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 min. 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.

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.

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

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

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.

<table>
<thead>
<tr>
<th>TSI Kit Lot</th>
<th>LoB (IU/L)</th>
<th>LoD (IU/L)</th>
<th>LoQ (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.03</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>2</td>
<td>0.01</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>3</td>
<td>0.03</td>
<td>0.06</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Reportable range: 0.10–40 IU/L

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 autoimmune diseases

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).

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

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

Sensitivity
Specificity
No discrimination
IMMULITE 2000 TSI

Samples:
GD Positive = 133
GD Negative = 297
Total = 430
Assay Cut-off = 0.55 IU/L
Area Under Curve = 1.00
Sensitivity = 100%
Specificity = 99.7%
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.

<table>
<thead>
<tr>
<th>THYRETAIN TSI Reporter BioAssay</th>
<th>IMMULITE 2000 TSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Positive Agreement: 100% (129/129)</td>
<td></td>
</tr>
<tr>
<td>Negative Agreement: 92.2% (106/115)</td>
<td></td>
</tr>
<tr>
<td>Overall: 96.3% (235/244)</td>
<td></td>
</tr>
</tbody>
</table>

Clinical sensitivity and specificity

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

<table>
<thead>
<tr>
<th>GD Diagnosis</th>
<th>IMMULITE 2000 TSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>232</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
</tr>
<tr>
<td>Clinical Sensitivity: 98.3% (232/236)</td>
<td></td>
</tr>
<tr>
<td>Clinical Specificity: 99.7% (338/339)</td>
<td></td>
</tr>
<tr>
<td>Overall: 99.1% (570/575)</td>
<td></td>
</tr>
</tbody>
</table>

Cross-reactivity

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>IU/L Added</th>
<th>Apparent Dose (IU/L)</th>
<th>At 0.0 IU/L TSI</th>
<th>At 0.4 IU/L TSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicle-stimulating hormone (FSH)</td>
<td>750</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Luteinizing hormone (LH)</td>
<td>500</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Thyroid-stimulating hormone (TSH)</td>
<td>0.14</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Human chorionic gonadotropin (HCG)</td>
<td>100,000</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Antithyroglobulin (aTg)</td>
<td>2,000,000</td>
<td>0.02</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Antithyroid peroxidase (aTPO)</td>
<td>2,000,000</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

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: 08I204.

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

References: