ASSAY SERVICE
THYROID STIMULATING AUTOANTIBODY BIOASSAY
Bioassay

General information
Description: Bioassay for the determination of TSHR stimulating autoantibodies (TSAb) in serum.
Disease reference: Graves’ disease
Advantages: Sensitive and specific bioassay
Literature:
- Y Ochi et al, Thyroid 2000 10:653-657
- Sensitive thyroid-stimulating antibody assay in whole serum containing five percent polyethylene glycol using porcine thyroid cells
- B Rees Smith et al, Thyroid 2007 17:923-938
- TSH receptor antibodies
- B Rees Smith et al, Horm Metab Res 2009 41:448-455
- TSH receptor – Autoantibody interactions

Sample requirement
See also Request form for TSAb
Assay service code: AS/TSA
Test samples: Serum from clotted blood, lipaemic or haemolysed samples are not suitable. Plasma should not be used.
Sample volume: 500µL per patient sample
Test results: 2 - 4 weeks from sample receipt.
**Assay method:** Bioassay

**Assay principle:**

1. **Cell Incubation Stage**
   - TSAb
   - CHO cells expressing TSHR
   - Cells lysed

2. **cAMP Assay Stage**
   - Goat anti-Rabbit IgG coated well
   - Sample cAMP
   - cAMP-AP conjugate
   - Rabbit polyclonal cAMP Ab
   - Detect cAMP-AP by addition of pNpp substrate and read OD at 405nm

Sample cAMP, cAMP-AP (AP = alkaline phosphatase) conjugate and rabbit polyclonal cAMP antibody are added to goat anti-rabbit IgG coated wells where they compete for binding. Sample cAMP is detected by decreased colour development after addition of substrate.

**Assay procedure:**

1. Test serum samples and controls diluted 1 in 10 in buffer and added to TSHR expressing CHO cells. 1 hr incubation at 37°C.
2. Samples removed from cell wells then cells lysed for 30 min.
3. Lysates transferred to goat anti-rabbit IgG coated wells with addition of cAMP-AP conjugate and rabbit polyclonal cAMP-Ab. 2 hr incubation.
4. Wash, add pNpp substrate. 1 hr incubation.
5. Stop reaction and read OD at 405nm.
6. Read cAMP levels off the standard curve.
7. Calculate % stimulation using the formula:
   
   \[
   \text{% stimulation} = \left( \frac{\text{test serum cAMP (pmol/mL) - pool of healthy blood donor sera cAMP (pmol/mL)}}{\text{pool of healthy blood donor sera cAMP (pmol/mL)}} \right) \times 100
   \]
**RSR**

**Assay performance**

**Sensitivity:** 89% for Graves’ disease (n = 44 treated and untreated patients positive for TRAb by ElisaRSR™ TRAb 3rd Generation and/or RiaRSR™ TRAb CT).

**Specificity:** 100% relative to healthy blood donors (n = 40).

**Detection range:** 0.2 – 50 IU/L (units: NIBSC 08/204: www.nibsc.ac.uk)

**Lower detection limit:** 124% stimulation (mean +3 standard deviations in assay of negative control; n = 36)

**Reference cut-off:**

- No detectable stimulating activity: <150% stimulation
- Positive for stimulating activity: ≥150% stimulation

**Cross reactivity:** Using 150% stimulation cut-off, 0/13 Addison’s disease patients, 0/20 rheumatoid arthritis patients and 0/19 type 1 diabetes mellitus patients were positive for TSAb activity

**Interference:**

- Serum TSH levels >10 mU/L (normal range approx. 0.4 – 4 mU/L) result in stimulation of cAMP production.
- Serum hCG levels >90,000 mU/mL result in stimulation of cAMP production (normal levels for males and non pregnant females 0–5 mU/mL, in pregnant females levels can reach >200,000 mU/mL).
- Serum LH concentrations up to 6,000 mU/mL (normal range approx. 5 -25 mU/mL) and serum FSH concentrations up to 10,000 mU/mL (normal range approx. 1.5 – 135 mU/mL) do not cause stimulation of cAMP production.
- Bilirubin (20 mg/dL), haemoglobin (500 mg/dL) and lipid (3,000 mg/dL) do not interfere with the BioassayRSR™TSAb assay.
- Plasma samples are not suitable for use in the BioassayRSR™TSAb assay.

**Inter assay precision:**

<table>
<thead>
<tr>
<th>Sample (n=20)</th>
<th>% stimulation</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>326</td>
<td>15.5</td>
</tr>
<tr>
<td>B</td>
<td>871</td>
<td>17.7</td>
</tr>
<tr>
<td>C</td>
<td>1185</td>
<td>17.6</td>
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</tbody>
</table>

**Intra assay precision:**

<table>
<thead>
<tr>
<th>Sample (n=25)</th>
<th>% stimulation</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>330</td>
<td>11.7</td>
</tr>
<tr>
<td>2</td>
<td>523</td>
<td>11.8</td>
</tr>
<tr>
<td>3</td>
<td>920</td>
<td>17.1</td>
</tr>
</tbody>
</table>

**NIBSC 08/204 curve:**

Dilution profile of the international standard for Thyroid Stimulating Antibody NIBSC 08/204 shows a wide dose-response range, similar to current TRAb assays based on inhibition of M22™ binding to the TSH receptor. 0.2 IU/L gives approximately 150%.
Measurements in different groups:

Samples from Graves’ disease patients (n = 44), healthy blood donors (n = 40), Addison’s disease patients (n = 13), rheumatoid arthritis patients (n = 20) and type 1 diabetes mellitus patients (n = 19) were tested for TSAb using BioassayRSR™ TSAb.

RESULTS:
39/44 (89%) patients positive for TRAb tested by ElisaRSR™ TRAb 3rd Generation and/or RiaRSR™ TRAb CT were positive for TSAb.
All 40 (100%) healthy controls were identified as being negative for TSAb.
None of the Addison’s disease patients (n = 13, positive for 21-OH Ab by RiaRSR™ 21-OH Ab), rheumatoid arthritis patients (n = 20, positive for rheumatoid factor) or the type 1 diabetes mellitus patients (n = 19, positive for GADAb by ElisaRSR™ GADAb) were positive for TSAb.

Other information
Significance: Thyroid stimulating autoantibodies are the cause of hyperthyroidism in Graves’ disease.