

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

A. 510(k) Number:

k083391

B. Purpose for Submission:

New Device

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 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 510(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 of thyroid stimulating autoantibodies to the thyroid stimulating hormones 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
Signal	Optical density	Radioactive
Detection instrument	Luminometer	Gamma counter set for ¹²⁵ I
Unit of measure	%SRR	U/L

Differences		
Item	Device	Predicate
Cut-off	Positive: $\geq 140\%$ of Reference Control Negative: $\leq 140\%$ pf Reference Control	Positive: $>15\%$ inhibition Indeterminate: 11-15% inhibition Negative: $<11\%$ inhibition

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

1. CLIA EP 17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline.
2. CLSI: EP 12-A: User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline.
3. Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests.

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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
 - a. Precision/Reproducibility:

Intra-assay Precision:

Intra-assay precision was conducted by a single user over a 20 day period, with each sample tested in triplicate. The resulting RLU values were averaged per sample. Each plate of cells analyzed per day contained 16 samples (3 high responding samples, 3 medium responding samples, 3 low responding samples, 3 normal samples, 2 reference controls, 1 positive control and 1 blank replicate). The average variation for each sample was calculated (CV%) across each plate. The result was summarized in the following table:

Sample	High	Mid	Low	Normal	Positive Control
N	3	3	3	3	1
Mean	525	310	184	62	291
%SRR					
SD	9	6	9	4	*
CV%	1.8	1.9	4.7	5.9	*

Average intra-plate variation was calculated at 4.7% (%CV).

Inter-Assay Precision (Intra-Day and Inter-Day):

Inter-Assay Precision was assessed using the same set of samples. Six replicates of each, high positive, medium positive, low positive, normal samples, 2 TSI positive control, and 4 reference control samples were tested per day, respectively. The results were summarized in the following table:

Sample (plate to plate)	High	Mid	Low	Normal	Positive Control
N	6	6	6	6	2
Mean (%SRR)	509	313	183	61	302
SD	19	8	8	3	15
CV%	3.6	2.6	4.2	5.0	5.0
Sample (day to day)					
N	120	120	120	120	40
Mean (%SRR)	446	263	158	52	267
SD	51	34	23	8	33
CV%	11.5	12.8	14.5	15.7	12.3

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 triplicate over an eight day period. Two days were excluded from site 2 due to a technical failure. The total number of replicates for each sample is 180 (3x2x8x4 – 3x2x2x1). The data was summarized in the following table:

Reproducibility Average SRR% (%CV)				
Specimen	Site 1	Site 2	Site 3	
			T1	T2
<u>A</u>	270.09% (11.47%)	439.8% (10.51%)	280.10% (13.56%)	289.49% (10.55%)
<u>B</u>	293.11% (14.72%)	495.14% (14.59%)	374.26% (14.70%)	345.13% (11.8%)
<u>C</u>	48.23% (14.94%)	67.89% (14.66%)	44.33% (20.21%)	48.60% (22.46%)
<u>D</u>	158.6% 10.62%)	198% (10.96%)	143.6% (14.43%)	142.7% (12.12%)

The overall percents of coefficient of variation (CV%) for the four specimens were 23.7%, 23.7%, 24.6%, and 17.9% respectively.

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

Reproducibility Average SRR% (%CV)	
Site 2	Diagnostic Hybrids Inc.
<u>Specimen E</u>	<u>Specimen E</u>
194% (9.3%)	170% (17.7%)
<u>Specimen F</u>	<u>Specimen F</u>
106% (20.2%)	106% (20.7%)
<u>Specimen G</u>	<u>Specimen G</u>
102% (21.6%)	107% (19.4%)

Each site's data were analyzed to determine the Reproducibility and Repeatability of the panel samples. Sample E had a positive ratio (Number Positive/Total Number Tested) of 60/60, Samples F and G had negative ratios (Number Negative/Total Number Tested) of 60/60. The overall coefficient of variation (%CV) for the three samples was 15.0%, 20.3%, and 20.5%, respectively.

b. Assay temperature:

The effect of temperature on cell lysis was determined to be critical during the reproducibility study. The assay was tested at different temperature to confirm the effect of the temperature on assay result. No statistical difference was observed when the lysis occurred between 20 - 25°C; however, a decrease of approximately 20% in %SRR was observed if the lysis temperature was dropped from 20°C to 19°C. The following notation was added to the assay procedure in the package insert: **“Note: It is critical to maintain the cell lysis temperature above 20°C. The test result will be affected if the lysis temperature is dropped to 19°C or less.”**

c. Cell density:

Cell density was determined to be critical to the assay performance. The impact of cell density was studied on duplicate plates that were seeded with three concentrations of cells, which will reach to 90% confluent, 100% confluent and over-100% confluent (more densely packed) respectively, after 18 hours of incubation. Each plate was treated with a positive, reference and normal controls as well as with serum containing high, med and low levels of TSI. No difference was found on %SRR among three plates containing different cell confluent levels when high and med levels of TSI were tested; however, a 19% decrease in %SRR was observed in wells planted with densely packed cells when serum containing a low level of TSI was tested.

A following warning note was added into the package insert: **“Individual wells containing piled or layered cells should be disqualified from use based on over-confluence.”**

d. *Linearity/assay reportable range:*
Not applicable.

f. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Reference Control was the bovine Thyroid-Stimulating Hormone (bTSH) standardized against the rhTSH Standard (WHO Standard NIBSC 03/192, Thyroid-Stimulating Hormone, Recombinant, Human, for Bioassay).

Positive control:

Positive control values between lots provided in the kit are different due to differences in each manufactured antibody lot. An extra warning sentence was therefore added to the package insert as following:

“It is good practice to examine the results of the Positive and Normal Controls before examining the test results of the specimens. However different lots might have a different positive control range. Please check the positive control reference range label for the test range before the test. If one or both of the controls fail to perform as expected, review the steps and conditions under which the test was performed to determine the cause(s). Do not report results until controls perform as expected.”

Specimen stability:

Four specimens containing two positive (near the cut-off) and two high positive samples were stored at 2°C - 8°C (up to 72 hours), -20°C (up to 3 months) and compared to samples stored at -80°C. No apparent decrease of the signal was observed.

Freeze and thaw:

Four specimens, one negative and three positives, were tested repeatedly for up to 4 cycles. No statistical difference in the results was shown when a sample was repeatedly frozen and thawed for a maximum of three cycles.

g. *Detection limit:*

The Limit of Blank (LoB) was determined by 20 blank measurements and the average of the 19th and 20th ranked measurement was 62.75%. Twenty measurements of a low positive were carried out and the standard deviation was calculated at 16%.

The Limit of Detection (LoD) of 89.14%SRR was calculated based on the formula provided in CLSI EP17-A. [$LoD = LoB + c_{\beta} SD_S (62.75 + 1.649 * 16)$]. The LoD was verified in 25 measurements of a positive specimen diluted to the claimed LoD (5 measurements of 5 positive specimens over 5-days). 24 out of 25 (96%) measurements showed %SRR value higher than

LoB.

h. Analytical specificity:

Cross-Reactivity:

Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), and human Chorionic Gonadotropin (hCG) share the same α -chain with TSH. The cross-reactivity study was performed by testing different amounts of FSH, LH and hCG in normal human serum and TSI containing sera from confirmed Graves' Disease (GD) subjects with low positive and high positive %SRR. No cross-reactivity was observed with FSH up to 2000 mIU/mL, LH up to 625mIU/mL, and hCG up to 40,625 mIU/mL in the serum. No cross-reactivity was observed in studies of spiked sera with TSH up to 0.35mIU/mL (the normal physiological range in healthy adults is 0.0003 – 0.0030 mIU/mL).

Autoimmune Specimen Cross-Reactivity:

Serum from patients with auto-antibodies associated diseases [10 patients with Rheumatoid Arthritis (RA), 10 patients with Systemic Lupus Erythematosus (SLE), and 16 patients with Hashimoto's' thyroiditis (Hm)] were tested and found to have no increased cAMP induced luciferase in the TSI Reporter Assay except one Hm sample. This sample which had a SRR% of 150%, was found to have a TSH level at or above -0.35 mIU/mL. A statement that any specimens with TSH levels above this range need to be disqualified for use is included in the Package Insert.

Interference:

Normal sera and TSI positive sera (one in high positive and one in low positive) were tested in the presence of different amounts of hemoglobin, bilirubin and lipemic samples. No interference was seen in samples spiked with hemoglobin up to 250 mg/dL, bilirubin levels up to 36.6 mg/dL, or lipemic levels up to 1168 mg/dL.

i. Assay cut-off:

The assay cut-off was established using 74 samples from 30 patients with diagnosed GD and 44 normal patients with no known or clinically diagnosed thyroid disease. The %SRR is calculated by the ratio of RLU (Relative Light Unit) from sample to RLU from reference control. The SRR% data obtained for each of these subjects were analyzed using receiver operating characteristic (ROC) curve analysis. The cutoff was verified in pre-clinical testing with an additional 50 GD positive sera from non-treated patients and 140 normal sera. The patient serum is considered positive for the presence of TSI if the SRR% measured $\geq 140\%$ over the Reference Control.

Cutoff Analysis on the TSI Reporter				
		Diagnosis		
		Positive (Graves' Disease)	Negative (Healthy Controls)	Total
TSI Reporter	Positive	46	0	46
	Negative	4	140	144
	Total	50	140	190

Sensitivity: 92% (46/50)

Specificity: 100% (140/140)

2. Comparison studies:

a. *Method comparison with predicate device:*

An initial study was performed at two testing sites with a total of 312 specimens to be evaluated by both the subject and predicate devices. One specimen was excluded due to insufficient quantity for testing. Twelve of these specimens were excluded from statistical analysis due to indeterminate results on the predicate device. The remaining 299 specimens were analyzed for positive and negative percent agreement.

Sites 1 and 2 Results				
310 specimen results		Predicate Device		
		+	Indeterminate	-
TSI Reporter	+	120	7	18
	-	8	5	153
	total	128	12	171
<i>95% Confidence Interval</i>				
<i>Positive Percent Agreement</i>		93.8% (120/128)	88.25 – 96.8%	
<i>Negative Percent Agreement</i>		89.5% (153/171)	84.05 – 93.2%	
<i>Overall Percent Agreement</i>		91.3% (273/299)	87.5 – 94.2%	

An additional study was performed at a third testing site using 247 specimens. Sixteen of these specimens were excluded from statistical analysis due to indeterminate results on the predicate device. The remaining 231 specimens were analyzed for positive and negative percent agreement.

Site 3 Results				
247 specimen results		Predicate Device		
		+	Indeterminate*	-
TSI Reporter	+	53	2	4
	-	18	14	156
	total	71	16	160
<i>95% Confidence Interval</i>				
<i>Positive Percent Agreement</i>		74.6% (53/71)	63.5 to 83.3%	
<i>Negative Percent Agreement</i>		97.5% (156/160)	93.8 to 99.0%	
<i>Overall Percent Agreement</i>		90.5% (209/231)		

The predicate device detects autoantibodies to the TSHR, of which there are two classes, stimulating (TSI-hyperthyroidism) and blocking (TBI-hypothyroidism). The predicate device is unable to distinguish between the two antibody types, whereas the TSI Reporter is specific for the TSI class. The patient populations tested in site 1 and 2 are different from site 3. Site 3 is a reference laboratory that receives specimens from all medical disciplines. The difference in patient populations tested at sites 1 and 2 vs. site 3 accounts for the decreased positive percent agreement value that is caused by the specificity difference between TSI Reporter and the predicate device at site 3.

b. *Matrix comparison:*

Not applicable. Serum is the only matrix.

3. Clinical studies:

a. *Clinical Sensitivity and Specificity:*

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

		Diagnosis		
		Positives (Graves Disease)	Negative (Other autoimmune diseases and healthy controls)	Totals
TSI Reporter	Positive	46	1	47
	Negative	4	198	202
	Total	50	199	249

Clinical Sensitivity: 92.0% (46/50) 95%CI 81.2 – 96.9%

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

4. Clinical cut-off:

Same as Assay cut-off.

5. Expected values/Reference range:

A total of 140 normal samples with 72 female subjects and 68 male subjects were tested to determine the reference range of SRR%. 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 are 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.