EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
KRONUS Zinc Transporter 8 Autoantibody ELISA Assay
DECISION SUMMARY

A. DEN:

DEN140001

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation for the KRONUS Zinc Transporter 8 Autoantibody (ZnT8Ab) ELISA Assay

C. Measurand:

Zinc Transporter 8 Autoantibody (ZnT8Ab)

D. Type of Test:

The KRONUS Zinc Transporter 8 Autoantibody (ZnT8Ab) ELISA Assay is for the semi-quantitative determination of autoantibodies to Zinc Transporter 8 (ZnT8) in human serum. The KRONUS Zinc Transporter 8 Autoantibody (ZnT8Ab) ELISA Assay may be useful as an aid in the diagnosis of Type 1 diabetes mellitus (autoimmune mediated diabetes).

E. Applicant:

KRONUS Market Development Associates, INC.

F. Proprietary and Established Names:

KRONUS Zinc Transporter 8 Autoantibody (ZnT8Ab) ELISA Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR § 866.5670

2. Classification:

   Class II

3. Product code:

   PHF

4. Panel:

   82-Immunology

H. Intended Use:

1. Intended use(s):

   The KRONUS Zinc Transporter 8 Autoantibody (ZnT8Ab) ELISA Assay is for the semi-quantitative determination of autoantibodies to Zinc Transporter 8 (ZnT8) in human serum. The KRONUS Zinc Transporter 8 Autoantibody (ZnT8Ab) ELISA Assay may be useful as an aid in the diagnosis of Type 1 diabetes mellitus (autoimmune mediated diabetes). The ZnT8Ab assay is not to be used alone and is to be used in conjunction with other clinical and laboratory findings.

2. Indication(s) for use:

   Same as Intended use.

3. Special conditions for use statement(s):

   For prescription use only in accordance with 21 CFR 801.109.

4. Special instrument requirements:

   ELISA Plate Reader suitable for 96-well format and capable of measuring at 450 nm, and ELISA plate shaker capable of 500 shakes/ minute.

I. Device Description:

The KRONUS Zinc Transporter 8 Autoantibody (ZnT8Ab) ELISA Assay contains the following: strip wells coated with ZnT8 (96 wells in total) and supplied as 12 strips of 8 wells in a frame and sealed in a foil bag with desiccant; ready-to-use 4 levels of calibrators (10, 20, 75, and 500 U/mL, 5x0.7 mL each); one each ready-to-use positive I, positive II and negative control serum (1x0.7 mL); Zinc T8 Biotin (lyophilized) 3x5.5 mL (reconstituted); ready-to-use reconstitution buffer for Zinc T8 Biotin 2x15 mL (colored red); Streptavidin Peroxidase (SA-POD dilute before use) 1x0.7 mL; ready-to-use diluent for SA-POD 1x15 mL; ready-to-use peroxidase substrate (TMB) 1x15 mL; concentrated wash solution (dilute with deionized water before use) 1x125 mL and ready-to-use stop solution 1x12 mL.

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

Not applicable

K. Test Principle:

The KRONUS Zinc Transporter 8 Autoantibody (ZnT8Ab) ELISA Assay depends on the ability of ZnT8 autoantibodies to act divalently and form a bridge between ZnT8 coated on the ELISA plate wells and liquid phase ZnT8- biotin. The ZnT8-biotin bound is then quantitated by addition of streptavidin peroxidase and a colorgenic substrate (TMB) with reading of the final absorbances at 450 nm. The absorbance of each vial is directly proportional to the amount of antibody present. Calibrator values are plotted on linear graph paper and the antibody concentrations of the controls and patient specimens are interpolated from the curve.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Intra-assay

The intra-assay precision was determined by testing nine serum specimens which included one close to the assay cut-off (15.4 U/mL); three low (6.9, 7.8, and 10.2 U/mL); one moderate (25.9 U/mL), and four high (66.3, 222.2, 441.5 and 471.5 U/mL). Twenty five (20-25) replicates of each sample were assayed in a single assay on a single day. Results showed %CVs ranged from 1.7-5.6% (see table below).

Sample	1	2	3	4	5	6	7	8	9
n	20	20	20	25	25	20	20	20	20
Mean (U/mL)	6.9	7.8	10.2	15.4	25.9	66.3	222.2	441.5	471.4
SD (U/mL)	0.2	0.4	0.4	0.5	1.2	1.7	11.8	7.6	13.5
% CV	3.3	5.6	4.2	3.3	4.5	2.5	5.3	1.7	2.9

Inter-assay

The inter-assay precision was determined by testing eleven samples: five serum specimens once a day for twenty days, two serum specimens once a day for 18 days and four sera for once a day for 10 days. The serum specimens consisted of two specimens with anti-ZnT8Ab concentration of 16.1 and 16.3 U/mL close to the assay 15 U/mL cut-off; four low levels (5.8, 7.2, 10.9 and 14.2 U/mL); one moderate level (28.7 U/mL) and three high levels (73.7, 250.3, 446.5 and 465.1 U/mL). The %CVs ranged from 2.9-17.7% (see table below).

Sample	1	2	3	4	5	6	7	8	9	10	11
n	18	18	10	10	20	10	10	20	20	20	20
Mean (U/mL)	5.8	7.2	10.9	16.1	28.7	73.7	250.3	14.2	16.3	446.5	465.1
SD (U/mL)	0.9	0.9	0.8	1.6	2.9	3.4	44.2	0.5	0.5	14.7	16.9
% CV	16.0	12.3	7.6	9.8	10.2	4.7	17.7	3.5	2.9	3.3	3.6

Lot-to-lot reproducibility:

Ten kit lots were tested using two control samples (48.4 and 156.3 U/mL) over a period of 96 weeks. The %CV for lot-to-lot reproducibility ranged from 7.6-9.2%.

Lab to Lab reproducibility:

Thirty-five samples (ranging from 0 to 1299 U/mL) and kit controls were assayed at two different laboratories. The correlation between laboratories was $R^2 = 0.984$ (y = 0.9199x +0.3183).

b. Linearity/assay reportable range:

Three positive samples were diluted (10 dilutions ranged from neat to 1:500). The observed values were graphed against the calculated values and a linear regression was performed. Results are summarized below:

Sample	Dilution range (U/mL)	Slope (95%CI)	Y- Intercept (95% CI)	R^2
A	75.4 – 485.3	0.9539	-59.55	0.9499
B	7.0 - 57.8	0.9556	- 0.5562	0.9783

The assay is linear for concentrations of 7.0 to 485 U/mL. *The Package Insert Calculation Section states: "For samples that result in values greater than the 500 U/mL (greater than the highest calibrator) KRONUS recommends reporting the value as "greater than 500 U/mL"*

High dose hook effect:

No hook effect was observed for ZnT8Ab concentration up to estimated 1980 U/mL.

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

There is no recognized reference standard available. The ZnT8 calibrator and controls (positive and negative) are prepared in-house and arbitrary units are assigned during the development process.

Stability

A real-time stability study was performed on three lots of ZnT8 Ab ELISA kit using 2 positive controls, a negative control and 11 samples (5 positive and 6 negative samples). Data support a shelf life of 9 months.

d. Detection limit:

The limit of blank (LoB) was determined by sequentially testing the negative control included with the kit sixty (60) times. A calibration curve of absorbance$_{450nm}$ vs. concentration was constructed. The mean and SD for the absorbance$_{450nm}$ of the negative control were calculated and the mean + 2 SDs was read off the calibration curve to give a U/mL value. The LoB was computed to be 2.1 U/mL.

The limit of detection (LoD) was determined by sequentially testing twenty five (25) replicates each of four healthy blood donor samples for a total of 100 samples. The LoD determination used a non-parametric statistical calculation. The 95th percentile corresponds to the 95.5 ordered observation of results placed in ascending order which yields a LoD estimate of 5.6 U/mL.

e. Analytical specificity:

Endogenous Interference

No significant interference was observed in 10 specimens (with ZnT8Ab levels ranging from 2.1 - 356 U/mL) spiked with hemoglobin (at 500 mg/dL), bilirubin (at 20 mg/dL) and Intralipids (at 1000 and 3000 mg/dL). The Package Insert 'Specimen Collection and Handling Section' states: "*Sera should be clear and grossly lipemic or hemolyzed samples should not be used*".

Cross-reactivity:

The KRONUS ZnT8Ab ELISA Assay Kit was tested with 246 sera from other autoimmune diseases and conditions. These samples comprised of 24 Graves' Disease, 9 Myasthenia gravis, 26 Rheumatoid arthritis, 23 Addison Disease, 3 Neuromyelitis optica, 24 Hashimoto's Disease; 60 Type 2 Diabetes mellitus; 10 Celiac Disease; 9 Systemic Lupus Erythematosus; 6 Testicular Cancer; 37 Metabolic syndrome; 10 Kidney Disease and 5 Urinary Tract Infection.

Two hundred forty one (241) of 246 specimens were negative with the KRONUS ZnT8Ab ELISA Assay Kit. The five positive sera were as follows: 1 Graves' Disease at 15.6 U/mL; 2 Addison Disease at 48.1 U/mL and 23.5 U/mL; 1 Type 2 Diabetes at >500 U/mL and 1 Kidney Disease at 65 U/mL.

Patient Group	No. of Samples Positive for ZnT8Ab	%
Graves' Disease	1/24	4
Hashimoto's Thyroiditis	0/24	0
Addison's Disease	2/23	9
Myasthenia Gravis	0/9	0
Neuromyelitis Optica	0/3	0
Type 2 Diabetes	1/60	2
Rheumatoid Arthritis	0/26	0
Celiac Disease	0/10	0
Systemic Lupus Erythematosus	0/9	0
Testicular Cancer	0/6	0
Metabolic Syndrome	0/37	0
Kidney Disease	1/10	10
Urinary Tract Infection	0/5	0

f. Assay cut-off:

The assay cut-off (greater than or equal to 15 U/mL is positive) for the KRONUS ZnT8Ab Assay Kit was determined by testing specimens from 397 US healthy blood donors (291 Black and 6 Caucasian males as well as 98 Black, 1 Hispanic and 1 Asian females). Ninety-nine percent (394/397) were negative for ZnT8Ab. The ZnT8Ab concentrations in the three positive samples were 19, 41 and 45 U/mL. The mean result was 1.90 U/mL with a SD of 3.84.

2. Comparison studies:

a. Method comparison with predicate device:

Refer to Clinical studies.

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

The clinical performance was evaluated on 569 clinically defined patient samples. These samples had the following diagnosis: 323 patients with Type 1 diabetes mellitus ("T1D" or "T1 DM"), 60 Type 2 diabetes mellitus ("T2 DM") and 186 other diseases (24 Graves' Disease, 9 Myasthenia Gravis, 26 Rheumatoid Arthritis, 23 Addison's Disease, 3 Neuromyelitis Optica, 24 Hashimoto's Disease; 10 Celiac Disease; 9 Systemic Lupus Erythematosus; 6 Testicular Cancer; 37 Metabolic syndrome; 10 Kidney Disease and 5 Urinary Tract Infection). The clinical sensitivity, specificity and overall agreement are summarized below:

		Diagnosis		
		Positive (TID)	Negative (Non-Target Diseases)	Totals
ZnT8Ab ELISA Assay Kit	Positive	220	5	225
	Negative	103	241	344
	Total	323	246	569

Sensitivity: 68 % (220/323) (95% C.I.: 63-73%)
Specificity: 98 % (241/246) (95% C.I.: 95-99%)
Overall Agreement: 81% (461/569) (95% C.I.: 76-85 %)

The rate of positivity was found to decrease with increasing age for the T1D population. Positivity rates based on age strata in the T1D population is shown below.

Age	n	# Positive	Positive (%)	Negative (%)
11-20	215	166	77	23
21-25	27	18	67	33
26-37	73	33	45	55
38+	8	3	38	62
Total	323	220	68	32

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

Not applicable.

4. Clinical cut-off**:**

Refer to assay cut-off.

5. Expected values/Reference range:

The expected value from 394 of the total 397 healthy individual blood donors was <15 U/mL (3 donors had >15 U/mL values).

M. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 801, 21 CFR Part 809, 21 CFR 801.109, and the special controls.

N. Identified Risks and Required Mitigations:

Identified Risks	**Required Mitigations**
Inaccurate test results that provide false positive or false negative results can lead to improper patient management.	Special controls (1), (2), and (3)
Failure to correctly interpret test results can lead to false positive or false negative results	Special controls (1) (iii), (2), and (3)

O. Benefit/Risk Analysis:

Summary	
Summary of the Benefit(s)	Patients with Type 1 diabetes mellitus ("T1D" or "T1DM") often have antibodies to pancreatic islet cell antigens. This assay represents the first to target antibodies to the zinc transporter, expressed in pancreatic islet cells. The assay can help in the diagnosis of T1D patients. In the clinical study, the assay demonstrated a clinical sensitivity of 68% and specificity of 98%. The study identified 11 of 323 T1D patients (3.4%) who were positive only for ZnT8Ab, and negative for other existing pancreatic autoantibodies, such as insulin antibodies (IAA), insulinoma-associated protein 2 autoantibodies (IA-2A), and glutamic acid decarboxylase autoantibodies (GADA). This highlights the clinical utility of adding ZnT8 to the existing islet cell autoantibodies to increase the capture of patients who potentially have T1D.
Summary of the Risk(s)	The risks are related to the consequences of decisions made based on false negative and false positive results due to inaccurate test results, or failure to correctly interpret test results. The combination of general and special controls would mitigate these risks. A false negative result could lead to a missed or delayed diagnosis of T1D, and a false positive result could lead to additional, unnecessary evaluation and testing. The false negative risk of 32% found in the clinical study is sufficiently mitigated by language in the Intended Use statement in the package insert (labeling) that the assay is not to be used alone and is to be used in conjunction with other clinical and laboratory findings. The false positive risk of 2% is extremely low.
Conclusions Do the probable benefits outweigh the probable risks?	The probable benefit of aiding in the increased detection of T1D patients outweighs the probable risk of a false negative assay result in 32% of patients with T1D and a false positive result in 2% of patients without T1D. The combination of general controls and special controls, including product labeling (package insert), adequately mitigates the risks posed by use of the assay. The addition of ZnT8Ab to the existing islet cell autoantibodies increases the capture of patients who potentially have T1D and outweighs risks of inaccurate test results, which are adequately described and sufficiently mitigated by statements in the Intended Use and Limitations sections of the package insert (product labeling).

P. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 866.5670 with special controls. FDA believes that special controls, along with the applicable general controls, provide reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code: PHF
Device Type: Zinc Transporter 8 Autoantibody immunological test system

Class: II (special controls)
Regulation: 21 CFR 866.5670

Identification. A Zinc Transporter 8 Autoantibody immunological test system is a device that consists of reagents used to measure, by immunochemical techniques, the autoantibodies in human serum samples that react with Zinc Transporter 8 (ZnT8). The measurements aid in the diagnosis of Type 1 diabetes mellitus (autoimmune mediated diabetes) in conjunction with other clinical and laboratory findings.

(a) *Classification*. Class II (special controls). Zinc Transporter 8 Autoantibody immunological test system must comply with the following special controls:

1) Premarket notification submissions must include the following information:
   i. A detailed description of the device that includes:
      A) A detailed description of all components in the test system, including a description of the assay components in the kit and all required ancillary reagents.
      B) A detailed description of instrumentation and equipment, and illustrations or photographs of non-standard equipment or methods if applicable.
      C) Detailed documentation of the device software, including, but not limited to, standalone software applications and hardware-based devices that incorporate software where applicable.
      D) A detailed description of appropriate internal and external quality controls that are recommended or provided. The description must identify those control elements that are incorporated into the recommended testing procedures.
      E) Detailed specifications for sample collection, processing and storage.
      F) A detailed description of methodology and assay procedure.
      G) Detailed specification of the criteria for test results interpretation and reporting.

   ii. Information that demonstrates the performance characteristics of the device, including:
      A) Device precision/reproducibility data generated from within-run, between-run, between-day, between-lot, between-operator, between-instruments, between-site, and total precision for multiple nonconsecutive days as applicable. A well characterized panel of patient samples or pools from the intended use population that covers the device measuring range must be used.
      B) Device linearity data generated from patient samples covering the assay measuring range if applicable.
      C) Information on traceability to a reference material and description of value assignment of calibrators and controls if applicable.
      D) Device analytical sensitivity data, including limit of blank, limit of detection and limit of quantitation if applicable.

E) Device analytical specificity data, including interference by endogenous and exogenous substances, as well as cross-reactivity with samples derived from patients with other autoimmune diseases or conditions.

F) Device instrument carryover data when applicable.

G) Device stability data including real-time stability under various storage times and temperatures.

H) Specimen stability data, including stability under various storage times, temperatures, freeze-thaw and transport conditions where appropriate.

I) Method comparison data generated by comparison of the results obtained with the device to those obtained with a legally marketed predicate device with similar indication of use. Patient samples from the intended use population covering the device measuring range must be used.

J) Specimen matrix comparison data if more than one specimen type or anticoagulant can be tested with the device. Samples used for comparison must be from patient samples covering the device measuring range.

K) A description of how the assay cut-off (the medical decision point between positive and negative) was established and validated as well as supporting data.

L) Clinical performance must be established by comparing data generated by testing samples from the intended use population and the differential diagnosis groups with the device to the clinical diagnostic standard. The diagnosis of Type 1 diabetes mellitus must be based on clinical history, physical examination, and laboratory tests, such as one or more pancreatic or insulin autoantibody test. Because the intended use population for Type 1 diabetes mellitus includes subjects less than 18 years old, samples from representative numbers of these subjects must be included. Representative numbers of samples from all age strata must be also be included. The differential diagnosis groups must include, but not be limited to, the following: Type 2 diabetes mellitus; metabolic syndrome; latent autoimmune diabetes in adults; other autoimmune diseases such as celiac disease (without a concomitant diagnosis of Type 1 diabetes mellitus), systemic lupus erythematosus, rheumatoid arthritis, and Hashimoto's thyroiditis; infection; renal disease; and testicular cancer. Diseases for the differential groups must be based on established diagnostic criteria and clinical evaluation. For all samples, the diagnostic clinical criteria and the demographic information must be collected and provided. The clinical validation results must demonstrate clinical sensitivity and clinical specificity for the test values based on the presence or absence of Type 1 diabetes mellitus. The data must be summarized in tabular format comparing the interpretation of results to the disease status.

M) Expected/ reference values generated by testing an adequate number of samples from apparently healthy normal individuals.

iii Identification of risk mitigation elements used by the device, including description of all additional procedures, methods, and practices incorporated into the directions for use that mitigate risks associated with testing.

2) Your 21 CFR 809.10(a) compliant label and 21 CFR 809.10(b) compliant labeling must include warnings relevant to the assay including:
   - *i.* A warning statement that reads "The device is for use by laboratory professionals in a clinical laboratory setting."
   - *ii.* A warning statement that reads "The test is not a stand-alone test but an adjunct to other clinical information. A diagnosis of Type 1 diabetes mellitus should not be made on a single test result. The clinical symptoms, results on physical examination, and laboratory tests (e.g., serological tests), when appropriate, should always be taken into account when considering the diagnosis of Type 1 diabetes mellitus and Type 2 diabetes mellitus."
   - *iii.* A warning statement that reads "Absence of Zinc T8 autoantibody does not rule out a diagnosis of Type 1 diabetes mellitus."
   - *iv.* A warning statement that reads "The assay has not been demonstrated to be effective for monitoring the stage of disease or its response to treatment."

3) Your 21 CFR 809.10(b) compliant labeling must include a description of the protocol and performance studies performed in accordance with special control (1)(ii) and a summary of the results.