510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K220085

B Applicant

KRONUS, Inc.

C Proprietary and Established Names

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

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
OIF	Class II	21 CFR 866.5660 - Multiple Autoantibodies Immunological Test System	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

Modification to previously cleared device (k171731) to allow for qualitative determination of IA-2 antibodies in human serum in addition to the previously cleared quantitative measurement functionality. Modifications include the addition of calculated index values in the determination of qualitative results and associated labeling changes to include instructions for calculating and reporting results qualitatively.

B Measurand:

Islet Antigen-2 (IA-2) Autoantibodies

C Type of Test:

Quantitative enzyme-linked immunosorbent assay (ELISA)

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:
The KRONUS IA-2 Autoantibody (IA-2Ab) ELISA Kit is for the quantitative or qualitative determination of antibodies to Islet Antigen-2 (IA-2) in human serum. The KRONUS IA-2 Autoantibody (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.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D Special Instrument Requirements:
- Microtiter plate reader capable of measuring a 96 well plate at 450 nm
- ELISA plate shaker, capable of 500 shakes/min (not an orbital shaker)

IV Device/System Characteristics:

A Device Description:
The following components of the KRONUS IA-2 Autoantibody (IA-2Ab) ELISA Kit are unchanged from K171731:
- Human recombinant Insulinoma Antigen-2 (rhIA-2) -coated ELISA strip wells and rhIA-2 biotin,
- Five levels of ready to use calibrators at concentrations of 0.75, 7.5, 35,120, 350 U/mL IA-2 (NIBSC 97/550 Units),
- Positive and negative controls,
- IA-2 Reaction Enhancer,
- rhIA-2 Biotin,
- IA-2 Biotin Reconstitution Buffer,
- Streptavidin-Peroxidase (SA-POD),
- Streptavidin-Peroxidase Diluent,
- Tetramethylbenzidine Peroxidase Substrate (TMB),
- Stop solution,
- Concentrated wash solution.

B Principle of Operation:
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 quantitatively from a standard curve and expressed in U/mL or as IA-2 Ab qualitative levels that are derived as a ratio of the mean value of duplicate wells and the value of the 7.5 U/mL calibrator. The ratio is described as an index value as follows:

$$\text{Index Value} = \frac{\text{test sample absorbance at 450 nm}}{\text{7.5 U/mL calibrator at 450 nm}} \times 100$$

The qualitative results are assessed against an index value of 100 that is associated with the previously established quantitative cut-off of 7.5 U/mL calibrator and reported as "Negative", "Positive", or "Indeterminate" relative to the index value cut-off of 100. A specimen with an index value ≥100 is considered positive for antibodies against IA-2 while an index value <100 is considered negative for IA-2 antibodies.

V Substantial Equivalence Information:

A Predicate Device Name(s):
KRONUS IA-2 Autoantibody (IA-2Ab) ELISA Kit

B Predicate 510(k) Number(s):
K171731

C Comparison with Predicate(s):

Device & Predicate Device(s):	K220085	K171731
Device Trade Name	KRONUS IA-2 Autoantibody (IA-2Ab) ELISA Kit	Same
General Device Characteristic Similarities		
Intended Use/Indications For Use	Aid in the diagnosis of Type 1 diabetes mellitus (autoimmune mediated diabetes).	Same
Method/Principle	Enzyme-linked immunosorbent assay (ELISA)	Same
Analyte	IA-2 Autoantibodies	Same
Sample Matrix	Serum	Same
General Device Characteristic Differences		
Assay format	Qualitative	Quantitative
Cut-off	Positive: Index value ≥100 Negative: Index value < 100	Positive: ≥ 7.5 U/mL Negative: <7.5 U/mL

VI Standards/Guidance Documents Referenced:

CLSI EP12-A2-User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline - Second Edition.

CLSI GP44-A4 (Formerly H18-A4) - Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline—Fourth Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

The precision of the KRONUS IA-2Ab ELISA Kit was assessed using six serum samples with mean index values that cover the complete range of results (i.e., <100=negative, ≥100=positive). The samples were assayed twice a day for twenty days by two operators. The reproducibility data for all samples was analyzed to generate a summary of the qualitative agreement as shown in the table below:

Panel Member			Qualitative Agreement (N=40 per sample)
#	Mean Index Value	Sample Characterization	Number of Positive Test Results / N
1	79.1	Negative	0/40
2	98.2	Negative (near to cut-off)	19/40
3	109.7	Positive (near to cut-off)	39/40
4	302.0	Positive	40/40
5	1086.5	Positive	40/40
6	3420.0	Positive	40/40

2. Linearity:

Not applicable for the qualitative test. Linearity for the quantitative test was previously established in k171731.

3. Analytical Specificity/Interference:

Previously established in k171731.

4. Assay Reportable Range:

Not applicable for the qualitative test. The reportable range for the quantitative test was previously established in k171731.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Traceability: IA-2 Ab levels are calculated qualitatively using the 7.5 U/mL calibrator as a cut-off calibrator. The calibrators for the KRONUS IA-2 Autoantibody (IA-2Ab) ELISA Kit are traceable to NIBSC 97/550 reference material as in the predicate (K171731).

6. Detection Limit:

Not Applicable.

7. Assay Cut-Off:

The cut-off for the assay is set to an index value of 100 using the 7.5 U/mL calibrator as in the predicate (K171731). Index values ≥ 100 are considered positive and index values <100 are considered negative.

B Comparison Studies:

1. Method Comparison with Predicate Device:

A method comparison study was performed, comparing the results obtained from the KRONUS IA-2Ab ELISA Kit (qualitative assay) to the results from predicate device (k171731). Testing included sera from patients with type 1 diabetic patients (T1DM) and non-target samples (Type 2 diabetes (T2DM), Latent Autoimmune Diabetes in Adults (LADA) and other auto-immune diseases) using KRONUS IA-2Ab ELISA Kit (quantitative assay).

The Index values used in the qualitative method are calculated from the mean value of duplicate wells, using the 7.5 U/mL calibrator as the reference preparation and the results are reported as "Negative" or "Positive". Qualitative agreement between the two methods was determined to be 100%. Results are summarized in the table below:

		KRONUS IA-2Ab ELISA Kit (Quantitative assay)		
KRONUS IA-2Ab ELISA Kit (Qualitative assay)	Result	+	-	Total
	+ ≥ 100	168	0	168
	- ≤ 100	0	411	411
	Total	168	411	579

Based on the results, the IA-2Ab ELISA qualitative and quantitative calculation methods have a positive percent agreement of 100 x 168/168 = 100% (95% CI = 97.8 to 100), a negative % Agreement: 100 x 411/411 = 100% (95% CI = 99.1 to 100), and an overall % Agreement: 100 x 579/579 = 100% (95% CI = 99.3 to 100%).

2. Matrix Comparison:

Not Applicable. The assay is only intended for use with serum samples.

C Clinical Studies:

1. Clinical Sensitivity and Specificity:

The performance of KRONUS IA-2Ab ELISA Kit (Qualitative Assay) was compared to clinical diagnosis of T1DM using samples consisting of 239 T1DM (target disease); 57 T2DM; 45 LADA and 210 other autoimmune diseases. The Index values used in the qualitative method are calculated from the mean value of duplicate wells, using the 7.5 U/mL calibrator as the reference preparation and the results are reported as "Negative" or "Positive". Results are summarized in the table below:

Clinical sensitivity and specificity in this sample cohort are summarized in the following table:

		Diagnosis			
		Target (T1DM)	Non-Target (T2DM)	Non-Target (Autoimmune Disease)	Total
KRONUS IA-2Ab ELISA Kit	Positive ≥100	139	2	6	147
	Negative <100	100	55	204	359
	Total	239	57	210	506

Clinical sensitivity: 58.2% (139/239) 95% C.I. = 51.8% to 64.2%
Clinical specificity (T2DM): 97.0% (2/55) 95% C.I. = 94.2% to 98.5%
*Clinical specificity (Autoimmune Disease): 78.7% (6/210) 95% C.I. = 74.9% to 82.0%

**Results from patients diagnosed with Latent Autoimmune Diabetes in Adults (LADA) are not included in the analysis*.

The Positive Predictive & Negative Predictive Values for this assay are 94.6% (95% C.I. = 89.6% – 97.2%) & 72.1% (95% C.I. = 67.3% – 76.5%) respectively.

2. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not applicable.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

Previously established in k171731

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.