IMMULITE and all associated marks are trademarks of Siemens Healthcare Diagnostics Inc., or its affiliates. All other trademarks and brands are the property of their respective owners.

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

References:
The role of autoantibody detection in Graves’ disease diagnosis

Introduction

Graves’ disease (GD) is the most common type of hyperthyroidism and can cause profound metabolic disruption. It is an autoimmune disorder in which stimulating autoantibodies (TSAs) targeting the TSH receptor (TSHR) induce production of excess T3 and T4, despite low or absent TSH. Diagnosis can often be made on the basis of hormonal testing and clinical presentation alone, however when GD is suspected but not supported by thyroid hormone results, or when confirmation is needed, testing for TSAs can play a crucial role in confirming the diagnosis since they are highly specific for GD. The absence of TSAs can also help to differentiate between GD and other pathologies causing thyroidosis (such as multinodular toxic goiter or thyroid hormone—secreting adenomas) when clinical presentation and other biomarkers do not provide sufficient information.

Currently, two types of tests are used to detect TSAbs: bioassays and immunoassays. Bioassays expose specially modified cells in culture to patient serum and TRAb assays are immunoassays that use a labeled ligand to detect TSHR antibodies. Commercial TRAB assays use a competitive format in which either labeled TSAbs, TRAb, or both species are located in the area of the binding pocket closer to the hinge region of the protein. The chimeric TSH receptor stimulating antibody; stimulates T3 and T4 release in the absence of TSH. The chimeric capture receptor used in the IMMULITE 2000 TSI assay is composed of the N-terminal region of TSHR containing the TSAb binding sites spliced to an amino acid sequence from the rat LH/GCG hormone receptor. This format results in an assay whose clinical sensitivity and specificity are at least as good as—and in some cases superior to—commercial TRAB assays and bioassays.

Several published studies evaluating and confirming the high analytical performance and diagnostic accuracy of the IMMULITE 2000 TSI assay are presented in this compendium. Each article is presented as a brief abstract followed by Siemens Healthineers’ interpretation of the significant work. The authors’ own conclusions. We hope that these synopses encourage you to read each article in its entirety for a more complete understanding of these highly relevant works in the field.

Glossary of terms

GD: Graves’ Disease; an autoimmune disease that causes hyperthyroidism and is marked by some combination of tachycardia, fatigue, muscle weakness, heat intolerance, sleep disturbance, weight loss despite increased appetite and caloric consumption, ocular changes, visual disturbances, nervousness, irritability, depression, and mood swings.

TSH: Thyroid stimulating hormone; hormone released from the pituitary that stimulates the thyroid to produce and release thyroid hormones regulating metabolic homeostasis.

T3: Triiodothyronine; a thyroid hormone.

T4: Thyroxine; a thyroid hormone.

TSAb: TSH receptor stimulating antibody; stimulates T3 and T4 release in the absence of TSH.

TBAbs: TSH receptor blocking antibody; prevents TSH binding and stimulation of the TSH receptor, reducing the release of T3 and T4 by the thyroid.

HT: Hashimoto’s thyroiditis; an autoimmune disease that causes hypothyroidism and is marked by goiter, weight gain, cold intolerance, fatigue, and constipation.

TSAb bioassay: Cell-based assay designed to preferentially detect TSAs.

Bridge assay: The IMMULITE 2000 TSI immunoassay, which uses genetically engineered chimeric TSH receptors lacking the primary TAb binding site to capture and label TSAs in patient serum samples. The Bridge assay is designed to reduce TAb detection with the objective of more specifically detecting TSAs.
Comparison of the bridge assay to 2nd and 3rd generation TRAb assays

Evaluation of the first fully automated immunoassay method for the measurement of stimulating TSH receptor autoantibodies in Graves' disease.


Objective
• Evaluate the ability of the IMMULITE 2000 TSI assay (bridge assay) to differentiate untreated Graves' disease patients from patients with other thyroid diseases and nonthyroid autoimmune diseases.
• Compare the IMMULITE 2000 TSI assay to the second-generation TRAK Human TRAB radioimmunoassay (BRAHMS Thermo Scientific) and the third generation Elecsys/CoBAS Anti-TSH Receptor electrochemiluminescence IMA (TRAB ECLIA, Roche Diagnostics).

Methods
• Retrospective evaluation of patients (age not specified) with untreated Graves' disease diagnosed according to American Thyroid Association guidelines.
• Also tested patients with other thyroid disease (autoimmune thyroiditis, multinodular non-toxic goiter), patients with non-thyroid autoimmune diseases (rheumatoid arthritis, systemic lupus, chronic autoimmune gastritis, celiac disease), and healthy controls <30 years meeting the NACB guidelines for normal thyroid function.
• Used ROC analysis to determine the best cut-off level.
• Correlation and agreement were used to compare the three assays.

Results
• The difference between median TSI in patients with and without Graves' disease was statistically significant.
• Passing & Bablok analysis indicated good correlation between IMMULITE 2000 TSI assay results for the two assays, however, with a negative bias. The two TRAb assays correlated well with minimal bias.

Authors’ conclusions

The diagnostic performance of fully automated IMMULITE 2000 TSI assay in GD patients is at least comparable to that of current TRAb assays, with a trend toward a better accuracy. As a consequence, it may be adopted in clinical practice for the differential diagnosis of hyperthyroidism...and to assess patients with Graves’ orbitopathy.

Sensitivity
• The diagnostic sensitivity of the IMMULITE 2000 TSI assay determined in this study was 100%, which is higher than the sensitivity reported in other studies for the two compared TRAb assays.

Figure 2. IMMULITE 2000 TSI results demonstrating high sensitivity and specificity for diagnosing Graves’ disease.

Figure 3. Performance characteristics of the IMMULITE 2000 TSI assay based on contrived cohorts.


Objective
Retrospectively compare the performance of the IMMULITE 2000 TSI assay (bridge assay) to the Roche anti-TSHR (TRAb) assay in clinical practice.

Methods
• Samples were analyzed from patients diagnosed with Graves’ disease, autoimmune thyroiditis, nonautoimmune nodular thyroid disease, thyroid cancer (differentiated, poorly differentiated, or anaplastic), as well as from patients with no history of thyroid disease.
• Approximately 13% of the Graves’ disease samples were from newly diagnosed patients. The remainder were from patients already receiving therapy. Samples were collected between 3 and 12 months of initial diagnosis.
• Decision thresholds were determined using receiver operator characteristics (ROC) analysis.
• Sensitivity was calculated using samples taken from patients diagnosed with Graves’ disease.
• Specificity was calculated using samples taken from healthy individuals and patients with different thyroid diseases (excluding GD patients).

Results
• Confirmed the 0.55 IU/L cutoff determined by Siemens Healthineers recommended cutoff.
• PPV and NPV (Figure 3) were greater than 93% in all analyses, indicating that the 0.55 IU/L cutoff is effective for both rule-in and rule-out of Graves’ disease.
• The IMMULITE 2000 TSI assay detected 9% more patients with new or existing Graves’ disease than the Roche anti-TSHR assay.
• Overall correlation between the IMMULITE 2000 TSI assay and the Roche anti-TSHR assay was high but less than 90%.

Authors’ conclusions

Our results demonstrate the new automated bridge assay to detect TRAb with high sensitivity (in diagnosing GD) and specificity (in discriminating it from other thyroid diseases).

Significance
• Confirms the high clinical sensitivity and specificity of the IMMULITE 2000 TSI assay.
• Suggests that the IMMULITE 2000 TSI assay is a little more sensitive than the Roche anti-TSHR assay for diagnosing Graves’ disease patients.
Stimulating TSH receptor autoantibodies immunoassay: analytical evaluation and clinical performance in Graves’ disease.


Objective
• Evaluate the analytical and clinical performance of the IMMULITE 2000 TSI assay (bridge assay) for diagnosing Graves’ disease and detecting relapse following treatment, and compare it to the performance of Roche Elecsys/Cobas Anti-TSH Receptor (TRAb) electro-chemiluminescence immunoassay.

Methods
• Prospective evaluation of patients (age not specified) with suspected Graves’ disease or other thyroid disorder treated in a single clinic over the course of one year. Results were compared to remnant donated blood from apparently healthy subjects.
• Final diagnosis was made based on the American Thyroid Association guidelines. Patients were diagnosed with Graves’ disease, atrophic thyroiditis, chronic autoimmune thyroiditis (CAT), or multinodular non-toxic goiter.
• LoD, LoQ, and LoB were determined for each assay according to the CLSI EP17-A protocol.
• ROC analysis was used to determine the best cut-off for differentiating patients with Graves’ disease from patients with other thyroid diseases and healthy individuals with the highest possible diagnostic sensitivity and specificity.
• The method comparison was conducted using the ROC-determined cut-off for IMMULITE 2000 TSI assay (bridge assay) and the Roche recommended cut-offs for the Cobas anti-TSHR (TRAb) assay.

Results
• The LoD, LoQ, and %CVs for intra- and inter-assay precision determined in the study and by Siemens Healthineers were almost identical (Table 1).
• Only one false-negative (individual with mild hyperthyroidism) and two false-positive results were generated using the study cut-off. The authors point out that at least one of the false-positive results could reflect accurate detection of stimulating antibodies as other studies support the presence of stimulating antibodies in CAT.
• Passing & Bablok analysis indicated 98% correlation between IMMULITE 2000 TSI assay and the Roche TRAb assay, with a small but negative IMMULITE bias. This was attributed to the difference in assay formats (bridge vs TRAb).
• More false-negative and false-positive results were observed using the Roche assay at its manufacturer-recommended cut-off than with the IMMULITE 2000 TSI assay.

Authors’ conclusions
“[T]he test allows to accurately detect very low values of analyte, apart from identifying GD patients correctly. The highest analytical sensitivity that has emerged could make this method the elective one...”

Significance
• The study offers additional confirmation of the IMMULITE 2000 TSI assay’s superior sensitivity, specificity, and clinical diagnostic accuracy over the Roche anti-TSHR assay.
• The high functional and clinical sensitivity make the IMMULITE 2000 TSI assay a valuable tool both for initial diagnosis and for diagnosing recurrence in the patient who is no longer being treated.

Table 1. Similarity between assay characteristics determined in the study and reported in the Siemens Healthineers IFU.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Cut-off (IU/L)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>LoD (IU/L)</th>
<th>LoQ (IU/L)</th>
<th>LoB (IU/L)</th>
<th>Intra-assay % CV</th>
<th>Interassay % CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMMULITE 2000 TSI IFU</td>
<td>0.55</td>
<td>98.6</td>
<td>98.5</td>
<td>0.03</td>
<td>0.06</td>
<td>0.10</td>
<td>3.5–7.0</td>
<td>5.0–8.1</td>
</tr>
<tr>
<td>IMMULITE 2000 TSI Study</td>
<td>0.57</td>
<td>98.0</td>
<td>99.9</td>
<td>0.04</td>
<td>0.07</td>
<td>0.14</td>
<td>4.2–5.9</td>
<td>4.5–7.2</td>
</tr>
<tr>
<td>Roche anti-TSHR</td>
<td>1.75</td>
<td>96</td>
<td>99</td>
<td>NR*</td>
<td>NR</td>
<td>0.9</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

*NR=Value not reported in study article.
Diagnostic accuracy of a new fluoroenzyme immunoassay for the detection of TSH receptor autoantibodies in Graves’ disease.


Objective
• Evaluate the diagnostic accuracy of a new third generation automatic fluorescence enzyme immunoassay, the ELIA anti-TSHR assay (Thermo Fischer Scientific) for TSHR antibody measurement in GD, in comparison to two current immunoassays: the BRAHMS TRAK RIA (TRAb) and the IMMULITE 2000 TSI assay (bridge assay).

Methods
• Evaluated sera from patients with untreated GD, treated GD (1–12 months of treatment), GD and Graves’ orbitopathy (GD/GO), non-toxic multinodular goiter (NTMG), Hashimoto’s thyroiditis (HT), toxic adenoma or toxic multinodular goiter (TA/NTMG), non-thyroid autoimmune diseases (NTAD: systemic lupus erythematosus, rheumatoid arthritis, autoimmune gastritis, celiac disease), and normal controls (NC).
• Determined clinical sensitivity and specificity for all assays.
• Used ROC analysis to determine cut-off for the ELIA assay.

Results (Table 2)
• Cut-off for the ELIA assay (3.8 IU/L) is higher than any other 2nd or 3rd generation TRAb assay, and almost 7 x greater than the IMMULITE 2000 TSI assay cut-off.
• The Clinical sensitivity of the ELIA assay was 94.7% for untreated GD patients, 76% for treated GD patients, and 86.7% for GD/GO. Specificity was 99.6%
• The IMMULITE 2000 TSI assay sensitivity determined in the study was 100% and the specificity was 98.2% using the recommended 0.55 IU/L cut-off.

Authors’ conclusions
“The diagnostic sensitivity of ELIA™–TSH-R assay for GD resulted high, though slightly lower than those of the TRAK™ and TSI™ Immulite [sic] assays. In all probability, this is associated to the lower analytical sensitivity of the ELIA™–TSH-R assay, as shown by the high cut-off (3.8 IU/L).”

Significance
• This study was done to support the ELIA assay, however the results indicate that it is not as sensitive as the IMMULITE 2000 TSI assay and confirms the very high sensitivity and specificity of the IMMULITE 2000 TSI assay.
• The high cutoff indicates that the ELIA assay has lower functional sensitivity.
• While the ELIA assay appears to have slightly higher specificity than the IMMULITE 2000 TSI assay, this difference is likely not statistically significant. In addition, several other studies have demonstrated higher specificity for the IMMULITE 2000 TSI assay than reported in this study.

Table 2. Summary of IMMULITE 2000 TSI assay clinical sensitivity and specificity results determined in the above four studies.

<table>
<thead>
<tr>
<th>Reference</th>
<th>GD patients (n)</th>
<th>Patients with other thyroid or autoimmune diseases (n)</th>
<th>Healthy individuals (n)</th>
<th>Assay Cut-off (IU/L)</th>
<th>Clinical Sensitivity (%)</th>
<th>Clinical Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMMULITE 2000 TSI US IFU</td>
<td>361a</td>
<td>404</td>
<td>0</td>
<td>0.55</td>
<td>98.6</td>
<td>98.5</td>
</tr>
<tr>
<td>Tozzoli et al. 2017</td>
<td>72b</td>
<td>191</td>
<td>120</td>
<td>0.54c</td>
<td>100</td>
<td>98.7</td>
</tr>
<tr>
<td>Allelein et al. 2016</td>
<td>266b</td>
<td>180</td>
<td>41</td>
<td>0.55</td>
<td>100</td>
<td>99</td>
</tr>
<tr>
<td>Autilio et al. 2018</td>
<td>46b</td>
<td>49</td>
<td>50</td>
<td>0.57c</td>
<td>98.0</td>
<td>99.9</td>
</tr>
<tr>
<td>Villalta et al. 2018</td>
<td>57b</td>
<td>213</td>
<td>120</td>
<td>0.55</td>
<td>100</td>
<td>98.2</td>
</tr>
</tbody>
</table>

a. Treated and untreated patients
b. Untreated patients, only
c. Determined in study using ROC analysis
Objective
• Compare the clinical performances of the Thyretain TSI bioassay and two automated immunoassays: the Roche Anti-TSHR antibody (TRAb) assay (performed on Cobas e601) and the IMMULITE 2000 TSI assay (bridge assay).
• Evaluate the analytical performance of the two automated assays.

Methods
• Prospective evaluation of patients (age not specified) referred for TSI testing using the Thyretain assay.
• Automated assay results were obtained on site. Thyretain assay results were generated at a reference laboratory.
• Approximately 65% of samples were evaluated by all three methods.
• An extended sample set was used to compare the Thyretain TSI bioassay and the Siemens Healthineers IMMULITE 2000 TSI assay against the final diagnosis.
• Discordant samples (at least one automated assay not aligned with Thyretain result) were compared to clinical history if available. For samples without clinical history, biochemical thyroid status was assessed by determining TSH and free T4 (FT4) levels, and in some cases, anti-thyroid peroxidase (aTPO).
• None of the assays were susceptible to HCG interference for the concentrations tested. Both immunoassays demonstrated good imprecision in line with what is reported in the manufacturers’ IFUs.

Results
• All strongly positive samples according to Thyretain were also positive according to both automated assays.
• There were fewer discrepant results between the Thyretain bioassay and the IMMULITE 2000 TSI assay employing a bridge assay format (16%) when the bioassay results were negative (Table 4). Following review of clinical history, the authors noted that all of the Thyretain-negative/IMMULITE-positive samples were collected from patients with a diagnosis of Graves’ disease who were being treated or previously treated with methimazole or propylthiouracil.
• In comparison, using clinical history for resolution, only 50% of Thyretain-negative/Roche-positive samples were found to come from patients with a history of Graves’ disease.

Authors’ conclusions
“The 3 commercially available anti-TSHR autoantibody measurement methods demonstrated equivalent performance in patients with untreated Graves’ disease.”

Significance
• The IMMULITE 2000 TSI assay (bridge assay) appears to be more sensitive for detecting stimulating antibodies associated with Graves’ disease.
• The IMMULITE 2000 TSI assay results might align more reliably with clinical presentation and patient history than results obtained with either the Thyretain bioassay or the automated Roche anti-TSHR assay.

Table 3. Agreement between each immunoassay and the bioassay.

<table>
<thead>
<tr>
<th>Immunoassays</th>
<th>Positive (%)</th>
<th>Negative (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche Anti-TSHR assay (TRAb)</td>
<td>84.4</td>
<td>86.9</td>
<td>85.2</td>
</tr>
<tr>
<td>IMMULITE 2000 TSI assay (Bridge)</td>
<td>91.1</td>
<td>84.2</td>
<td>88.0</td>
</tr>
</tbody>
</table>

Table 4. Resolution of discordant results for patients with known clinical history. Results in agreement with clinical history or biomarker results are highlighted in orange, uncertain interpretation in gray.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Thyretain bioassay</th>
<th>Roche Anti-TSHR assay (TRAb)</th>
<th>IMMULITE 2000 TSI assay (Bridge)</th>
<th>Interpretation based on clinical history or TSH and FT4 results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Negative</td>
<td>Negative/Positive</td>
<td>Positive</td>
<td>GD receiving therapy</td>
</tr>
<tr>
<td>2</td>
<td>Negative</td>
<td>Negative/Positive</td>
<td>Positive</td>
<td>GD receiving therapy / TPO positive</td>
</tr>
<tr>
<td>3</td>
<td>Negative</td>
<td>Positive/Negative</td>
<td>Negative</td>
<td>Thyroiditis</td>
</tr>
<tr>
<td>4</td>
<td>Negative / Not tested</td>
<td>Positive/Previous GD</td>
<td>Positive /Thyroiditis</td>
<td>GD receiving therapy / euthyroid</td>
</tr>
<tr>
<td>5</td>
<td>Negative</td>
<td>Positive/Previous GD</td>
<td>Positive</td>
<td>GD receiving therapy / euthyroid</td>
</tr>
<tr>
<td>6</td>
<td>Negative / Not tested</td>
<td>Positive/Previous GD</td>
<td>Positive</td>
<td>GD receiving therapy / euthyroid</td>
</tr>
<tr>
<td>7</td>
<td>Negative</td>
<td>Negative/Positive</td>
<td>Previous GD / GD receiving therapy</td>
<td>GD receiving therapy / euthyroid</td>
</tr>
<tr>
<td>8</td>
<td>Negative</td>
<td>Positive/Previous GD</td>
<td>Positive /Thyroiditis</td>
<td>GD receiving therapy / euthyroid</td>
</tr>
<tr>
<td>9</td>
<td>Negative / Not tested</td>
<td>Positive/Previous GD</td>
<td>Positive /Thyroiditis</td>
<td>GD receiving therapy / euthyroid</td>
</tr>
<tr>
<td>10</td>
<td>Negative</td>
<td>Not tested/Positive</td>
<td>Positive /Thyroiditis</td>
<td>GD receiving therapy / euthyroid</td>
</tr>
<tr>
<td>15</td>
<td>Positive</td>
<td>Negative/Positive</td>
<td>Previous GD / GD receiving therapy</td>
<td>GD receiving therapy / euthyroid / TPO positive, Hashimoto’s Thyroiditis</td>
</tr>
</tbody>
</table>