

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

A. 510(k) Number:

k092229

B. Purpose for Submission:

Modification of k083391: number of replicates decreased from three to two

C. Measurand:

Thyroid Stimulating Hormone Receptor (TSHR) Autoantibodies

D. Type of Test:

Cell-based qualitative chemiluminescent assay

E. Applicant:

Diagnostic Hybrids, Inc.

F. Proprietary and Established Names:

Thyretain™ TSI Reporter BioAssay

G. Regulatory Information:

1. Regulation section:

21 CFR §866.5870, Thyroid autoantibody immunological test system

2. Classification:

Class II

3. Product code:

JZO, System, test, thyroid autoantibody

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

The Thyretain™ TSI Reporter BioAssay is intended for the qualitative detection in serum of thyroid stimulating autoantibodies to the thyroid stimulating hormone receptors (TSHRs) on the thyroid. The detection of these stimulating autoantibodies, in conjunction with other clinical and laboratory findings, may be useful as an aid in the differential diagnosis of patients with Graves' disease.

2. Indication(s) for use:

Same as Intended Use.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

Luminometer; Humidified, 5% CO₂, 35°C to 37°C incubator; Bio-safety cabinet Class II; -70°C or lower freezer or liquid nitrogen Dewar; Light microscope

I. Device Description:

The Thyretain™ TSI Reporter BioAssay consists of cryovials containing CHO Mc4 cells cryogenically preserved in cryoprotective medium containing DMSO (stored at ≤ -70°C); 100 mL bottle of Cell Attachment Solution; 100 mL growth medium (Hamm's F12 culture medium with 10% FBS); 500 mL reaction buffer; positive, reference and normal control set (0.5 mL vial each); and a luciferase reagent set (1

vial luciferase substrate and 10 mL vial luciferase assay buffer solution).

J. Substantial Equivalence Information:

1. Predicate device name(s):
KRONUS TSH Receptor Antibody (TRAb) Coated Tube (CT) Assay Kit
2. Predicate K number(s):
k032134
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	The Thyretain™ TSI Reporter BioAssay is intended for the qualitative detection in serum of thyroid stimulating autoantibodies to the thyroid stimulating hormone receptors (TSHRs) on the thyroid. The detection of these stimulating autoantibodies, in conjunction with other clinical and laboratory findings, may be useful as an aid in the differential diagnosis of patients with Graves' disease.	KRONUS TSDH Receptor Antibody (TRAb) Coated Tube (CT) Assay Kit is designed to measure human serum autoantibodies to the thyroid stimulating hormone (TSH or thyrotropin) receptor. The TRAb CT kit is useful as an aid in the differential diagnosis of Graves' disease.
Sample matrix	serum	same

Differences		
Item	Device	Predicate
Assay Format	Qualitative	Qualitative and quantitative
Assay Principle	Cell-based Chemiluminescent Assay	Radioreceptor assay
Solid Phase	CHO Mc4 cell monolayer (96-well microplate)	TSHR-coated tube
Analyte	Autoantibodies to Thyroid stimulating hormone (TSI-hyperthyroidism)	Autoantibodies to Thyroid stimulating hormone (TSI-hyperthyroidism) and thyroid blocking immunoglobulins (TBI-hypothyroidism)
Calibration	NISBC Standard 03/192 or similar standard	NIBSC Standard 90/672 or similar calibrator

Differences		
Item	Device	Predicate
Signal	Optical density	Radioactive particles
Detection method	Luminometer	Gamma counter set for ¹²⁵ I
Unit of measure	% SRR	U/L

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

Not applicable.

L. Test Principle:

In this assay, patient sera, reference control, positive and normal controls are added to the genetically engineered Chinese hamster ovary (CHO) cells which express chimeric form of the human TSHR and a cyclic adenosine monophosphate (cAMP) induced luciferase reporter gene. The cells are seeded and grown for 15-18 hours to a confluent monolayer in a 96-well plate prior to the addition of the samples. CHO cell monolayer is incubated with patient serum for 3 hours to allow the binding of TSI immunoglobulin to the chimeric human TSHR on the cell surface. This binding induces a signaling cascade resulting in increased production of intra-cellular cAMP which induces the production of luciferase. After incubation, the cells are lysed and luciferase levels are measured with a luminometer. A significant increase in luminescence over the Reference Control indicates the presence of TSI antibodies in the sample. Patient samples are tested in duplicate and averaged.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

A set of serum samples for testing was manufactured by diluting a single TSI positive sample into normal human serum that had been previously screened with the Thyretain bioassay. The sample set consisted of a sample that tests above 300%, one that tests near 200%, one that tests positive near 140%, one that tests near 100% (high normal) and one that tests near the limit of detection. Each sample was tested in three duplicate runs per plate on two assay plates per day for a total of 15 days:

Intra- and Inter-Assay Precision Results

Sample	High Positive	Mid Positive	Low Positive	High Normal	Normal
Intra-Assay					
N	3	3	3	3	3
Mean (%SRR)	362	181	147	106	77
SD	10	7	6	7	5
CV%	2.8	3.6	3.8	6.7	6.3
Intra-Day					

Sample	High Positive	Mid Positive	Low Positive	High Normal	Normal
N	6	6	6	6	6
Mean (%SRR)	361	175	145	102	71
SD	7	9	5	8	7
CV%	1.9	5.0	3.4	7.5	10.0
Inter-Day					
N	90	90	90	90	90
Mean (%SRR)	345	195	146	86	58
SD	31	17	10	12	11
CV%	8.9	8.6	6.8	14.6	19.2

Site-to-Site Reproducibility:

Site-to-site reproducibility was assessed at 3 trained sites (with 2 technicians at site 3) that performed 2 runs per day with each of four samples in duplicate over an eight day period. The total number of replicates for each sample is 180 (2x2x8x4 – 3x2x2x1). The data was summarized in the following table:

Proficiency Training Period Study Site Results							
Site 1		Site 2		Site 3 - Tech 1		Site 3 -Tech 2	
Mean %SRR	%CV	Mean %SRR	%CV	Mean %SRR	%CV	Mean %SRR	%CV
<u>Sample A</u>		<u>Sample A</u>		<u>Sample A</u>		<u>Sample A</u>	
273%	11.7%	442%	10.8%	282%	13.4%	291%	10.6%
<u>Sample B</u>		<u>Sample B</u>		<u>Sample B</u>		<u>Sample B</u>	
294%	14.5%	492%	14.8%	374%	14.5%	344%	12.1%
<u>Sample C</u>		<u>Sample C</u>		<u>Sample C</u>		<u>Sample C</u>	
48%	14.2%	69%	15.6%	44%	20.8%	48%	22.6%
<u>Sample D</u>		<u>Sample D</u>		<u>Sample D</u>		<u>Sample D</u>	
158%	10.7%	199%	10.9%	145%	14.4%	143%	12.2%

All Study Sites performed the study using manufactured panel samples. Each site's data was analyzed cumulatively to determine the reproducibility of the panel samples. Samples A and B both had a positive ratio (Number Positive/Total Number Tested) of 180/180, Sample C had a negative ratio (Number Negative/Total Number Tested) of 180/180 and Sample D had a positive ratio (Number Positive/Total Number Tested) of 140/180. The overall co-efficient of variation (%CV) for Samples A, B, C and D were 23.6%, 23.5%, 25.4% and 17.9% respectively.

An additional smaller study was performed using three samples near the cut-off was performed at two sites twice a day for five-days:

Reproducibility			
Study Site 2		Diagnostic Hybrids, Inc.	
Mean %SRR	%CV	Mean %SRR	%CV
<u>Specimen E</u>		<u>Specimen E</u>	
191%	9.4%	172%	18.0%
<u>Specimen F</u>		<u>Specimen F</u>	
104%	20.3%	107%	21.4%
<u>Specimen G</u>		<u>Specimen G</u>	
100%	21.7%	108%	20.0%

Each site's data were analyzed cumulatively to determine the reproducibility of the panel samples when duplicate wells 1 and 2 were used. Sample E had a positive ratio (Number Positive/Total Number Tested) of 55/60 and Samples F and G had negative ratios (Number Negative/Total Number Tested) of 55/60 and 56/60, respectively. The overall co-efficient of variation (CV) % for Samples E, F and G were 14.8%, 20.7%, and 21.0%, respectively.

Performance of the assay around the assay cut-off of 140% using duplicate samples rather than triplicate samples was assessed by testing 20 samples within $\pm 25\%$ of the cut-off (10 above, 10 below) in quintuplicate. The first three readings were averaged to replicate the original assay format results while the fourth and fifth replicates were averaged to replicate the proposed assay format. The difference between triplicate and duplicate results ranged from 2 – 13%; the %CV between all five replicates ranged from 1.2% to 6.4%. Nineteen (19) of the 20 specimens (95%) had positive and negative agreement between the two methods of calculation.

- b. *Linearity/assay reportable range:*
Not applicable.
 - c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
The traceability and stability of this assay were discussed in k083391.
 - d. *Detection limit:*
The detection limit of this assay was determined in k083391.
 - e. *Analytical specificity:*
The analytical specificity of this assay was determined in k083391.
 - f. *Assay cut-off:*
The assay cut-off of this assay was determined in k083391. The patient serum is considered positive for the presence of TSI if the SRR% measured $\geq 140\%$ over the Reference Control.
2. Comparison studies:
- a. *Method comparison with predicate device:*
Because of the complexity of the studies, the data from the original method comparison studies in k083391 was re-analyzed using the first and second

wells and compared to the comparator device (KRONUS TRAb) and then to the result obtained by the triplicate reading in the original submission (k083391). All specimens were handled in accordance with the procedure in the Instructions for Use for TSI Reporter and the comparator device Product Insert.

Comparison to Original Predicate:

As described in k083391, an initial study was performed at two testing sites with a total of 299 specimens analyzed for positive and negative percent agreement:

Sites 1 and 2 Combined Result Summary (Duplicate Subject Device)				
		Comparator Device (KRONUS TRAb)		
		+	-	Indeterminate
Thyretain, Duplicate	+	120	18	7
	-	8	153	5
	Null	0	0	0
		<i>95% Confidence Interval</i>		
<i>Positive Percent Agreement</i>	93.8%	88.2% to 96.8%		
<i>Negative Percent Agreement</i>	89.5%	84.0% to 93.2%		

An additional study was performed at a third testing (Study Site 3) using 247 specimens evaluated by both the subject and comparator devices. All specimens were handled in accordance with the procedure in the Instructions for Use for TSI Reporter and the comparator device Product Insert. Sixteen (16) of these specimens were excluded from statistical analysis due to indeterminate results on the comparator device. Twenty-four (24) of the remaining specimens were excluded from statistical analysis due to invalid (Null) results using the Thyretain bioassay. [A null value is defined as a CV greater than 15% between the replicates; the directions for use caution not to report the assay results and suggest repeating the assay]. The remaining 207 specimens were analyzed for positive and negative percent agreement:

Site 3 Result Summary (Duplicate Subject Device)				
		Comparator Device (KRONUS TRAb)		
		+	-	Indeterminate
Thyretain, Duplicate	+	53	4	2
	-	16	134	12
	Null	2	22	2
		<i>95% Confidence Interval</i>		
<i>Positive Percent Agreement</i>	76.8%	65.6% to 85.2%		
<i>Negative Percent Agreement</i>	97.1%	92.8% to 98.9%		

Comparison to Original Device:

The performance of the duplicate wells was compared to the triplicate wells for all study sites. There was 100% agreement between the result obtained by the original triplicate method and the result obtained by proposed duplicate method at all sites. However, the number of null (invalid) samples was different between the methods at Site 3 where there were 20 null results by the triplicate method and 26 null results by the duplicate method. A null value is defined as a CV greater than 15% between the replicates; the directions for use caution not to report the assay results and suggest repeating the assay.

b. Matrix comparison:

Not applicable. Serum is the only matrix.

3. Clinical studies:

a. Clinical Sensitivity:

Sera from 50 Graves disease patients and 199 normal subjects were tested for assay sensitivity and specificity. The results are summarized in the following table:

Clinical Sensitivity and Specificity (Null Results Excluded)				
		Diagnosis		
		Positives (Graves Disease)	Negative (Other autoimmune diseases and healthy controls)	Totals
Thyretain™ TSI Reporter	Positive	46	1	47
	Negative	4	179	183
	Null	0	19	19
	Total	50	199	249

Clinical Sensitivity: 92.0% (46/50) 95th CI: 81.2 – 96.8%

Clinical Specificity: 99.4% (179/180) 95th CI: 96.9 – 99.9%

Invalid Results (Null): 7.6% (19/249)

Clinical Sensitivity and Specificity (Null Results as Positive)				
		Diagnosis		
		Positives (Graves Disease)	Negative (Other autoimmune diseases and healthy controls)	Totals
Thyretain™ TSI Reporter	Positive	46	20	66
	Negative	4	179	183
	Total	50	199	249

Clinical Sensitivity: 92.0% (46/50) 95th CI: 81.2 – 96.8%

Clinical Specificity: 89.9% (179/199) 95th CI: 85.0 – 93.4%

Clinical Sensitivity and Specificity (Null Results as Negative)				
		Diagnosis		
		Positives (Graves Disease)	Negative (Other autoimmune diseases and healthy controls)	Totals
Thyretain™ TSI Reporter	Positive	46	1	47
	Negative	4	198	202
	Total	50	199	249

Clinical Sensitivity: 92.0% (46/50) 95th CI: 81.2 – 96.8%

Clinical Specificity: 99.5% (198/199) 95th CI: 97.2 – 99.9%

b. *Clinical specificity:*

See above.

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

4. Clinical cut-off:

Same as assay cutoff.

5. Expected values/Reference range:

As described in k083391, a study of 140 normal samples found the average SRR% for the female population is 34% (ranged from 9% to 116%) and the average SRR% for the male population is 37% (ranged from 5% to 116%). There were no apparent differences in the reference ranges for male and female populations. Expected value in normal population is negative.

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.