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White Paper

Analytical and Clinical Performance of the IMMULITE 2000 TSI Assay

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Abstract

Background: In Graves' disease (GD) hyperthyroidism, thyroid-stimulating immunoglobulins (TSI) bind to the TSH receptor and mimic TSH stimulation of the thyroid gland. The TSH receptor contains a large extracellular domain that presents epitopes for a variety of autoantibodies, including TSI and thyroid-blocking immunoglobulins (TBI). In contrast to TSI, TBI inhibit TSH stimulation of thyroid cells, leading to hypothyroidism. The IMMULITE® 2000 TSI assay from Siemens Healthcare Diagnostics is designed for the specific, quantitative detection of TSI in serum and plasma. The clinical utility of a TSI assay includes a determination of the autoimmune etiology of thyrotoxicosis, monitoring Graves' patient therapy, prediction of remission or relapse, confirmation of Graves' ophthalmopathy, and prediction of hyperthyroidism in neonates.

Methods: The IMMULITE 2000 TSI assay is an automated chemiluminescent immunoassay with time to first result of 65 minutes. It employs a pair of recombinant human TSH receptor chimeras in a bridging format. The assay is traceable to WHO NIBSC 08/204.

Results: The detection limits of the assay were determined in accordance with CLSI EP17-A2 as follows: LoB = 0.03 IU/L; LoD = 0.06 IU/L; LoQ = 0.10 IU/L. A total of 842 serum samples from apparently healthy males and females were analyzed. The results demonstrate a nonparametric upper 97.5th percentile of 0.07 IU/L. The assay precision was evaluated according to CLSI EP5-A2. The repeatability %CV varied from 3.5 to 7.0% across the assay range. The IMMULITE 2000 TSI assay was compared to the THYRETAIN TSI Reporter BioAssay using 244 serum samples from GD and other thyroid or autoimmune disease patients with the following results: positive agreement: 100% (129/129); negative agreement: 92.2% (106/115); overall agreement: 96.3% (235/244). Serum samples from 236 treated and untreated GD patients, 138 individuals with other thyroid or autoimmune diseases, and 200 apparently healthy individuals were evaluated. At 0.55 IU/L cutoff, the clinical sensitivity and specificity were 98.3% (232/236) and 99.7% (338/339), respectively.

Conclusions: The IMMULITE 2000 TSI assay is a sensitive quantitative immunoassay for the specific detection of TSI in the routine diagnosis and assessment of GD patients.

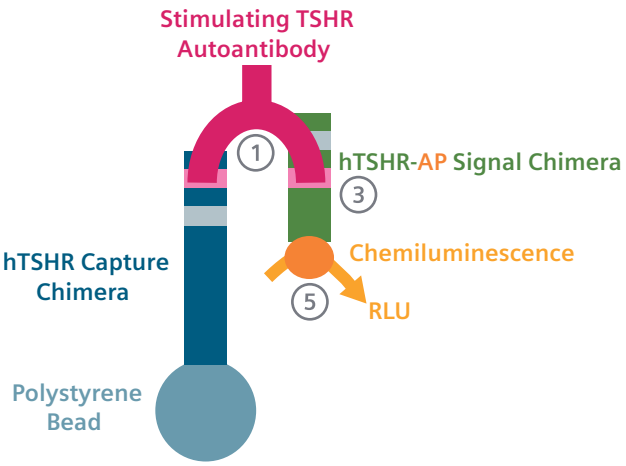
Background

Toxic diffuse goiter, also known as Graves' disease (GD), is an autoimmune disorder and the most common cause of hyperthyroidism. In GD, thyroid-stimulating immunoglobulins (TSI) bind to the TSH receptor (TSHR) and mimic TSH stimulation of the thyroid gland. Because TSI-induced thyroid hormone secretion is not controlled by negative feedback, such stimulation leads to GD hyperthyroidism. The TSH receptor has a large extracellular domain that presents epitopes for a variety of autoantibodies, including TSI and thyroid-blocking immunoglobulins (TBI).[1] In contrast to TSI, TBI bind to the TSH receptor and inhibit TSH stimulation of thyroid cells, leading to hypothyroidism. TSHR autoantibody (TRAb) assays do not distinguish between TSI and TBI. The IMMULITE® 2000 TSI assay from Siemens Healthcare Diagnostics utilizes recombinant human TSH receptors (hTSHR) for the specific detection of thyroid-stimulating autoantibodies.

The clinical utility of a TSI assay includes a determination of the autoimmune etiology of thyrotoxicosis, monitoring Graves' patient therapy,[2] prediction of remission or relapse,[3] confirmation of Graves' ophthalmopathy,[4] and prediction of hyperthyroidism in neonates.[5]

Methods

Principle of the assay



1. Add sample, incubate with capture receptor coated bead 30-min
2. Wash
3. Add signal receptor, incubate 30-min
4. Wash
5. Add AP-substrate, incubate 5-min, measure chemiluminescence

Figure 1. The IMMULITE 2000 TSI bridge immunoassay format with time to first result of 65 min.

LoB, LoD, and LoQ

- CLSI protocol EP17-A2
- LoB: Five negative samples, four replicates per run, 3 days
- LoD: Five low-positive samples, four replicates per run, 3 days
- Three IMMULITE 2000 and two IMMULITE 2000 XPi Immunoassay systems
- Three reagent lots per system
- LoQ: Six low-positive samples, three replicates per run, 3 days
- One IMMULITE 2000 system
- Three reagent lots

Precision

- CLSI protocol EP5-A2
- Six patient sample pools in duplicates, two runs/day for 20 days
- Two IMMULITE 2000 and two IMMULITE 2000 XPi systems
- Three reagent lots per system
- Reference intervals
- CLSI guideline C28-A3c
- Serum samples from 842 apparently healthy individuals
- One IMMULITE 2000 and one IMMULITE 2000 XPi system
- Three reagent lots

Assay cutoff

- CLSI protocol EP24-A2
- 133 GD patient samples
- 297 patient samples with other thyroid or autoimmune diseases

Method comparison

- CLSI protocol EP9-A3
- 244 serum samples from patients with Graves' and other thyroid or autoantibody diseases

Diagnostic accuracy

- CLSI protocol EP12-A2
- 236 serum samples from treated and untreated Graves' disease patients, 139 individuals with other thyroid or autoimmune diseases, and 200 apparently healthy individuals

Cross-reactivity

- CLSI protocol EP7-A2
- Six compounds spiked into zero- and low-TSI patient samples

Alternate sample type

- 40 matched sets of samples from patients with Graves' disease drawn into lithium heparin, EDTA, and BD SST Vacutainer® tubes

Results

LoB, LoD, and LoQ

Table 1. The low end of the IMMULITE 2000 TSI assay measuring range, as defined by LoQ, is 0.10 IU/L.

TSI Kit Lot	LoB (IU/L)	LoD (IU/L)	LoQ (IU/L)
1	0.03	0.05	0.05
2	0.01	0.05	0.05
3	0.03	0.06	0.10
Reported Value	0.03	0.06	0.10

Reportable range: 0.10–40 IU/L

Assay cutoff

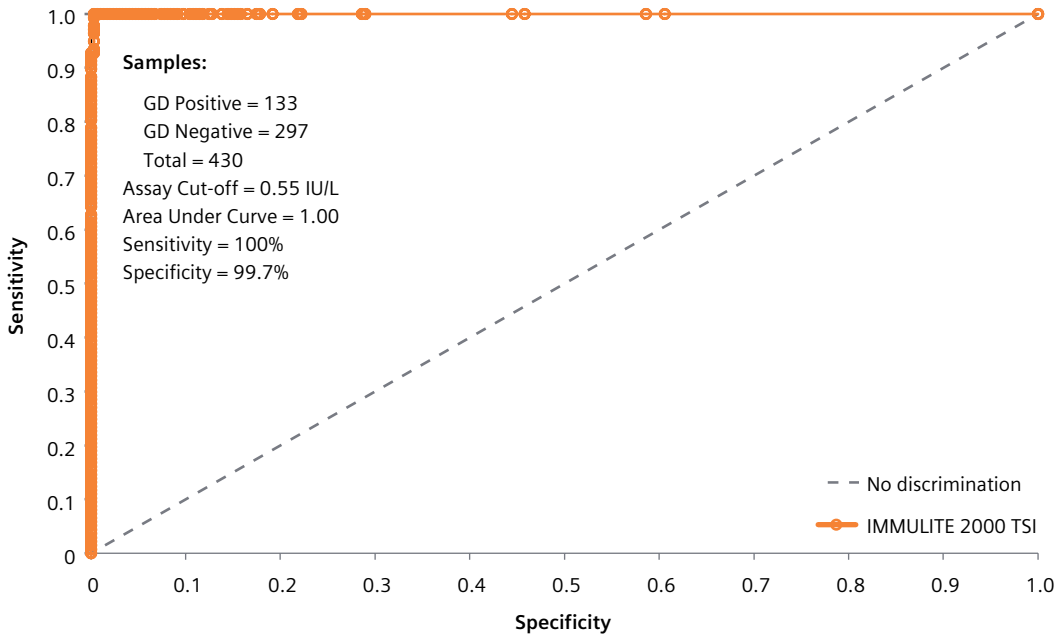


Figure 2. ROC analysis of one lot of the IMMULITE 2000 TSI assay showing AUC =1.00 with 0.55 IU/L cutoff.

Precision

Table 2. The assay demonstrates good precision with repeatability CVs ≤7.0% and within-lab CVs ≤8.3% across the measuring range.

Sample	Mean (IU/L)	Repeatability		Within-lab	
		SD	%CV	SD	%CV
Sample 1	0.34	0.02	7.0	0.03	8.3
Sample 2	0.69	0.03	4.1	0.03	5.0
Sample 3	1.57	0.07	4.4	0.08	5.3
Sample 4	4.43	0.18	4.0	0.26	5.9
Sample 5	7.80	0.27	3.5	0.42	5.4
Sample 6	29.09	1.91	6.6	2.11	7.3

Expected values

Table 3. The 97.5% upper limit of the reference interval for apparently healthy patients was 0.07 IU/L, below the assay's LoQ (0.10 IU/L).

Sample	n	97.5% Upper Limit (IU/L)
Apparently healthy	842	0.07

Method comparison

Table 4. The method comparison of IMMULITE 2000 TSI to THYRETAIN Reporter assays showed 100% positive agreement and 92.2% negative agreement. Seven of the nine discrepant samples (IMMULITE TSI positive, THYRETAIN TSI Reporter negative) were from confirmed Graves' disease patients. Therefore, the IMMULITE TSI assay shows higher clinical sensitivity than the THYRETAIN TSI Reporter BioAssay.

		THYRETAIN TSI Reporter BioAssay	
		Positive	Negative
IMMULITE 2000 TSI	Positive	129	9
	Negative	0	106

Positive Agreement: 100% (129/129)

Negative Agreement: 92.2% (106/115)

Overall: 96.3% (235/244)

Clinical sensitivity and specificity

Table 5. The assay demonstrates high clinical sensitivity and excellent clinical specificity.

		GD Diagnosis	
		Positive	**Negative**
IMMULITE 2000 TSI	Positive	232	1
	Negative	4	338

Clinical Sensitivity: 98.3% (232/236)

Clinical Specificity: 99.7% (338/339)

Overall: 99.1% (570/575)

Cross-reactivity

Table 6. The assay demonstrated no cross-reactivity to the six compounds listed below.

Compound	IU/L Added	Apparent Dose (IU/L)	
		At 0.0 IU/L TSI	At 0.4 IU/L TSI
Follicle-stimulating hormone (FSH)	750	0.01	0.00
Luteinizing hormone (LH)	500	0.01	0.01
Thyroid-stimulating hormone (TSH)	0.14	0.00	0.00
Human chorionic gonadotropin (hCG)	100,000	0.01	0.00
Antithyroglobulin (aTG)	2,000,000	0.02	0.00
Antithyroid peroxidase (aTPO)	2,000,000	0.00	0.01

Alternate sample type

Li heparin = 0.98 (serum) – 0.00 IU/L	r = 0.995
EDTA = 1.04 (serum) – 0.01 IU/L	r = 0.995
SST = 0.99 (serum) – 0.01 IU/L	r = 0.991

Standardization

This assay is traceable to WHO 2nd International Standard for Thyroid Stimulating Antibody, NIBSC Code: 08/204.

Conclusion

The IMMULITE 2000 TSI assay demonstrated:

- High analytical sensitivity and good precision across the measuring range
- No cross-reactivity to six other hormones or autoantibodies
- 100% positive agreement to THYRETAIN TSI Reporter assay
- Excellent clinical sensitivity and specificity
- Ease of use for routine diagnosis in clinical setting

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