboratory findings.

3. Special conditions for use statement(s):

   Federal Law restricts this device to sale by or on the order of a licensed practitioner. The device is for use by laboratory professionals in a clinical laboratory setting.

4. Special instrument requirements:

   - Microtiter plate reader capable of measuring a 96 well plate at 450 nm. In this submission, a Bio-Tek ELx800 plate reader was used to generate all data.
   - ELISA plate shaker, capable of 500 shakes/min (not an orbital shaker)

I. Device Description:

The KRONUS IA-2Ab ELISA Kit consists of the following components:

1. rhIA-2-coated ELISA stripwells: The human recombinant Insulinoma Antigen-2 (rhIA-2) used in this kit is an intracellular fragment containing aa 605-979 and produced in an E. coli expression system.

2. Calibrators (5 concentrations): The kit calibrators are prepared by diluting rabbit serum, containing antibodies against human IA-2, in pooled IA-2 antibody-negative human serum containing sodium azide (0.5 mg/mL). Calibrators are supplied ready to use (5 x 0.7 mL) at concentrations of 0.75, 7.5, 35,120, 350 U/mL (NIBSC 97/550 Units)

3. Positive Control: The positive control is prepared by the same procedure as the calibrators and supplied ready to use.

4. Negative Control: The negative control is pooled normal human serum (negative for IA-2 antibodies) and supplied ready to use.

5. IA-2 Reaction Enhancer: The reaction enhancer contains Red Dye (E122), Polyethylene

Glycol 6000, Oxypyrion and NMethylisothiazolone in a Phosphate Buffered Saline solution.

6. rhIA-2 Biotin: IA-2 Biotin is prepared as described above and biotinylated using EZ-LinkTM Sulfo NHS-LCLC- Biotin (Life Technologies) using standard procedures. High purity rhIA-2 biotin is obtained by size exclusion chromatography.

7. IA-2 Biotin Reconstitution Buffer: The reconstitution buffer for IA-2-biotin contains Sodium Chloride, Di-Sodium Hydrogen Phosphate, Sodium Azide, Bovine Serum Albumin, Potassium Di-Hydrogen Phosphate, Potassium Chloride, Tween 20 and blue dye (E131) in HPLC grade water.

8. Streptavidin-Peroxidase (SA-POD): The streptavidin-peroxidase conjugate is purchased from Roche and then diluted in a manufacturer's formulation of Stabilzyme HRP Conjugate Stabilizer from Surmdodics.

9. Streptavidin-Peroxidase Diluent: Streptavidin-peroxidase conjugate diluent contains potassium chloride, sodium chloride, synperonic F68, Byco C, Chloroacetamide, N-methylisothiazolone, 0.5M phosphate buffer (pH 7.0) in HPLC grade water.

10. Tetramethylbenzidine Peroxidase Substrate (TMB): The peroxidase substrate is purchased from Neogen Corporation and is the manufacturer's formulation of tetramethylbenzidine.

11. Stop solution: 0.25M sulfuric acid

12. Concentrated wash solution: The concentrated wash solution contains sodium chloride, Tris buffer pH adjusted to 7.60 with hydrochloric acid and Tween 20 in HPLC grade water.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   KRONUS IA-2Ab RIA Assay Kit

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

   k073590

3. Comparison with predicate:

Similarities		
Item	KRONUS IA-2Ab ELISA Assay Kit (Candidate Device)	KRONUS IA-2Ab RIA Assay Kit (K073590) Predicate Device
Intended Use	Aid in the diagnosis of Type 1 diabetes mellitus (autoimmune mediated diabetes).	Same
Analyte	IA-2 Autoantibodies	Same
Test Matrix	Serum	Same

Differences		
Item	Device	Predicate
Test Platform	Autoantibodies to IA-2 bind to IA-2-biotin, are detected by a colorimetric reaction with streptavidin-peroxidase and tetramethyl benzidine and read off a calibration curve.	Autoantibodies to IA-2 react with 125I-labeled IA-2, are precipitated with Protein A, and read off a calibration curve
Test System Principle	ELISA	Radioimmunoassay
Detection Equipment	Plate Reader	Gamma Counter
Traceability	Traceable to NIBSC 97/550	No claim for tracability. This assay pre-dates the standard material.
Cut-off	7.5 U/mL	1U/mL
Measuring Range	5.9 -350 U/mL	0.6 -50 U/mL

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

- CLSI EP5-A3, Evaluation of Precision of Quantitative Measurement Procedures, "Third Edition"
- CLSI EP17-A2, "Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guidelines – 2nd Edition"
- CLSI EP-6A, "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline"
- CLSI EP28-A3c, "Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition"

L. Test Principle:

The KRONUS IA-2 Autoantibody (IA-2Ab) ELISA Kit depends on the ability of IA-2 autoantibodies to act divalently and form a bridge between IA-2 coated on ELISA plate wells

and liquid phase IA-2-biotin. The resulting antigen-antibody-antigen complexes are then quantitated by the addition of streptavidin peroxidase (SA-POD) and tetramethylbenzidine (TMB) to produce a colorogenic reaction. Stop solution is added to halt the reaction and absorbance is read using an ELISA plate reader. The absorbance of each well is directly proportional to the amount of antibody present. IA-2 antibody (Ab) levels are derived from a standard curve, traceable to the NIBSC 97/550 WHO reference standard, and expressed in U/mL.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Lot-to-Lot Reproducibility
All samples used in the lot to lot reproducibility study were patient samples diluted with pooled normal human serum. Eight samples ranging from 6.7 U/mL to 302.3 U/mL were tested in 18 different lots of the KRONUS IA-2Ab ELISA Kit. Results are summarized in the table below:

	Sample (U/mL)						
	1	2	3	4	5	6	7
Mean	21.6	42.8	59.6	101.6	180.1	6.7	302.3
SD	2.20	4.43	6.01	8.55	14.60	0.44	25.18
%CV	10.19	10.33	10.08	8.41	8.11	6.52	8.33
Number of Lots tested	17	18	18	18	18	18	18

Lab-to-Lab Reproducibility

Twenty seven samples (patient samples diluted with pooled normal human serum) were assayed at two different laboratories. The correlation between laboratories was:

$Y=0.94x+ 1.38$ $R^2 = 0.9829$

Within-Lab Reproducibility Study:

Variability of the KRONUS IA-2Ab ELISA Kit was assessed using a protocol based on the single site protocol described in CLSI EP5-A3. Five samples with IA-2 autoantibodies concentrations ranging in mean values of 5.5 – 281.6 U/mL were assayed with one kit lot, twice a day. Assays were performed by two operators using one plate reader. The study was conducted over the course of twenty days. Results of the analysis using the result from a single well per sample are summarized in the table below:

		Within-Run		Between-Run		Between-Day		Within-Device	
Sample	Mean (U/mL)	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	5.5	0.43	7.82	0.62	11.27	0.60	10.91	0.96	17.45
2	7.3	0.49	6.69	0.69	9.41	0.71	9.69	1.10	15.01
3	29.1	1.47	5.05	3.99	13.72	1.88	6.46	4.65	15.99
4	82.9	2.40	2.89	4.67	5.63	4.08	4.92	6.65	8.02
5	281.6	10.55	3.75	42.98	15.26	30.88	10.96	53.96	19.16

b. Linearity/assay reportable range:

Linearity and recovery characteristics of the KRONUS IA-2Ab ELISA Kit were evaluated according to the methods described in CLSI EP-6A, "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline". Kit calibrators and negative control material were pooled and used to prepare four dilution schemes ranging from 0.75 – 360 U/mL and samples were assayed in duplicate and assessed for linearity (Table 45). Values below the LoQ were not reported. Additionally, recovery rates were evaluated by comparing concertation values of each sample to its theoretical value. The results are summarized below:

Sample pool	Dilution Range (U/mL)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	% Recovery Range (Mean Recovery)
1	40.7 - 360	0.930 (0.843– 1.018)	-5.14 (-24.8– 14.50)	0.989	83.7– 96.8 (90.6)
2	10.7 - 100	0.919 (0.818– 1.021)	5.13 (-1.17– 11.43)	0.982	91.6– 121.9 (104.2)
3	5.7– 50	1.022 (0.939 - 1.105)	-1.20 (-3.80– 1.39)	0.990	81.1 – 104.1 (95.3)
4	5.6 - 25	0.986 (0.898 - 1.075)	-0.17 (-1.30– 1.63)	0.990	95.2– 106.6 (99.9)

This evaluation supports the claimed measuring of 5.4 to 350 U/mL for the IA-2Ab ELISA Kit

Hook Effect:

A patient sera sample containing high levels of IA-2 Ab (estimated at 14,075 U/mL), was sequentially diluted in negative human serum to a 1/512 dilution. No hook effect was observed for any of the dilutions of this sample.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
The calibrators for the KRONUS IA-2 Autoantibody (IA-2Ab) ELISA Kit are traceable to NIBSC 97/550 reference material. The reference material is used to assign values of the human master calibrators. The rabbit master calibrators are then prepared and the concentration of the secondary calibrators are read off the human master calibration curve and adjusted as required.

d. *Detection limit:*

Limit of Blank (LoB)

The KRONUS IA-2Ab ELISA Kit LoB was determined to be 1.3 U/mL, calculated as the 95th percentile from 30 determinations of 4 IA-2 Ab negative samples.

Limit of Detection (LoD)

The LoD was determined to be 2.0 U/mL, calculated from the pooled standard deviation of 28 replicates of 4 low IA-2 Ab samples.

Limit of Quantitation (LoQ)

LoQ was determined for the KRONUS IA-2Ab ELISA Kit using the method described in EP17-A2, "Evaluation of Detection Capability for Clinical Laboratory

Measurement Procedures; Approved Guidelines – 2nd Edition" Section 6, "Protocol for Evaluation of the Limit of Quantitation". The LoQ was calculated using data generated from the IA-2Ab LoD study and a method similar to the "Precision Profile Approach" from EP17-A2 to determine the assay's LoQ. Specifically, the LoQ was estimated for two separate kit lots. Each lot evaluation consisted of four low positive samples run in replicates over three days (n=60/kit lot). LoQ was defined as the concentration that met the precision goal of 20% CV. The LoQ was selected from the higher determination of the 2 lots and calculated to be 5.4 U/mL.

e. Analytical specificity:

Interference

Potential interferents were evaluated for their impact on the assay. Specifically, samples containing varying concentrations of IA-2 Ab (including one close to the cut-off value of 7.5 U/mL) were spiked with insulin (up to 10 mg/mL), 5 mg/mL hemoglobin, 20 mg/dL bilirubin, 14 µg/mL biotin or varying concentrations of Intralipid and compared to their unspiked reference. Compounds were determined, by the sponor, not to interfere in the assay if recovery rates did not deviate more than 20% from the reference value. Based on this criteria, no interference was observed from insulin, hemoglobin, bilibrubin or biotin.

Significant interference from Intralipid was observed, so an additional Intralipid dose response study was carried out to determine the concentration at which significant interference begins with the IA-2Ab ELISA Kit. It was determined that Intralipid interferes with the assay at concentrations >500 mg/dL. The sponsor includes the following warning in their labeling:

Visibly turbid samples will have interference in this assay.

f. Assay cut-off:

The cut-off for the assay is set to 7.5 U/mL.

2. Comparison studies:

a. Method comparison with predicate device:
Sera from pediatric (21 years of age or younger) and adult type 1 diabetic patients (T1DM), as well as patients with other auto-immune diseases (non-target samples, see details in clinical specificity section below) were tested using both the KRONUS IA-2Ab RIA Assay Kit and the KRONUS IA-2Ab ELISA Kit. Values obtained from the predicate device are arbitrary units/mL (u/mL) while the ELISA values are reported as Units/mL (U/mL) and based on the WHO NIBSC 97/550 reference standard, as such, RIA and ELISA values cannot be directly compared. Samples with a concentration ≥7.5 U/mL in the IA-2 Ab ELISA Kit are considered positive. Samples

with a concentration >1 u/mL in the IA-2 Ab RIA Assay Kit are considered positive.

Of the 118 Adult T1DM patient sera, 73 (61.9%) were positive by the RIA assay and 70 (59.3%) were positive by the ELISA assay. Method comparison agreement for this cohort resulted in a positive percent agreement (PPA) of 93.1% (68/73 samples) a negative percent agreement (NPA) of 95.5 % (44/45 samples) and an overall percent agreement (OPA) of 94.1% (111/118). Two RIA negative samples were positive in the ELISA and five ELISA negative samples resulted in positive RIA values.

Summary of results for adult T1DM samples evaluated in the candidate device and predicate device.

Adult T1DM Samples		**Predicate Device - RIA**		
Candidate Device - ELISA		+	-	Total
	+	68	2	70
	-	5	43	48
	Total	73	45	118

Of the 121 pediatric T1DM patient sera, 61 (50.4%) were positive in the RIA and 69 (57.0%) were positive in the ELISA. Method comparison agreement for this cohort resulted in a PPA of 95.1% (58/61 samples), an NPA of 81.7% (49/60 samples) and an OPA of 88.4% (107/121 samples). Eleven RIA negative samples were positive in the ELISA and three ELISA negative samples resulted in positive RIA values.

Summary of results for pediatric T1DM samples evaluated in the candidate device and predicate device.

Pediatric T1DM Samples		**Predicate Device - RIA**		
Candidate Device - ELISA		+	-	Total
	+	58	11	69
	-	3	49	52
	Total	61	60	121

Of the non-target samples, 18 (6.6%) were positive in the RIA and 14 (5.1%) were positive in the ELISA. Method comparison agreement (ELISA vs RIA) for this group of samples resulted in a PPA of 61.1% (11/18 samples), an NPA of 98.8% (252/255 samples) and an OPA of 96.3% (263/273samples).

Summary of results for non-target samples evaluated in the candidate device and predicate device.

Non-Target Samples		Predicate Device - RIA		
Candidate Device - ELISA		+	-	Total
	+	11	3	14
	-	7	252	259
	Total	18	255	273

Based on the results of all 239 samples, the IA-2Ab RIA and IA-2Ab ELISA have a PPA of 94.0% (126/134samples), an NPA of 87.6% (92/105 samples), and an overall percent agreement OPA of 91.2% (218/239 samples) for positive samples.

b. Matrix comparison:

Not Applicable.

3. Clinical studies:

a. Clinical Sensitivity:

As part of a clinical study to validate the performance of the KRONUS IA-2Ab ELISA Kit, serum samples were collected from a total of 239 individuals (both adults and pediatrics) previously diagnosed with Type 1 diabetes mellitus and evaluated using the KRONUS IA-2Ab ELISA Kit. (These samples were also used in the method comparison study).))Of the 121 pediatric T1DM patient sera, 69 (57.0%) (95% C.I.= 48.1% - 65.5%) were positive in the ELISA. Of 118 Adult T1DM patient sera, 70 (59.3%) (95% C.I.= 50.3% - 67.8%) were positive in the ELISA.

Utilizing the negative cut-off of <7.5 U/mL and based on the performance testing of Type 1 diabetes mellitus patient samples, Type 2 diabetes patient samples, and patients with other autoimmune diseases (not including patients with Latent Autoimmune Diabetes in Adults (LADA)), the KRONUS IA-2Ab ELISA Kit has a clinical sensitivity, specificity, and overall agreement of 58.2% (95% C.I. = 51.8% to 64.2%), 97.0% (95% C.I. = 94.2% to 98.5%), and 78.7% (95% C.I. = 74.9% to 82.0%) respectively.

The positive predictive and negative predictive values for this assay are 94.6% (95% C.I. = 89.6% – 97.2%) and 72.1% (95% C.I. = 67.3% – 76.5%) respectively.

b. Clinical specificity:

Sera from patients with various autoimmune disease states, including LADA and Type 2 diabetes were evaluated for cross-reactivity in the KRONUS IA-2Ab ELISA

Kit. Six of 210 patients (2.9%) with other autoimmune diseases were positive in the IA2-Ab ELISA. Furthermore, 10 of 45 (22.2%) LADA patients and 2 of 57 (3.5%) of patients diagnosed with Type 2 diabetes were found to be positive in the IA-2Ab ELISA.

Condition	Number of Samples Evaluated	Number of IA-2Ab ELISA Negative Results	Number of IA-2Ab ELISA Positive Results	IA-2Ab ELISA Percent Positive
LADA	45	36	10	22.2
Type 2 Diabetes	57	55	2	3.5
Rheumatoid Arthritis	20	19	1	5.0
Hashimoto's Thyroiditis	20	19	1	5.0
Grave's Disease	19	19	0	0.0
Celiac Disease	50	50	0	0.0
SLE	14	14	0	0.0
AChR Ab Positive	8	8	0	0.0
NMOSO	3	3	0	0.0
Metabolic Syndrome	35	34	1	2.9
Urinary Tract Infection	5	5	0	0.0
Kidney Disease	9	9	0	0
Addison's Disease	27	24	3	11.1

c. *Other clinical supportive data (when a. and b. are not applicable):*

Not applicable.

4. Clinical cut-off**:**

Not applicable.

5. Expected values/Reference range:

Samples from 718 healthy blood donors were evaluated using the KRONUS IA-2Ab ELISA Kit to establish the reference range for the assay. The blood donor samples were obtained from a population that was largely male and African American and included

women and subjects of other ethnicities.

The adult reference range was evaluated using the non-parametric calculations described in CLSI EP28-A3c. The lower limit of the reference range (equal to the 2.5th percentile) was below the LoQ of the device. The upper limit was based on the 701th ranked sample (equal to the 97.5th percentile) with a value of 4.6 U/mL (90% C.I. = <3.9 – 10.2 U/mL).

In addition, a total of 197 healthy pediatric blood donor samples were evaluated in the KRONUS IA- 2Ab ELISA Kit to establish a pediatric reference range. Samples were obtained from pediatric patients of both genders and a representative distribution of ethnicities. Samples were obtained from patients in the following age ranges:

Pediatric Reference Range Age Distribution		
	Number	Percent
<7 Years	16/197	8.08%
7-12 Years	67/197	33.84%
13-17 Years	38/197	19.19%
18-21 Years	76/197	38.89%

The pediatric reference range was evaluated using the non-parametric calculations described in CLSI EP28-A3c. The lower limit of the reference range (equal to the 2.5th percentile) was below the LoQ of the device. The upper limit was based on the 194th ranked sample (equal to the 97.5th percentile) with a value of 2.7 U/mL (90% C.I. = <2.4 - 7.8 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.