TSH receptor antibodies testing:
The potent benefits of new generation assays

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« Thinking out of the box »

500 000 Belgian citizen lifetime substituted with Thyroxin
Because thyroid disorders represent a worldwide burden
Thyroid diseases

Approximately 750 million people worldwide are affected by thyroid disorders.
HYPERTHYROIDISM

- Intolerance to Heat
- Fine, Straight Hair
- Bulging Eyes
- Facial Flushing
- Enlarged Thyroid
- Tachycardia
- ↑ Systolic BP
- Breast Enlargement
- Weight Loss
- Muscle Wasting
- Localized Edema
- Menstrual Changes (Amenorrhea)
- ↑ Diarrhea
- Tremor
- Finger Clubbing
Thyroid diseases

Madariaga et al.; 2014

Figure 3. Prevalence of undiagnosed hyperthyroidism.

P= 98.88%, P < 0.001
Who are they?

Robert James Graves

Carl Adolph von Basedow
Because of the need of the differential diagnosis of hyperthyroidism
Case Report

Mrs. CW, 33 yrs

<table>
<thead>
<tr>
<th>Tests</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH</td>
<td>&lt;0.01 IU/L</td>
</tr>
<tr>
<td>FT4</td>
<td>35 pmol/L</td>
</tr>
<tr>
<td>FT3</td>
<td>8.5 pmol/L</td>
</tr>
</tbody>
</table>

Non-Autoimmune Hyperthyroidism?
(e.g. toxic multi-nodular goiter, toxic thyroid adenoma)

Autoimmune Hyperthyroidism?
(Graves’ Disease)
Graves disease

Bones, heart, liver, and other organs are affected by the excess thyroid hormone.
Some high risk populations
Graves disease and pregnancy

- The prevalence of hyperthyroidism in pregnancy ranges from 0.1 to 0.4%
- Graves’ disease accounting for 85% of cases
- Activity level of Graves’ disease may fluctuate during gestation, with exacerbation during the first trimester and improvement by late gestation

Inadequately treated maternal thyrotoxicosis is associated with an increased risk of:

- preterm delivery, intrauterine growth restriction and low birth weight, pre-eclampsia, congestive heart failure, and fetal death

Skuza KA, J Pediatr, 1996, 128:264-268
Graves disease and pregnancy

Fetal hyperthyroidism due to the transplacental passage of maternal TSH receptor stimulating antibody (TRAb) levels is rare (0.01% of pregnancies)

but it should be considered in any woman with a past or current history of Graves’ disease and may require treatment with maternal antithyroid medications.
Pediatric Graves’ disease

- Graves’ disease is the most common cause of hyperthyroidism in the pediatric age range.

- Frequently needs to be distinguished from other causes of thyrotoxicosis, particularly the toxic phase of subacute or chronic lymphocytic thyroiditis, in which antithyroid drug therapy is not required.

- Extrathyroidal manifestations of Graves disease in children and adolescents are normally mild and self-limited and characteristically occur during the acute hyperthyroid state.
Since autoimmune thyroid disease is common after alemtuzumab treatment for MS, pretreatment screening and careful follow-up may allow for early diagnosis and treatment.

Graves’ disease following immune reconstitution or immunomodulatory treatment: should we manage it any differently?

Graves’ disease and other disorders of thyroid function may occur following treatment with novel anticancer agents or during periods of lymphocyte recovery after lymphopenia.
For a better understanding of the pathophysiology
Pathogenesis of Graves disease

TSH belongs to the glycoprotein hormone family and binds to the TSHR on the surface of thyroid follicular cells.

TSH regulates production and secretion of the thyroid hormones thyroxine and triiodothyronine via a negative feedback system involving a hypothalamic–pituitary–thyroid axis.

TSH-R belongs to the GPCRs family.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Molecular weight (kDa)</th>
<th>Number of AA</th>
<th>Distribution in tissue</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSHR</td>
<td>85</td>
<td>764</td>
<td>Thyroid, lymphocytes, fibroblast, adipocytes (retroorbital)</td>
<td>Transduction of TSH signal</td>
</tr>
</tbody>
</table>
In Graves’ disease autoantibodies to the TSHR mimic the actions of TSH and cause thyroid overactivity characterized by high serum thyroid hormone levels and low serum TSH levels.

Space filling representation of the TSH LRD interactive surface: green, residues interacting with M22 and orange, residues interacting with TSH, pink those interacting with both ligands.
Because Physicians rely on TSH receptor auto-antibodies testing
Laboratory tests

Symptoms of thyrotoxicosis

Check TSH, free thyroxine

Normal TSH, normal free thyroxine

- Normal

Fully suppressed TSH, high free thyroxine

- Primary hyperthyroidism

Check TSH receptor antibodies

- Present: Graves' disease
- Absent: Perform radionuclide uptake scan

  - Diffuse increased uptake: Graves' disease
  - Patchy or single nodule: Toxic multinodular goitre/toxic nodule
  - No uptake: Thyroiditis

Fully suppressed TSH, normal free thyroxine

- Check free triiodothyronine
  - High free triiodothyronine: Triiodothyronine toxicosis
  - Normal free triiodothyronine: Subclinical hyperthyroidism

Normal/high TSH, high free thyroxine

- Assay problem
  - Resistance to thyroid hormone
  - TSH secreting pituitary adenoma

Vaidya et al., BMJ 2014

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- 147 ETA members participated.
- In addition to serum TSH and free T4 assays, most respondents would request TSH-receptor autoantibody (TRAb) measurement (85·6%) and thyroid ultrasound (70·6%) to confirm aetiology, while isotopic studies were selected by 37·7%.
- Antithyroid drug (ATD) therapy was the preferred first-line treatment (83·8%).
- Compared to the previous European survey, Europeans currently more frequently use TRAb measurement and thyroid ultrasound for diagnosis and evaluation, but first-line treatment remains ATDs in a similar percentage of respondents.

*Bartalena et al., 2014*
## Physician expectations – A quick poll

<table>
<thead>
<tr>
<th>Physicians</th>
<th>Clinical expectations</th>
<th>« Services » expectations</th>
</tr>
</thead>
</table>
| **Pr. C. Daumerie**<br>**CUSL - Brussels** | a) Diagnosis of Graves disease  
b) Monitoring the end of ATS treatment  
c) Green light for pregnancy  
d) Third trimester of a non desired pregnancy | a. Short TAT to avoid scintigraphy.  
b. Reproductibility / precision  
c. Cost  
d. Specific tests for stimulating and blocking |
| **Dr. V. Preumont**<br>**CUSL - Brussels** | a) Diagnosis of Graves disease  
b) Prognosis of Graves before stopping ATS  
c) Graves ophtalmopathy | a. Reproductibility / precision  
b. Short T.A.T |
| **Dr. O. Alexopoulou**<br>**CUSL - Brussels** | a. Diagnosis of Graves disease  
b. In anti TPO + pregnant women  
c. Testing of blocking Abs in Graves with hypothyridism | a. Short T.A.T  
b. Reliability |
Because we still need to improve our assays
**TRAb Nomenclature**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TRab</strong></td>
<td>TSH receptor antibody assays can detect both blocking and stimulating antibodies (TBII) or stimulating only antibodies (TSI)</td>
</tr>
<tr>
<td><strong>Anti-TSHR:</strong></td>
<td>TSH receptor antibodies. Two types can exist - stimulating antibodies (TSAb/TSI) or blocking antibodies (TBAb)</td>
</tr>
<tr>
<td><strong>TBII:</strong></td>
<td>TRab assay that can detect both blocking and stimulating antibodies</td>
</tr>
<tr>
<td><strong>TSI:</strong></td>
<td>TRab assay that can detect stimulating-only antibodies</td>
</tr>
<tr>
<td><strong>TBAb:</strong></td>
<td>Blocking TSHR antibodies</td>
</tr>
<tr>
<td><strong>TSAb:</strong></td>
<td>Stimulating TSHR antibodies</td>
</tr>
</tbody>
</table>

**Competition for ligand binding to the TSHR**

**Bioassays measure bio-response to IgG or TSH stimulation (cAMP or luciferase)**

**McLachlan et al., 2013**
Clinical performances

Rees et al., 2005

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<table>
<thead>
<tr>
<th>Manufacturer / Assay type</th>
<th>Time</th>
<th>Format</th>
<th>F.A.S</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>STD</th>
<th>Cut-point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche Elecsys, cobas anti-TSHR</td>
<td>27 min</td>
<td>Automated</td>
<td>0.9</td>
<td>97</td>
<td>99</td>
<td>NIBSC 90/672</td>
<td>1.75</td>
</tr>
<tr>
<td>Thermo Brahms Kryptor</td>
<td>~1 hr</td>
<td>Automated</td>
<td>0.82</td>
<td>96.3</td>
<td>98.1</td>
<td>WHO NIBSC 90/672</td>
<td>1.8</td>
</tr>
<tr>
<td>Medipan: 1&lt;sup&gt;st&lt;/sup&gt;, 2&lt;sup&gt;nd&lt;/sup&gt; Gen RRA, ELISA; 3&lt;sup&gt;rd&lt;/sup&gt; Gen ELISA</td>
<td>&gt;3 hrs</td>
<td>Manual</td>
<td>0.8</td>
<td>80, 95, 99 (gen 1, 2, 3)</td>
<td>100, 99, 99 (gen 1, 2, 3)</td>
<td>WHO NIBSC 90/672</td>
<td>1.5</td>
</tr>
</tbody>
</table>
Intermethod Variability

Differences may depend on:

- different types of patients studied
- analytical methods used
- source of TSHR (recombinant human or purified porcine)
- assay procedure (times of incubation, positivity thresholds, reference values)

Massart et al.; Ann Bio Clin 2009
Standardization?

Report of the IFCC Working Group for Standardization of Thyroid Function Tests; Part 1: Thyroid-Stimulating Hormone
Linda M. Thienpont,1* Kathleen Van Uytvanckhe,2 Graham Beastall,2 James D. Faix,2 Tamio Ieiri,4 W. Greg Miller,5 Jerald C. Nelson,6 Catherine Ronin,7 H. Alec Ross,6 Jos H. Thijsen,8 and Brigitte Toussaint,9,10 for the IFCC Working Group on Standardization of Thyroid Function Tests

Special Reports

NIBSC 90/672
Effects of the international standard NIBSC 90/672 in different TRAb assays

WHO International Standard
2nd International Standard for Thyroid Stimulating Antibody
NIBSC code: 08/204
Instructions for use (Version 2.0, Dated 28/03/2013)

1. INTENDED USE
The World Health Organization (WHO) Expert Committee on Biological Standardization (ECBS) has recognized (2006) the need for a replacement International Standard for Thyroid-stimulating antibody (TSAb) for the calibration of TSAb assays. The 2nd IS, coded 08/204 was established at the 61st Meeting of the ECBS. This material replaces the 1st IS coded 90/672, which is discontinued.

Rees et al., 2005

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Specificity

YOU ARE UNIQUE
...just like everybody else.
Changes of TSH-stimulation blocking antibody (TSBAb) and thyroid stimulating antibody (TSAb) over 10 years in 34 TSBAb-positive patients with hypothyroidism and 98 TSAb-positive Graves’ patients with hyperthyroidism: reevaluation of TSBAb and TSAb in TSH-receptor-antibody (TRAb)-positive patients.


McLachlan et al., 2013
Clinical relevance of thyroid-stimulating autoantibodies in pediatric graves' disease-a multicenter study.
Why do we need to be specific?

Relevance of TSH-receptor antibody levels in predicting disease course in Graves’ orbitopathy:

comparison of the third-generation TBII assay and Mc4-TSI bioassay

Table 2 Cutoff value for the prediction of severe Graves’ orbitopathy course of the third-generation TBII and Mc4-TSI bioassay using Youden method

<table>
<thead>
<tr>
<th></th>
<th>TBII assay</th>
<th>TSI bioassay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥10.67 IU/l</td>
<td>≥555.10%</td>
</tr>
<tr>
<td>% (95% CI)</td>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>66.7 (51.9-81.5)</td>
<td>69.2 (54.7-83.7)</td>
</tr>
<tr>
<td></td>
<td>84.9 (76.7-93.1)</td>
<td>89.0 (81.9-96.2)</td>
</tr>
</tbody>
</table>

Abbreviations: AUC, area under the curve; CI, confidence interval; TBII, thyrotropin-binding inhibitory immunoglobulin; TSI, thyroid-stimulating immunoglobulin.

Clinical characteristics of Graves’ orbitopathy in patients showing discrepancy between levels from TBII assays and TSI bioassay

**Bioassays**

- **Thyroid Stimulating Ab (TSAb)**
- **Thyroid Stimulation Blocking Ab (TSBAb)**
- **Thyroglobulin Ab**
- **Thyroid peroxidase Ab (anti TPO)**

**Graves’ Disease**

**Autoimmune Hypothyroidism (Hashimoto’s)**

**Standardization of a bioassay for thyrotropin receptor stimulating autoantibodies.**

*Diana et al; Thyroid 2015*
Assay improvement

IMMULITE 2000 XPi TSI Assay is Engineered to Detect Only TSAbs

*Product is currently under development. Due to regulatory requirements, future availability cannot be guaranteed in all countries.*
The IMMULITE® 2000/XPi TSI Assay Format

Chimeric TSH receptor

Anti-human TSH receptor anchoring antibody

Solid substrate (bead)

Chimeric TSH receptor fused with alkaline phosphatase

TSAb in patient serum

Signal

TRAb concentration
The IMMULITE® 2000/XPi TSI

Performance Summary (Siemens)

- Serum samples (plasma heparin)
- Storage: 7 days at 2-8°C and at least 12 months at -20°C

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Time to First Result</th>
<th>Sensitivity LoQ</th>
<th>Assay Range</th>
<th>Cut-off</th>
<th>Calibration Interval</th>
<th>Onboard Stability</th>
<th>Sample Volume</th>
<th>Clinical Sensitivity</th>
<th>Clinical Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum, Plasma (Li Heparin, EDTA)</td>
<td>65 min</td>
<td>0.10 IU/L</td>
<td>0.10-40 IU/L</td>
<td>0.55 IU/L</td>
<td>4 weeks</td>
<td>90 days</td>
<td>50 µL</td>
<td>98.3%</td>
<td>99.7%</td>
</tr>
</tbody>
</table>

Traceable to the WHO Standard, NIBSC code: 08/204
IMMULITE TSI Assay Performance (Evaluation CUSL)

IMPRECISION

The test panel was blinded and the test sequence was randomized within each run. A total of 10 reproducibility runs was performed over the 5 days of the study. One new aliquot of pool was used for each of the 10 runs.

<table>
<thead>
<tr>
<th>Sample</th>
<th>TSI (IU/L)</th>
<th>Between run CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>0.23</td>
<td>2.3%</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.44</td>
<td>3.0%</td>
</tr>
<tr>
<td>Sample 3</td>
<td>0.55</td>
<td>2.1%</td>
</tr>
<tr>
<td>Sample 4</td>
<td>0.71</td>
<td>3.5%</td>
</tr>
<tr>
<td>Sample 5</td>
<td>1.04</td>
<td>2.4%</td>
</tr>
<tr>
<td>QC1</td>
<td>1.06</td>
<td>3.4%</td>
</tr>
<tr>
<td>QC2</td>
<td>22.3</td>
<td>3.0%</td>
</tr>
</tbody>
</table>

The graph shows the relationship between TSI Immulite (IU/L) and CV(%). The data points are plotted on a scatter plot with a line of best fit indicating a decrease in CV as TSI Immulite increases.
Method comparison (1)

- Sample size 151
- Concordance correlation coefficient = 0.62
- Pearson ρ (precision) = 0.8938
- Bias correction factor $C_b$ (accuracy) = 0.6987
IMMULITE TSI Assay Performance (Evaluation CUSL)

Method comparison (2)

Sample size 35

Concordance correlation coefficient = 0.6798

Pearson $\rho$ (precision) = 0.9320

Bias correction factor $C_b$ (accuracy) = 0.7294
IMMULITE TSI Assay Performance (Evaluation CUSL)

Method comparison (2)

Sample size 35
Concordance correlation coefficient 0.8181

Pearson $\rho$ (precision) = 0.9003

Bias correction factor $C_b$ (accuracy) = 0.9087
IMMULITE TSI Assay Performance (Evaluation CUSL)
Reference values

Healthy volunteers (n= 90; males; mean age: 35 yrs, 19 - 55)
IMMULITE TSI Assay Performance (Evaluation CUSL)

Clinical performances

TRAb Medizym

TSI Immulite
Clinical performances

Weighted Kappa 0.746

<table>
<thead>
<tr>
<th></th>
<th>Immulite</th>
<th>Medizym</th>
</tr>
</thead>
<tbody>
<tr>
<td>GD+ (pos/neg)</td>
<td>20 (18/2)</td>
<td>20 (16/4)</td>
</tr>
<tr>
<td>GD- (neg/pos)</td>
<td>50 (48/2)</td>
<td>50 (45/5)</td>
</tr>
</tbody>
</table>
IMMULITE TSI Assay Performance (Evaluation CUSL)

Clinical performances

Receiver operator curve plot analysis, including the data from patients with Graves’ disease and patients without Graves’ disease

Sensitivity: 88.6
Specificity: 94.5
Criterion: >0.293

AUC Immulite: 0.956 (0.912 to 0.982)
AUC Medizym: 0.908 (0.852 to 0.948)
DEFINITION OF THE MEASUREMENT OF TSH RECEPTOR STIMULATING AUTOANTIBODIES: THE HOLY GRAIL FOR THE LABORATORY DIAGNOSIS OF GRAVES’ DISEASE?

F. D’Aurizio¹, D. Villalta², L. Giovanella³, F. Scattolin⁴, P. Metus¹, R. Tozzoli¹
Because we can offer more perspectives
- Accurate and reliable assay
- High clinical sensitivity and specificity

- Consolidation of testings
- Improved process and decreased blood volume

- Automation, improved TAT and faster clinical decision

- Improved specificity and clinical performances
Compared with non-TSI algorithms, 100% use of algorithms that include the TSI test result in

- 46% less time to diagnosis (5.3 weeks earlier)
- 47% annual payer cost savings ($698,892 or $760/person)
  - Fewer misdiagnoses
- Faster referral from primary care physicians to endocrinologists
  - Fewer specialist visits
  - Increased patient productivity
COMMUNICATION

THE 3 FASTEST MEANS OF COMMUNICATION

telephone, television and tell a woman
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Dr. D. Maisin
B. Ferracin
Journée du Département des Laboratoires Cliniques
23 avril 2016

« Maladies chroniques »

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Thank you very much….  

…for your attention !

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