510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k080159

B. Purpose for Submission:
New Device

C. Measurand:
Thyroid Stimulating Hormone Receptor (TSHR) Auto-antibody

D. Type of Test:
Semi-quantitative ELISA

E. Applicant:
KRONUS Market Development Associates, INC.

F. Proprietary and Established Names:
KRONUS TSH Receptor Autoantibody (TRAb) ELISA Assay

G. Regulatory Information:
1. Regulation section:
   21 CFR § 866.5870, Thyroid autoantibody immunological test system
2. Classification:
   Class II
3. Product codes:
   JZO, System, test, thyroid autoantibody
4. Panel:
   Immunology (82)

H. Intended Use:
1. Intended use(s):
   The KRONUS TRAb ELISA Assay Kit is for the semi-quantitative determination of antibodies to the thyroid stimulating hormone receptor in human serum. The KRONUS TRAb ELISA Assay is useful as an aid in the diagnosis of Graves' Disease 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 only.
4. Special instrument requirements:
   ELISA plate reader, plate shaker

I. Device Description:
The device consists of: ELISA strip wells coated with TSH Receptor (12x8), four levels calibrators (1, 2, 8, and 40 IU/L), M22 peroxidase, positive and negative controls, M22-peroxidase reconstitution buffer, start buffer, concentrated wash solution, peroxidase (TMB) substrate, and stop solution.

J. Substantial Equivalence Information:
1. Predicate device name(s):
   KRONUS TRAb Coated Tube (CT) RIA Assay
2. Predicate K number(s):

k032134
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Trade name	KRONUS TSH Receptor Autoantibody (TRAb) **ELISA** Assay	KRONUS TRAb Coated Tube **RIA** Assay
Intended Use	Measurement of TSH receptor autoantibody	Same
Indication for Use	Aid in differential diagnosis of Graves' Disease	Same
Analyte	TSH receptor autoantibodies	Same
Assay principle	TSH receptor immuno-inhibition	Same
Sample Matrix	Human serum	Same
Calibrator Levels	4 Levels (1, 2, 4, 40 U/L)	Same
Controls	Positive and negative	Same
Reference Standard Calibration	NIBSC 90/672	Same

Differences		
Item	Device	Predicate
Test principles	Enzyme-linked immunosorbent assay	Radioimmunoassay
Antibody capture	Microwell coated with $F(ab)_2$ fragment of mouse monoclonal anti-TSHR Ab bind to TSHR	^{125}I- labeled TSHR coated tube
Assay Format	Semi-quantitative	Qualitative and quantitative
Solid phase	Microtiter wells	Tubes
Shaker	Elisa plate shaker (500 shakes/ minute)	Orbital rotator
Enzyme/Substrate	TSH human monoclonal autoantibody M22 peroxidase/ TMB	N/A
Incubation periods	60-25-25 minutes	120-60 minutes
Signal	Optical density	Radioactivity
Detection instrument	Elisa microplate reader (spectrophotometer) set with 450 nm filter	Gamma counter set for ^{125}I
Units of measure	IU/L	U/L

Differences		
Item	Device	Predicate
Analytical sensitivity	0.06 IU/L	0.3 U/L
Cut-off	Positive: >1 IU/L Negative: ≤ 1 IU/L	Positive: >1.5 U/L (15% inhibition) Equivocal: 1.1-1.5 U/L (11-15% inhibition) Negative: ≤1.0 U/L (10% inhibition)

K. Standard/Guidance Document Referenced (if applicable):
None

L. Test Principle:
In the KRONUS TRAb ELISA, TSH receptor autoantibodies in patient sera, calibrators and controls are allowed to interact with TSH receptor that are bound to ELISA plate wells by . After 1 hour incubation, the samples are discarded leaving TRAb bound to the immobilized TSH receptor. A thyroid stimulating human monoclonal autoantibody conjugated to peroxidase (M22-peroxidase) is added in a second incubation step, where it interacts with immobilized TSH receptors, which have not been blocked by bound TRAb. The M22-peroxidase bound to the plate is then semi-quantitated by the addition of a colorgenic substrate, TMB, with reading of the final absorbance at 450 nm. A lower absorbance indicates the presence of TRAb in the test sample. Calibrator values are plotted on semi-log graph paper and the antibody concentrations of the controls and patient specimens are interpolated from the curve.

M. Performance Characteristics (if/when applicable):
1. Analytical performance:
   a. *Precision/Reproducibility:*
      Intra-assay
      The intra-assay precision was determined by testing six serum samples 15-22 times. The serum samples consisted of 2 high anti-TSHR samples (4.93 and 7.14 IU/L), 2 samples close to the cut-off (1.13 and 1.23 IU/L) and 1 negative sample (1.00 IU/L). Results showed %CV ranged from 3.1% to 11.7% (see table below).

Intra-assay Performance of TRAB Elisa

Sample	A	B	C	D	E	F
n	20	20	20	20	22	15
Mean (IU/L)	4.93	1.96	1.00	7.14	1.23	1.13
SD (IU/L)	0.15	0.14	0.08	0.28	0.15	0.09
CV %	3.1	7.2	7.7	3.9	11.7	8.1

Inter-assay
The inter-assay precision was determined by testing seven serum samples 9-25 independent runs. The serum samples consisted of 4 samples with high

anti-TSHR concentrations (3.51-18.57 IU/L), 2 samples close to the cut-off (1.89-1.99 IU/L) and 1 negative sample (1.04 IU/L). %CV ranged from 3.3% to 8.5% (see table below).

Inter-assay Performance of TRAB Elisa

Sample	1	2	3	4	5	6	7
N	20	20	25	25	10	9	10
Mean	4.56	18.57	3.51	5.38	1.04	1.99	1.89
SD	0.15	1.4	0.17	0.43	0.07	0.17	0.15
CV %	3.3	7.6	4.8	8	7.1	8.5	7.9

b. *Linearity/assay reportable range:*
Eight serum samples of varying levels of TSHR autoantibodies (from 6.94 to >40 IU/L) were serially diluted and assayed. All diluted in a linear fashion. Due to the nature of the autoantibodies, affinities, and avidities, linearity for each patient sample is variable; therefore, sample dilutions are not advisable. The measuring range for the assay is from 1-40 IU/L. Results above the highest calibrator should be reported as >40 IU/L.
Hook-effect
Three high serum samples (from 3.89 to >40 IU/L) were serially diluted and no hook effect was observed for samples up to >143.0 IU/L.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
The TSHR ELISA calibrators are standardized against the National Institute for Biological Standards and Control (NIBSC) 90/672 Thyroid Stimulating Hormone Antibody reference preparation.
Shelf life
Data demonstrated the assay has a shelf life of 8 months.

d. *Detection limit:*
The lower detection limit of the assay was determined by sequentially testing the negative control 20 times. A calibration curve of absorbance (450 nm) vs. concentration was constructed. The mean and standard deviation were calculated and the mean +2 SD, +3 SD and +10 SD were read off the calibration curve to give an IU/L value. The lower detection limit was computed to be 0.56 IU/L which is the mean of 20 determinations +10 SD.

e. *Analytical specificity:*
Interference by endogenous substances: No interference was observed in samples spiked with hemoglobin up to 50 mg/dL, bilirubin up to 20 mg/dL, lipids up to 3000 mg/dL, human chorionic gonadotropin up to 16.3 U/mL, follicle stimulating hormone up to 5 U/mL, luteinizing hormone up to 5 U/mL, and thyroid stimulating hormone up to 0.335 mU/mL.
HAMA interference study: Four blood donor samples containing human anti-mouse antibodies did not result in falsely elevated levels of TSHR antibodies with the assay. In addition, six different TRAb positive sera were spiked with three HAMA positive blood donor samples and no interferences were observed.
Crossreactivity with other autoantibodies: The TRAb ELISA kit was tested

with 36 sera on other auto immune diseases: 18 autoimmune hypothyroidism, 8 systemic lupus erythematosus (SLE) and 10 rheumatoid arthritis (RA). All samples were negative with the TRAb ELISA kit.

   f. Assay cut-off:
The assay cut-off was established by testing sera from 104 individual healthy blood donors (44 females) in the KRONUS TRAb ELISA. All 104 sera gave values of less than 1 IU/L.

2. Comparison studies:

   a. Method comparison with predicate device:
Testing was performed on 182 samples from 82 patients with Graves Disease, 39 non-autoimmune thyroid diseases, 17 autoimmune hypothyroidism, and 44 healthy blood donor samples. The Positive Percent Agreement was 100.0% (64/64); the Negative Percent Agreement was 95% (112/118) and the Overall Agreement was 97% (176/182). Refer to table below:

		Predicate device: TRAb CT RIA		
		Positive	Negative	Total
New deviceTRAb ELISA	Positive	64	6	70
	Negative	0	112	112
	Total	64	118	182

Positive Percent Agreement: (64/64) = 100%   (95% CI: 95-100)
Negative Percent Agreement: (112/118) = 95% (95% CI: 90-98)
Overall Agreement: (176/182) = 97%   (95% CI: 93-99)

   b. Matrix comparison:
Not applicable.

3. Clinical studies:

   a. Clinical Sensitivity and specificity:
The clinical sensitivity and specificity were evaluated on 261 clinically defined samples from patients with the following diagnosis: 82 Graves Disease, 75 disease controls (39 non-autoimmune thyroid diseases, 18 autoimmune hypothyroidism, 8 SLE, 10 RA, and 104 healthy individuals). The TRAb ELISA assay sensitivity and specificity were 85% (70/82) and 100% (179/179) respectively (refer to table below).

		Diagnosis		
		Positives (Graves Disease)	Negative (Disease Controls and Healthy Controls)	Totals
TRAb ELISA	Positive	70	0	70
	Negative	12	179	191
	Total	82	179	261

Sensitivity: (70/82) = 85%
Specificity: (179/179) = 100%

  b. Other clinical supportive data (when a. is not applicable):
    Not applicable.
 4. <u>Clinical cut-off</u>**:**
   Refer to assay cut-off.
 5. <u>Expected values/Reference range:</u>
   The expected value for normal population is ≤1 IU/L.

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.