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White Paper

Clinical Evaluation of the IMMULITE 2000 TSI Assay

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IMMULITE® 2000 XPi

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 is designed for the quantitative detection of TSI in serum and plasma. This study reports the analytical performance of the IMMULITE 2000 TSI assay and compares its diagnostic accuracy to Roche's Anti-TSHR assay and THYRETAIN TSI Reporter Bioassay.

Methods

The IMMULITE 2000 TSI assay from Siemens Healthcare Diagnostics 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.

*Under FDA review. Not available for sale in the U.S. Product availability varies by country

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 to establish a reference range. 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. Two positive samples showed linear dilution across the tested measuring range. Serum samples from 125 untreated GD patients, 265 individuals with other thyroid or autoimmune diseases, and 150 apparently healthy individuals were evaluated by IMMULITE 2000 TSI, Roche's Anti-TSHR, and THYRETAIN TSI Reporter BioAssay. The clinical sensitivity of IMMULITE TSI was 96.8%, Anti-TSHR was 96.8%, and THYRETAIN 91.2%. The clinical specificity of the IMMULITE TSI was 98.6%, Anti-TSHR 97.3% and THYRETAIN 99.3%.

Conclusions

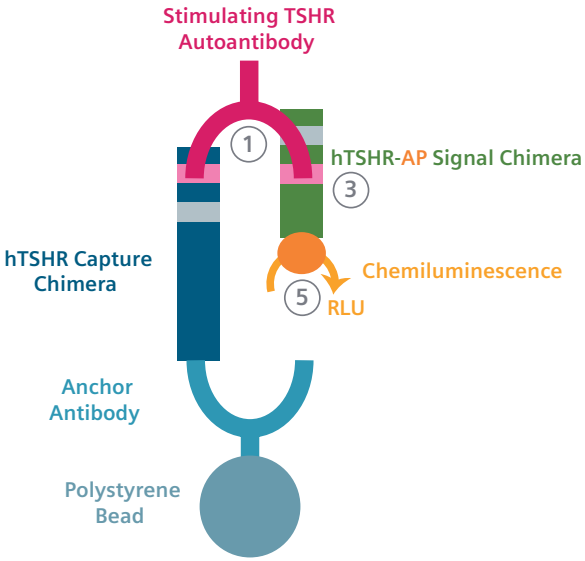
The IMMULITE 2000 TSI assay is a sensitive quantitative immunoassay for the specific detection of TSI in the routine diagnosis and assessment of GD patients.

Background

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 utilizes two recombinant human TSH receptor (hTSHR) chimeras for the capture and detection of thyroid-stimulating autoantibodies. The capture receptor is constructed by replacing the major epitope for TBI binding with amino acid sequence from rat luteinizing hormone-choriogonadotropin (LH-CG) receptor.[2,3] The signal chimera uses N-terminus amino acids of hTSHR conjugated to alkaline phosphatase (AP).[4] 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

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 Systems
  - Three reagent lots per system
- LoQ: Six low-positive samples, three replicates per run, 3 days
- One IMMULITE 2000/XPi Immunoassay 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 systems

Linearity

- CLSI protocol EP6-A
- Two TSI positive samples diluted with two negative samples

Reference Intervals

- CLSI guideline C28-A3c
- Serum samples from 842 apparently healthy individuals

Assay cutoff

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

Diagnostic accuracy

- CLSI protocol EP12-A2
- 125 serum samples from untreated Graves' disease patients, 265 individuals with other thyroid or autoimmune diseases (toxic multinodular goiter, nontoxic goiter, sub-acute thyroiditis, thyroid cancer, Hashimoto's thyroiditis, Systemic Lupus Erythematosus, rheumatoid arthritis, Crohn's disease, Sjögren's syndrome, Celiac disease, Addison's disease), and 150 apparently healthy individuals

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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

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	1.19	6.6	2.11	5.7

Linearity

Table 3. Two TSI positive samples diluted linearly across the tested measuring interval.

Sample Dilution	Sample 1			Sample 2		
	Observed (IU/L)	Predicted (IU/L)	% Bias	Observed (IU/L)	Predicted (IU/L)	% Bias
TSI Positive	3.83	3.81	1%	42.98	43.21	-1%
Dilution 1	3.30	3.34	-1%	37.76	37.85	0%
Dilution 2	2.89	2.87	1%	32.94	32.44	2%
Dilution 3	2.40	2.40	0%	26.92	27.05	0%
Dilution 4	1.88	1.92	-2%	21.36	21.61	-1%
Dilution 5	1.45	1.45	0%	16.71	16.26	3%
Dilution 6	1.03	0.98	5%	10.77	10.86	-1%
Dilution 7	0.53	0.51	4%	5.48	5.47	0%
TSI Negative	0.01	0.04	N/A	0.01	0.17	N/A

Expected values

Table 4. 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

Assay cutoff

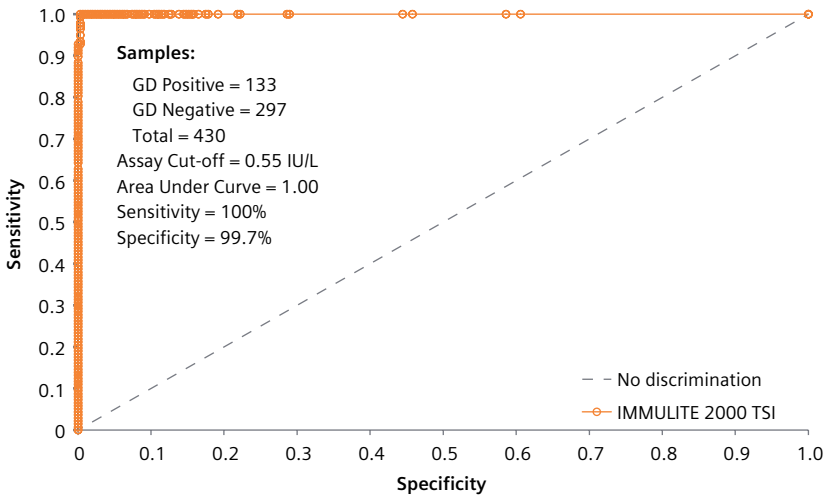

Figure 2. ROC analysis of one lot of the IMMULITE 2000 TSI assay showing AUC =1.00 with 0.55 IU/L cutoff.

Clinical sensitivity and specificity

Table 5. The IMMULITE 2000 assay demonstrates high clinical sensitivity and specificity.

		GD Diagnosis		Clinical	
		Positive	Negative	Sensitivity (95% Confidence Interval)	Specificity (95% Confidence Interval)
IMMULITE 2000 TSI	Positive	121	6	96.8% (92.0%–99.1%)	98.6% (96.9%–99.5%)
	Negative	4	409		
Roche Anti-TSHR	Positive	121	11	96.8% (92.0%–99.1%)	97.3% (95.3%–98.7%)
	Negative	4	404		
Thyretain TSI	Positive	114	3	91.2% (84.8%–95.5%)	99.3% (97.9%–99.9%)
	Negative	11	412		

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
- Excellent clinical sensitivity and specificity
- Ease of use for routine diagnosis in clinical setting

References

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Product availability may vary from country to country and is subject to varying regulatory requirements. Please contact your local representative for availability.

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