510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

K152061

B. Purpose for Submission:

New Device

C. Measurand:

Thyroid stimulating immunoglobulins (TSI)

D. Type of Test:

Semi-Quantitative Chemiluminescent Immunoassay

E. Applicant:

Siemens Laboratory Diagnostics, Inc.

F. Proprietary and Established Names:

IMMULITE® 2000 TSI (thyroid-stimulating immunoglobulins) Assay
IMMULITE® 2000 TSI CVM (Calibration Verification Material)

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
   JJX – Quality Control Material (Assayed and Unassayed)

4. Panel:

Immunology (82)
Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

The IMMULITE® 2000 TSI (thyroid-stimulating immunoglobulins) Assay is an in vitro diagnostic immunoassay for the semi-quantitative determination of thyroid stimulating autoantibodies specific to thyroid stimulating hormone receptors (TSHR) in human serum (including Serum Separator tubes) or plasma (K_2-EDTA or lithium heparin). The IMMULITE® 2000 TSI Assay is for use on the IMMULITE® 2000 system. The measurement of thyroid stimulating autoantibodies, in conjunction with other clinical and laboratory findings, is used as an aid in the diagnosis of patients suspected of having Graves' disease.

The IMMULITE® TSI Calibration Verification Material (CVM) is for in vitro diagnostic use in the verification of calibration of the IMMULITE® TSI Assay on the IMMULITE® 2000 Systems.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

For Prescription Use Only

4. Special instrument requirements:

IMMULITE® 2000 Analyzer (K970227)

I. Device Description:

1. The IMMULITE 2000 TSI assay kit consists of the following components:

   a. TSI bead-pack coated with monoclonal antibody (mAb) 3D7 anti-TSHR anchor antibody and hTSHR Capture Chimera
   b. TSI reagent wedge containing hTSHR-Chimera alkaline phosphatase conjugate
   c. TSI adjustors, negative, low and high, containing TSI negative heat-inactivated bovine serum and TSI positive serum or thyroid stimulating human mAb M22
   d. TSI controls, negative and positive, containing TSI negative human serum and TSI positive serum or thyroid stimulating mAb M22
   e. Autoantibody sample diluent containing protein/buffer matrix

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2. The IMMULITE® TSI CVM kit consists of the following components:

Calibration Verification Material, four vials, lyophilized human monoclonal thyroid stimulating antibody in bovine serum with preservative.

J. Substantial Equivalence Information:

1. Predicate device name(s)and Predicate 510(k) number(s):

Thyretain TSI Reporter BioAssay, K092229
Elecsys Anti-TSHR Immunoassay, K080092
Elecsys Anti-TSHR CalCheck (control material), K080643

2. Comparison with predicates:

IMMULITE® 2000 TSI (thyroid-stimulating immunoglobulins) Assay:

Similarities with Thyretain TSI Reporter BioAssay		
Item	Device IMMULITE® 2000 TSI	Predicate Thyretain TSI Reporter BioAssay K092229
Intended Use	The IMMULITE® 2000 TSI (thyroid-stimulating immunoglobulins) Assay is an in vitro diagnostic immunoassay for the semi-quantitative determination of thyroid stimulating autoantibodies specific to thyroid stimulating hormone receptors (TSHR) in human serum (including Serum Separator tubes) or plasma (K2-EDTA or lithium heparin). The IMMULITE® 2000 TSI Assay is for use on the IMMULITE® 2000 system. The measurement of thyroid stimulating autoantibodies, in conjunction with other clinical and laboratory findings, is used	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.

Similarities with Thyretain TSI Reporter BioAssay		
Item	Device IMMULITE® 2000 TSI	Predicate Thyretain TSI Reporter BioAssay K092229
	as an aid in the diagnosis of patients suspected of having Graves' disease.	
Analyte	Thyroid-stimulating immunoglobulins (TSI)	Same

Differences with Thyretain TSI Reporter BioAssay		
Item	Device IMMULITE® 2000 TSI	Predicate Thyretain TSI Reporter BioAssay K092229
Sample Matrix	Serum (including Serum Separator tubes) or plasma (EDTA or lithium heparin)	Serum
Assay format	Semi-quantitative	Qualitative
Assay Principle	Bead-based chemiluminescent immunoassay	Cell-based chemiluminescent assay
Solid Phase	The capture receptor, hTSHR Capture Chimera, is immobilized on polystyrene beads by mAb 3D7 directed against the C-terminus of hTSHR	CHO Mc4 cell monolayer (96-well microplate)
Detection Reagent	hTSHR-Chimera alkaline phosphatase conjugate	Luciferase
Traceability	NIBSC standard 08/204	NISBC Standard 03/192
Unit of Measure	IU/L	% SRR
Measuring range	0.10–40 IU/L	Not Applicable (qualitative)
Cut-off	≥ 0.55 IU/L	$\geq 140\%$
Calibrator	Two level adjustor linked to lot-specific master curve	Not Applicable
Controls	Three levels (negative, low and high)	Three levels (negative, reference, positive)
Sample Volume	50 μL	40 μL

Similarities with Elecsys Anti-TSHR Immunoassay		
Item	Device IMMULITE® 2000 TSI	Predicate Elecsys Anti-TSHR Immunoassay K080092
Intended Use	The IMMULITE® 2000 TSI (thyroid-stimulating immunoglobulins) Assay is an in vitro diagnostic immunoassay for the semi-quantitative determination of thyroid stimulating autoantibodies specific to thyroid stimulating hormone receptors (TSHR) in human serum (including Serum Separator tubes) or plasma (K2-EDTA or lithium heparin). The IMMULITE® 2000 TSI Assay is for use on the IMMULITE® 2000 system. The measurement of thyroid stimulating autoantibodies, in conjunction with other clinical and laboratory findings, is used as an aid in the diagnosis of patients suspected of having Graves' disease.	Elecsys Anti-TSHR Immunoassay is an immunoassay for the in vitro quantitative determination of autoantibodies to TSH receptor in human serum using a human thyroid stimulating monoclonal antibody. The anti-TSH receptor determination is used in the assessment of patients with suspect Graves' disease (autoimmune hyperthyroidism). The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers.
Unit of Measure	IU/L	Same
Calibrator	Two level adjustor linked to lot-specific master curve	Same
Sample Volume	50 μL	Same

Differences with Elecsys Anti-TSHR Immunoassay		
Item	Device IMMULITE® 2000 TSI	Predicate Elecsys Anti-TSHR Immunoassay K080092
Sample Matrix	Serum (including Serum Separator tubes) or plasma (EDTA or lithium heparin)	Serum
Assay format	Semi-quantitative	Quantitative

Differences with Elecsys Anti-TSHR Immunoassay		
Item	Device IMMULITE® 2000 TSI	Predicate Elecsys Anti-TSHR Immunoassay K080092
Assay Principle	Bead based chemiluminescent immunoassay	Microparticle based electro-chemiluminescence immunoassay
Solid Phase	The capture receptor, hTSHR Capture Chimera, is immobilized on polystyrene beads by mAb 3D7 directed against the C-terminus of hTSHR	The capture receptor, porcine TSHR (pTSHR), is immobilized to streptavidin-coated microparticles by a mAb directed against pTSHR
Detection Reagent	hTSHR-Chimera alkaline phosphatase conjugate	mAb M22 labeled with ruthenium complex
Analyte	thyroid stimulating autoantibodies	TSH receptor autoantibodies (blocking and stimulating)
Traceability	NIBSC standard 08/204	Standardized against NIBSC 1st IS 90/672 Standard
Measuring range	0.10–40 IU/L	0.8–40 IU/L
Cut-off	≥ 0.55 IU/L	> 1.75 IU/L
Controls	Three Levels (negative, low and high)	Two levels

IMMULITE® 2000 TSI CVM (Calibration Verification Material)

Differences		
Item	Device IMMULITE® TSI Calibration Verification Material (CVM)	Predicate Elecsys Anti-TSHR CalCheck (control material)
Form	Lyophilized	Same
Stability	Stable unopened until expiration date	Same
Use	Single use only	Same
Analyte	Thyroid Stimulating IgG	Same
Storage	2–8°C	Same

Differences		
Item	Device IMMULITE® TSI Calibration Verification Material (CVM)	Predicate Elecsys Anti-TSHR CalCheck (control material)
Intended Use	The IMMULITE® TSI Calibration Verification Material (CVM) is for in vitro	For use in the verification of calibration established by the Elecsys Anti-TSHR reagent on

Differences		
Item	Device IMMULITE® TSI Calibration Verification Material (CVM)	Predicate Elecsys Anti-TSHR CalCheck (control material)
	diagnostic use as a control for calibration verification of the IMMULITE® TSI Assay on the IMMULITE® 2000 System	the indicated Elecsys and co bas e immunoassay analyzers.
Sample Matrix	Bovine serum with preservatives	Human serum
Number of Levels	Four	Three

K. Standard/Guidance Documents Referenced:

- CLSI EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods, Approved Guideline - Second Edition
- CLSI EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline
- CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition
- CLSI EP09-A3, Measurement Procedure Comparison and Bias Estimation Using Patient Samples - Third Edition
- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition
- CLSI EP24-A2, Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves; Approved Guideline - Second Edition
- CLSI EP25-A, Evaluation of Stability of in vitro Diagnostic Reagents; Approved Guidance - First Edition
- CLSI EP28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory - Third Edition

L. Test Principle:

The IMMULITE 2000 TSI assay is an automated, two-cycle, chemiluminescent immunoassay. It employs two recombinant chimeric human TSH receptors (hTSHR) where the major epitope for the blocking antibody is replaced. The capture receptor is immobilized on the solid phase (polystyrene bead) by 3D7, a monoclonal antibody directed against the C-terminus of hTSHR. In the first 30-minute cycle, thyroid-stimulating antibodies in patient sample bind through one arm to the capture receptor on the polystyrene bead. Upon completion of the 1st cycle, the bead is washed four times with water onboard the instrument. In the second cycle, a liquid reagent containing the signal receptor is added to the reaction tube and incubated for 30 minutes. The immobilized TSI binds the signal receptor through the second arm forming a bridge. Unbound signal receptor is then removed by four centrifugal water washes. Finally, chemiluminescent substrate is added to the reaction tube and a signal is generated in direct relation to the amount of bound AP enzyme and TSI in the sample.

M. Performance Characteristics:

1. Analytical performance:

    a. *Precision/Reproducibility:*

    Total Precision Study: Six serum samples and the low and high kit controls were tested in two runs per day, two replicates per run for a total of 20 days (n = 80). The study was performed at one site with one lot of reagents. Serum samples were prepared by pooling TSI-positive and TSI-negative patient samples to reach the desired concentration. Study design and data analysis were performed according to CLSI document EP05-A2. All data met the manufacturer's predetermined acceptance criteria of total % CV < 11% and are presented in the table below:

Sample	Mean Value (IU/L)	Within-Run		Between-Run		Between-Day		Total	
		SD	CV	SD	CV	SD	CV	SD	CV
1	0.34	0.02	7.0%	0.00	0.0%	0.02	4.6%	0.03	8.3%
2	0.69	0.03	4.1%	0.03	4.3%	0.02	2.9%	0.03	5.0%
3	1.57	0.07	4.4%	0.03	1.9%	0.04	2.3%	0.08	5.3%
4	4.43	0.18	4.0%	0.05	1.1%	0.18	4.1%	0.26	5.9%
5	7.80	0.27	3.5%	0.00	0.0%	0.32	4.1%	0.42	5.4%
6	29.09	1.91	6.6%	0.85	2.9%	0.31	1.1%	2.11	7.3%
Low Control	1.04	0.05	4.9%	0.00	0.0%	0.03	2.9%	0.06	5.4%
High Control	21.27	0.96	4.5%	0.00	0.0%	0.75	3.5%	1.23	5.7%

    Site-to-Site Precision Study: Six serum samples and the negative, low, and high kit controls were tested in two runs per day, four replicates per run for a total of 10 days (n = 80). Samples were tested with two different lots at three different sites. The range of total % CV for all samples across the two lots was 4.3%–7.7% for Site 1, 3.5%–8.5% for Site 2, and 4.1%–7.5% for Site 3. The data for the site-to-site precision are presented for Lot 1 in the table below. The data for Lot 2 are similar, and all data met the manufacturer's predetermined acceptance criteria of total % CV < 11%.

Sample	Mean Value (IU/L)	Within-Run		Between-Run		Between-Day		Between-Site		Total	
		SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
1	0.33	0.01	3.8%	0.01	3.5%	0.01	3.3%	0.01	1.6%	0.02	6.3%
2	0.66	0.03	4.1%	0.01	1.9%	0.01	1.5%	0.04	6.0%	0.05	7.7%
3	1.49	0.08	5.4%	0.02	1.5%	0.02	1.7%	0.09	6.0%	0.13	8.4%
4	4.42	0.15	3.3%	0.14	3.1%	0.00	0.0%	0.04	0.8%	0.20	4.6%
5	8.06	0.34	4.2%	0.27	3.4%	0.00	0.0%	0.10	1.2%	0.44	5.5%
6	30.28	1.28	4.2%	0.74	2.4%	0.00	0.0%	0.71	2.3%	1.64	5.4%
Negative Control	0.23	0.01	5.8%	0.00	0.0%	0.01	2.3%	0.01	2.9%	0.02	6.9%
Low Control	0.97	0.06	6.0%	0.00	0.0%	0.03	2.6%	0.05	4.7%	0.08	8.1%
High Control	20.93	1.00	4.8%	0.09	0.4%	0.63	3.0%	0.24	1.1%	1.21	5.8%

Lot-to-Lot Precision Studies: Two lot-to-lot precision studies were performed. The first study followed CLSI document EP05-A2. Six serum samples and the negative, low, and high kit controls were tested in two runs per day, four replicates per run for a total of 10 days (n = 80). Samples were tested with two different lots at three different sites. The range of total % CV for all samples across the three sites was 3.5%–8.5% for Lot 1, and 4.0%–6.9% for Lot 2. The data for the lot-to-lot precision for Site 1 are presented in the table below. The data for Sites 2 and 3 are similar, and all data met the manufacturer's predetermined acceptance criteria of total % CV < 11%.

Sample	Mean Value (IU/L)	Within-Run		Between-Run		Between-Day		Between-Lot		Total	
		SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
1	0.34	0.01	2.2%	0.01	4.2%	0.01	3.3%	0.01	3.9%	0.02	7.0%
2	0.68	0.00	0.0%	0.01	1.1%	0.02	2.2%	0.03	4.6%	0.04	5.2%
3	1.53	0.04	2.7%	0.01	0.8%	0.03	1.9%	0.06	4.2%	0.08	5.4%
4	4.37	0.19	4.2%	0.00	0.0%	0.11	2.6%	0.18	4.0%	0.28	6.4%
5	7.99	0.33	4.1%	0.00	0.0%	0.10	1.2%	0.32	4.1%	0.47	5.9%
6	30.67	0.23	0.7%	0.00	0.0%	1.11	3.6%	1.36	4.4%	1.77	5.8%
Negative Control	0.23	0.01	5.7%	0.00	1.2%	0.00	0.0%	0.01	6.2%	0.02	8.6%
Low Control	0.97	0.03	2.9%	0.01	1.3%	0.00	0.0%	0.06	5.8%	0.06	6.6%
High Control	19.98	1.19	6.0%	0.41	2.1%	0.00	0.0%	1.14	5.7%	1.70	8.5%

A second lot-to-lot precision study was performed by testing 39 samples in a single replicate using three lots in four different matrix types (serum, SST tubes, K_2-EDTA plasma, and Li-Heparin plasma). Linear regression was performed to compare the results from the different lots and the data met the manufacturer's predetermined acceptance criteria of slope 1.0 ± 0.1 and correlation coefficient 0.9–1.0. The data are presented in the table below:

Matrix	Value Range (IU/L)	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient
Serum	0.10–32.9	1.05 (1.02–1.09)	0.14 (−0.22–0.50)	0.99
SST Tubes	0.10–33.4	0.96 (0.92–1.01)	0.27 (−0.18–0.73)	0.99
K2-EDTA Plasma	0.11–35.9	1.00 (0.96–1.04)	0.28 (−0.15–0.72)	0.99
Li-Heparin Plasma	0.10–34.0	0.93 (0.90–0.96)	0.26 (−0.09–0.60)	0.99

b. *Linearity/assay reportable range:*

Linearity: Six different patient serum samples as well as the calibrators were used to demonstrate assay linearity according to CLSI document EP6-A. For each sample, nine equally spaced dilutions were made by mixing a TSI-high patient sample with a TSI-negative patient sample. One dilution series was also prepared by mixing the high calibrator and the low calibrator. The patient-sample dilution series covered the claimed assay range of 0.10–40.0 IU/L. Each sample was tested in triplicate. Statistical linearity was established when none of the nonlinear terms in second and third-order polynomial models are statistically significant. For any sample determined to be statistically nonlinear, with at least 1 non-linear term having a p-value less than 0.05, the amount of nonlinearity (a percent of the predicted value) should be less than the acceptance target (15% or 0.5 IU/L whichever is greater) to be considered nominally linear. All data met the acceptance criteria and are presented in the table below:

Sample Pool	Range (IU/L)	Regression Equation	Slope 95% CI	Intercept 95% CI	Correlation Coefficient
1	0.51–3.81	y = 0.99x + 0.02	0.96–1.02	−0.05–0.09	1.00
2	1.10–9.16	y = 0.99x + 0.06	0.95–1.03	−0.19–0.32	1.00
3	3.68–32.87	y = 1.01x − 0.31	0.97–1.05	−1.11–0.50	1.00
4	4.12–40.79	y = 1.02x − 0.92	0.98–1.06	−2.04–0.20	1.00
5	3.89–36.54	y = 1.00x − 0.80	0.93–1.07	−2.47–0.88	1.00
6	5.47–37.85	y = 1.00x + 0.01	0.97–1.03	−0.70–0.72	1.00
High Calibrator	4.52–40.47	y = 1.01x − 0.37	0.98–1.05	−1.23–0.55	1.00

Hook Effect:

A serum sample with high TSI concentration (> 800 IU/L), was tested neat and diluted using the assay diluent MD2. The sample was diluted serially up to a 1:128 dilution factor. The sample was tested using two reagent lots and no dilutions fell below the signal of the highest assay calibrator. No hook effect was observed.

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

Traceability and Expected Values:

IMMULITE® 2000 TSI (thyroid-stimulating immunoglobulins) Assay

The IMMULITE® 2000 TSI assay is traceable to the WHO 2nd international standard for Thyroid Stimulating Antibody, NIBSC standard 08/204 and is manufactured using qualified materials and measurement procedures.

IMMULITE® 2000 TSI master calibrators are value-assigned against a set of standards prepared by gravimetric reconstitution and dilution of WHO international standard 08/204. The instrument-stored assay calibration curve is based on a series of calibrators covering the claimed assay range and is specific for each lot of the assay. Calibrators are used internally only, to generate a stored standard curve for individual lots of reagents. Adjustors are supplied in the assay kit and are used to conduct a two-point adjustment of the assay calibration.

Adjustors and Controls are prepared in-house and value assigned during the development process. Calibrator and Control values are summarized in the tables below. The listed values are approximate values, as the values are lot specific.

Adjustor	Dose Range IU/L
Low	0.20–0.40
High	10.00–15.00
Control	Target IU/L
Negative	0.20–0.30
Low	0.80–1.20
High	18.00–22.00

IMMULITE® 2000 TSI CVM (Calibration Verification Material)

The IMMULITE® TSI CVMs are traceable to the WHO international standard 08/204 that has been gravimetrically prepared. The CVMs are manufactured using qualified materials and measurement procedures.

Level	CVM	Target Mean (IU/L)	Range (IU/L)	
1	CVM1	0.02	≤ 0.02	
2	CVM2	0.52	0.47	0.57
3	CVM3	5.01	4.69	5.32
4	CVM4	39.86	34.89	44.83

Reagent Stability:

Real-time stability testing was performed for closed-vial and open-vial/in-use reagents, adjustors, controls and CVMs. Study design and data analysis were performed according to CLSI document EP25-A.

Closed-vial stability testing at 2–8°C was conducted in a real-time study at 0, 60, 120, 180, 270, 360, 450, 540, and 570 days. Eight samples were tested that covered the analytical measuring range of the assay: two pooled patient samples, the low and high adjustor, the low and high controls, the low CVM, and the high calibrator J. Four replicates were tested for the low and high adjustors and three replicates for each of the other samples were tested on each day of the study. Percent recovery was compared to the day zero mean value. The results are presented in the table below.

Open-vial stability testing at 2–8°C was conducted in a real-time study at 0, 7, 8, 14, 30, 45, 60, 90, and 97 days. Open-vial testing of aliquoted components stored at −20°C was conducted on Days 0, 30, 60, 90, 120, 150, 180, and 194 days. Open-vial testing was performed with the same eight samples and study design as in the closed-vial stability testing. The results are presented in the table below.

Component	Storage	Stability Claim
Kit, unopened	2–8 °C	12 Months
Bead-Pack, open	2–8 °C	90 Days
Reagent wedge, open	2–8 °C	90 Days
Sample diluent	2–8 °C	30 Days
Sample diluent	−20 °C	6 Months
Adjustors, open	2–8 °C	90 Days
Adjustors, open, frozen, aliquoted	−20 °C	4 Months
Controls, open	2–8 °C	90 Days
Controls, open, frozen, aliquoted	−20 °C	6 Months
CVM, unopened	2–8 °C	12 Months
CVM, opened and reconstituted	2–8 °C	30 Days

Sample Stability:

Sample stability testing was performed with serum and EDTA and lithium-heparin plasma. Five patient samples covering the analytical measuring range were tested for each matrix type at three different temperatures. The sample stability study supports the claim of stability for 24 hours at 20–25°C, seven days at 2–8°C, and 12 months at −20°C for all samples types.

Calibration Curve Stability:

Real time testing supported the recommended adjustment interval of four weeks.

d. *Detection limit:*

Detection limits for the IMMULITE® 2000 TSI Assay was determined as recommended in CLSI EP17-A2.

Limit of Blank (LoB): LoB was determined by testing five TSI negative serum samples on three different instruments using three lots of reagent. Each of the five samples was tested with two replicates per run and two runs per day for three days. A total of 180 measurements were made per lot. The LoB result was calculated as the value of the 95th ranked sample. The highest value for LoB was reported among the three different lots, and LoB was calculated as 0.03 IU/L.

Limit of Detection (LoD): To determine the LoD, five TSI serum sample pools with mean concentrations from 0.03–0.11 IU/L were tested with three different lots of reagents. Each sample was tested with four replicates per day over three days on three different instruments (n = 36 replicates per sample for each lot). The LoD was calculated for each lot using the methods described in EP17-A2 and the highest value among the three lots was chosen. LoD was calculated as 0.06 IU/L.

Limit of Quantitation (LoQ): To determine the LoQ, six serum samples with mean concentrations from 0.06–0.16 IU/L were tested using three different lots of reagents. Samples were tested on one instrument with three replicates per day for three days (n = 54 replicates per lot).

The LoQ was determined as the highest concentration with a total error of $\leq 45\%$ according to the Westgard model. The highest LoQ was chosen among the three lots. LoQ was calculated to be 0.10 IU/L.

e. *Analytical specificity:*

Endogenous Interferents:

Three serum pools with concentrations across the assay range (Sample pool 1: 0.3–0.5 IU/L, Sample pool 2: 8–12 IU/L, Sample pool 3: 24–40 IU/L) were prepared then

spiked separately with endogenous interferents. A control sample was prepared for each interfering substance by spiking the appropriate diluent at the same volume as the interfering substance; each sample was tested in triplicate. The interferents tested were:

Substance	Highest Concentration Tested
Intralipid	1000 mg/dL
Hemoglobin	200 mg/dL
Bilirubin, conjugated	40 mg/dL
Bilirubin, unconjugated	40 mg/dL
EDTA	9 mg/mL
HAMA	40 ng/mL
Rheumatoid Factor	200 IU/mL
LH	500 mIU/L
TSH	0.14 mIU/L
FSH	750 mIU/L
hCG	100,000 mIU/L
aTPO	2,000,000 mIU/L
aTG	2,000,000 mIU/L

No interference was found when the means of control samples were compared with the means of the spiked samples, except in the case of hemoglobin. The sponsor has included a limitation to this effect in the package insert: "*Presence of hemoglobin at a concentration of 200mg/dL may affect recovery.*"

In addition, samples spiked with a five-fold excess of EDTA anticoagulant were compared with normal serum to investigate the effect of a short blood draw on TSI assay results. Three samples were tested with three different lots of reagents (n = 9 samples). The percent interference for the nine samples ranged from −3.1%–−12.2%, with an average of −7.8%. Only one of the nine samples showed interference > 10%. The sponsor has included limitations to this effect in the package insert: "*Short Draw EDTA Plasma samples may result in under-recovery of IMMULITE 2000 TSI results.*"

Cross-reactivity:

Cross reactivity in related diseases was evaluated by testing samples from patients with similar diseases: Addison's disease, Type 1 Diabetes, Systemic Lupus Erythematosus, and Rheumatoid Arthritis. Bias was evaluated as percentage of samples with TSI values above the cut-off of 0.55 IU/L. None of the tested samples were cross reactive with TSI.

Disease State	# Tested	Percent Positive
Addison's Disease	18	0%
Type 1 Diabetes	10	0%
Systemic Lupus Erythematosus	29	0%
Rheumatoid Arthritis	19	0%

f. Assay cut-off:

The assay cut-off was determined by ROC analysis according to CLSI document EP24-A2. 434 serum samples were tested, consisting of 133 Graves' disease patient samples and 301 samples with other thyroid or autoimmune diseases. The cut-off was established by testing the 434 serum samples against the clinical diagnosis. A result ≥ 0.55 IU/L indicates that TSI was detected in the sample (hyperthyroid Graves' Disease). A result of < 0.55 IU/L indicates that the test is negative for Graves' Disease.

Disease	N
Graves' Disease	164
Autoimmune	45
Addison's Disease	45
Diabetes	24
Solid Tumor	6
Colonoscopy	10
Congestive Heart Failure	2
Hashimoto's Disease	123
Rheumatoid Arthritis	12
Thyroid Disorder	1
Thyroid Cancer	2
Total	434

2. Comparison studies:

a. Method comparison with predicate devices:

Comparison to the Thyretain TSI Reporter BioAssay (K092229):

A Method Comparison study was performed using 811 samples from patients with Graves' Disease, other thyroid diseases, and other autoimmune diseases. Samples were tested using the IMMULITE 2000 TSI assay at two external sites and one internal site. Samples were tested using the Thyretain TSI Reporter BioAssay device at one of the external sites. The following tables show the results of the Method Comparison study:

Disease	Count
Grave's Disease	444
Hashimoto's Disease	118
Other Thyroid Diseases	90
Other Autoimmune Diseases	159
Total	811

		Thyretain TSI Reporter BioAssay (K092229)		
		Positive	Negative	Total
IMMULITE 2000 TSI Assay	Positive	316	59	375
	Negative	14	422	436
	Total	330	481	811

% Positive Agreement	95.8%	95% CI: 93.0%–97.7%
% Negative Agreement	87.7%	95% CI: 84.5%–90.5%
% Overall Agreement	91.0%	95% CI: 88.8%–92.9%

Comparison to the Elecsys Anti-TSHR Immunoassay (K080092):

A Method Comparison study was performed using 569 samples from patients with Graves' Disease, other thyroid disease, and other autoimmune diseases. Samples were tested using the IMMULITE 2000 TSI assay at two external sites and one internal site. Samples were tested using the Elecsys Anti-TSHR Immunoassay (K080092) device at one of the external sites. All of the samples tested were in the analytical measuring range of both assays. The concordance of the two devices could be expected to be low given that the predicate device, Elecsys Anti-TSHR Immunoassay, measures both thyroid receptor stimulating and inhibitory autoantibodies and the new device, IMMULITE 2000 TSI Assay, measures only thyroid receptor stimulating antibodies. The following tables show the results of the Method Comparison study:

Disease	Count
Grave's Disease	555
Hashimoto's Disease	6
Other Thyroid Diseases	7
Other Autoimmune Diseases	1
Total	569

		Elecsys Anti-TSHR Immunoassay (K080092)		
		Positive	Negative	Total
IMMULITE 2000 TSI Assay	Positive	488	40	528
	Negative	15	26	41
	Total	503	66	569

% Positive Agreement	97.0%	95% CI, 95.1%–98.2%
% Negative Agreement	39.4%	95% CI, 28.5%–51.5%
% Overall Agreement	90.3%	95% CI, 87.6%–92.5%

b. *Matrix comparison:*

A matrix comparison study was performed using 107 matched serum and plasma samples collected in the following anti-coagulant tubes: serum clot I tube, lithium heparin plasma tube, serum separator tube (SST), and K_2-EDTA plasma tube. Sample concentrations ranged from 0.10 to 32.3 IU/L. Data was analyzed using Passing-Bablok regression analysis. All data met the manufacturer's predetermined acceptance criteria and the results are presented in the table below:

Serum vs.	Regression Equation	Slope 95% CI	Intercept 95% CI	Correlation Coefficient
K_2-EDTA Plasma	y = 1.03x – 0.01	1.01–1.05	−0.02–0.00	0.99
SST	y = 1.01x	0.99–1.03	−0.02–0.01	0.99
Lithium-Heparin Plasma	y = 0.99x − 0.01	0.98–1.01	−0.02–0.00	0.99

3. Clinical studies:

a. *Clinical Sensitivity and Specificity:*

A total of 765 patient serum samples were collected for the study. Normal samples were not included in the sensitivity and specificity data analysis as this population would not normally be tested for TSI. This population was separate from that used to establish the clinical cut-off.

The samples were collected from four clinical sites and two vendors (US, Canada, and Germany) and were tested at four different external test sites and one internal site. The samples were enrolled on the basis of medical diagnosis or if the patients were suspected of having the disease; their predicate autoantibody status was not used for inclusion or exclusion of any specimen. ~80% of the samples were from women, the median patient age was 44 years old and the age range for all patients was 11–94.

An assessment of thyroid status (Hyperthyroid/Euthyroid/Hypothyroid) was performed by a board-certified physician at the time of enrollment (for both treated and untreated Graves' disease patients) based on the clinical signs and symptoms. Clinical assessments were made based on nationally recognized guidelines (e.g., ATA and AACE). The clinical assessment was then complemented by laboratory tests (e.g., TSH, T3, T4, FT3, and FT4), RAID, and/or ultrasound scan. These tests may have been performed at the time of enrollment or collected from the medical records after the subjects were enrolled in the study. The combination of clinical assessment and biochemical status formed the basis of classification of subjects into various categories for the purpose of establishing Clinical Sensitivity and Specificity.

The data are summarized in the following tables:

Disease	Number	Number Positive (% Positive) with IMMULITE 2000 TSI Assay
Grave's Disease	361	356 (98.1%)
Hashimoto's Disease	111	4 (3.6%)
Other Thyroid Disease	107	1 (0.9%)
Other Autoimmune Disease	186	1 (0.5%)
Total	765	362 (47.3%)

		Clinical Diagnosis for Graves' Disease		
		Positive	Negative	Total
IMMULITE 2000 TSI Assay	Positive	356	6	362
	Negative	5	398	403
	Total	361	404	765

Clinical Sensitivity	98.6%	95% CI, 96.8%–99.5%
Clinical Specificity	98.5%	95% CI, 96.8%–99.5%

4. Clinical cut-off:

See assay cut-off.

5. Expected values/Reference range:

A total of 842 serum samples from apparently healthy males (n = 151), non-pregnant females (n = 155), first trimester (n = 169), second trimester (n = 191), and third trimester

(n = 176) pregnant donors were analyzed using the IMMULITE 2000 TSI assay. The results from this study demonstrate a nonparametric 97.5th percentile of < 0.10 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.