1. NAME AND INTENDED USE

THYROGLOBULINE IRMA is an immunoradiometric test for the assay of serum human thyroglobulin.

2. INTRODUCTION

The THYROGLOBULINE IRMA kit is an immunoradiometric test for the assay of serum human thyroglobulin. Thyroglobulin, an iodinated glycoprotein with a molecular weight of 660,000, is the principal constituent of vesicular colloid. It is synthesized exclusively by the thyroid cell for which it therefore constitutes a specific marker. Thyroglobulin plays an essential role in the biosynthesis, storage and secretion of thyroid hormones T3 and T4.

Its assay is useful:
- In oncology: to monitor the course of differentiated thyroid carcinomas. After total resection of the thyroid, thyroglobulin constitutes an early reliable marker of the development of metastases.
- In benign thyroid disease: the assay of thyroglobulin can be used to follow the course of Graves’ disease and to guide therapeutic withdrawal.
- The thyroglobulin levels are also increased in various forms of thyroiditis, especially in Hashimoto’s chronic thyroiditis.
- In the differential diagnosis of thyroid and parathyroid cysts by simultaneous assay of thyroglobulin and parathormone.
- For the differential diagnosis of thyroid agenesis and ectopia: in neonatal hypothyroidism, the presence of thyroglobulin suggests the diagnosis of ectopia, while thyroglobulin is absent in agenesis.
- For the differential diagnosis of thyrotoxicosis. In amiodarone-induced thyrotoxicosis, the thyroglobulin levels are high, while in pseudo-thyrotoxicosis, the thyroglobulin levels are undetectable.

Up until now, anti-thyroglobulin auto-antibodies present in the serum have affected the results of thyroglobulin assays. The THYROGLOBULINE IRMA kit uses monoclonal antibodies selected for their recognition of domains of thyroglobulin not recognised by the patient’s auto-antibodies, thereby allowing a reliable assay of thyroglobulin even in the presence of autoantibodies.

3. PRINCIPLE

The THYROGLOBULINE IRMA kit uses an immunoradiometric technique with the following characteristics:
- A mixture of four monoclonal anti-thyroglobulin antibodies, selected according to well defined criteria of specificity, avidity and complementarity, is coated onto the walls of the tubes.
- A fifth monoclonal antibody, iodine 125 labelled, recognizing a different epitope from those recognized by the antibodies bound to the tubes, is used as a tracer.
- These antibodies are directed against the epitopic zones not recognized by the majority of anti-thyroglobulin autoantibodies present in numerous thyroid diseases, thereby avoiding the systematic need for a spiked test.
- After incubation of the standards and the samples in the presence of the antibodies bound in excess to the tubes as well as with an excess of labelled antibodies, and elimination of the unbound fraction by washing, measurement of the activity bound to the tube allows calculation of the thyroglobulin concentration.

4. REAGENTS

Each kit contains enough reagents for 100 tubes. The expiry date is marked on the external label.

<table>
<thead>
<tr>
<th>REAGENTS</th>
<th>QUANTITY</th>
<th>STORAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>COATED TUBES (R1)</td>
<td>2 packs of 50 tubes</td>
<td>2-8°C until the expiry date. After packaging opening, unused antibodies coated tubes must be stored in the plastic bag, with the dessicant.</td>
</tr>
<tr>
<td>ANTI-THYROGLOBULIN 125I (R2)</td>
<td>1 vial of 42 ml</td>
<td>2-8°C until the expiry date.</td>
</tr>
<tr>
<td>WASHING SOLUTION (R3)</td>
<td>1 vial of 25 ml</td>
<td>2-8°C until the expiry date.</td>
</tr>
<tr>
<td>BUFFER SOLUTION (R4)</td>
<td>1 vial of 35 ml</td>
<td>2-8°C until the expiry date.</td>
</tr>
<tr>
<td>STANDARDS (S1 – S7)</td>
<td>7 vials of 1 ml</td>
<td>2-8°C until the expiry date.</td>
</tr>
<tr>
<td>CONTROL SERA (C1-C2)</td>
<td>2 vials qsp</td>
<td>After reconstitution: the control sera stored at +4°C can be used for 5 days. Any fraction not used can be frozen and stored at –20°C. Only freeze once. The reagents are stable under these conditions for 2 months.</td>
</tr>
</tbody>
</table>

(*) The values shown above are target values: they are shown on its label. The standards are calibrated against the CRM 457 (human thyroglobulin reference material).

(**) The acceptance range true values are printed on the vial label.
5. PRECAUTIONS FOR USE

5.1. Safety measures
Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBs antigen. However, as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all raw materials of human origin including the samples to be assayed must be treated as potentially infectious.

Do not pipette by mouth.
Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards.
Avoid splashing.
Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method of doing this is autoclaving for a minimum of one hour at 121.5°C.
Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.

5.2. Basic radioprotection rules
This radioactive product may only be received, purchased, stored or used by people so authorized, and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.
The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user's country.
The enforcement of the basic rules for handling radioactive products ensures adequate security.
A summary of these is given below:
Radioactive products must be stored in their original containers in a suitable area.
A record of the reception and storage of radioactive products must be kept up to date.
Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).
Do not eat, drink, smoke or apply cosmetics in a controlled zone.
Do not mouth-pipette radioactive solutions.
Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves.
Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes.
Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures.
All radioactive waste disposal must be carried out according to the regulations in force.

5.3. Handling precautions
Do not use kit components beyond their expiry date.
Do not mix reagents from different batches.
Avoid any microbic contamination of the reagents or of the water.
Fully respect the incubation conditions and the washing instructions indicated.

6. SPECIMEN COLLECTION AND PREPARATION

The test is performed on serum, or on plasma.
When the assay is performed on citrate samples, the results are 10% less than serum results. Citrate plasma = 0.86 x serum - 0.0075
\[ r^2 = 0.998 \]
The assay can be performed on samples stored at +2/+8°C for up to 5 days.
Beyond this time, store the serum samples at -20°C.
Avoid successive freezing and thawing.
Haemolysed or hyperlipemic samples should not be used.

Dilution
Should elevated thyroglobulin levels be suspected, dilution is performed with the buffer solution found in the kit.
It is recommended that disposable plastic tubes be used when carrying out dilutions.

7. ASSAY PROCEDURE

7.1. Material required
Precision micropipettes or similar with disposable tips, capable of dispensing 100 μl, 300 μl and 2 ml (± 1 %). Their calibration should be checked regularly. Graduate cylinder 1 l). Distilled or deionised water. Vortex-type mixer. Circular horizontal shaker. Parafilm® paper.
Aspiration system. Gamma scintillation counter calibrated for 125 iodine measurement. Equipment suitable for this assay is available from Schering CIS bio international; information on request.

7.2 Protocol
All reagents must be brought to room temperature (18-25°C) at least 30 minutes before their use.
Dispensing of the reagents into the tubes is also carried out at room temperature.
The assay requires the following groups of tubes:
T group, for the total activity determination,
Standard groups to establish the standard curve,
Control group for the control,
Sx groups for the samples to be assayed.
• It is recommended to perform the assay in duplicate for standards, control and samples.
• The preparation of the standard curve and the assay of the samples must be performed simultaneously.
a) STANDARD procedure

Observe the order in which reagents are to be added:

- Dispense 100 µl of standards, control serum or samples into the corresponding groups of tubes.
- Add 300 µl of the buffer solution into each tube, except tubes T.
- Cover the tubes with Parafilm® plastic film.
- Incubate 3 hours with agitation at room temperature (18-22°C).
- Wash the coated tubes as follows:
  Aspirate the contents of the tubes as completely as possible.
  Repeat the process once.
  Then, leave the tubes to stand 2 minutes or aspirate the contents of the tubes as completely as possible. There must be no residual volume in the coated tubes after washing.
- Dispense 400 µl of tracer $^{125}$I into each tubes.
- Cover the tubes with Parafilm® plastic film.
- Incubate overnight 16-20 hours at room temperature (18-22°C) without agitation.
- Wash the coated tubes as follows:
  Aspirate the contents of the tubes as completely as possible.
  Repeat the process once.
  Then, leave the tubes to stand 2 minutes or aspirate the contents of the tubes as completely as possible. There must be no residual volume in the coated tubes after washing.

To obtain reliable and reproducible results, the different washing steps have to be correctly performed. The addition of the washing solution must be carried out with an efficient speed in order to create turbulences into the tubes.

- Measure the remaining radioactivity bound to the tube with a gamma scintillation counter over 1 minute.

**STANDARD PROCEDURE (ASSAY FLOW CHART)**

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Standards (S1-S7) Controls (C1-C2))</th>
<th>Buffer solution µl</th>
<th>Agitate</th>
<th>Tracer $^{125}$I (R2) µl</th>
<th>Incubate 16 – 20 h at 18-22°C without agitation</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>-</td>
<td>-</td>
<td></td>
<td>400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standards</td>
<td>100</td>
<td>300</td>
<td>Incubate 3 hours at 18-22°C under agitation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>100</td>
<td>300</td>
<td>Wash 2 times</td>
<td>400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samples</td>
<td>100</td>
<td>300</td>
<td></td>
<td>400</td>
<td></td>
<td>Wash 2 times</td>
</tr>
</tbody>
</table>

b) Rapid procedure

Observe the order in which reagents are to be added:

- Dispense 100 µl of standards, control serum or samples into the corresponding groups of tubes.
- Add 400 µl of tracer $^{125}$I into each tube.
- Shake on a Vortex type shaker.
- Cover the tubes with Parafilm® plastic film.
- Incubate overnight (16-20 h) at room temperature (18-22°C) without agitation.
- Wash the coated tubes as follows:
  Aspirate the contents of the tubes as completely as possible.
  Add 2,0 ml of washing solution to each tube.
  Aspirate.
  Repeat the process once. Then, leave the tubes to stand 2 minutes or aspirate the contents of the tubes as completely as possible. There must be no residual volume in the coated tubes after washing.

To obtain reliable and reproducible results, the different washing steps have to be correctly performed. The addition of the washing solution must be carried out with an efficient speed in order to create turbulences into the tubes.

- Measure the remaining radioactivity bound to the tube with a gamma scintillation counter over 1 minute.
Rapid Procedure (Assay Flow Chart)

Order of addition and volume (in µl) of the reagents to be added to each tube

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Standards (S1-S7) Controls (C1 – C2)</th>
<th>Tracer (R2)</th>
<th>Agitate</th>
<th>Incubate 16 – 20 h at 18-22°C (without agitation)</th>
<th>Wash 2 times</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standards 100 µl</td>
<td>µl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls 100 µl</td>
<td>µl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Samples</td>
<td>µl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>“</td>
<td>400</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8. QUALITY CONTROL
Good laboratory practices require that quality control samples be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

9. RESULTS
Preparation of the standard curve:
Calculate the mean count for each pair. If necessary, calculate the percentage binding B/T (%). Draw the curve on linear-log paper, by plotting the cpm or B/T (%) on the y-axis and the concentration of the standards on the x-axis.
The thyroglobulin concentration is determined by interpolation of the cpm or B/T(%).

Typical standard curve (example only): these data must under no circumstances be substituted for results obtained in the laboratory.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>CPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1 0.2 ng/ml</td>
<td>112</td>
</tr>
<tr>
<td>S2 1.5 ng/ml</td>
<td>529</td>
</tr>
<tr>
<td>S3 5 ng/ml</td>
<td>1207</td>
</tr>
<tr>
<td>S4 15 ng/ml</td>
<td>3130</td>
</tr>
<tr>
<td>S5 50 ng/ml</td>
<td>9635</td>
</tr>
<tr>
<td>S6 200 ng/ml</td>
<td>37848</td>
</tr>
<tr>
<td>S7 500 ng/ml</td>
<td>84289</td>
</tr>
<tr>
<td>SC1 10.7 ng/ml</td>
<td>2708</td>
</tr>
<tr>
<td>SC2 110 ng/ml</td>
<td>23854</td>
</tr>
</tbody>
</table>

10. PROCEDURAL LIMITATIONS
Samples which show turbidity, haemolysis, hyperlipemia or contain fibrin may give misleading results.
Do not extrapolate sample values beyond the last standard. Dilute the concerned samples and re-assay.

11. EXPECTED VALUES
Each laboratory should establish its own range of normal values. The value given below are only indicative.
The values obtained in presumably healthy subjects of both sexes fall into the following concentration ranges (n = 149).

<table>
<thead>
<tr>
<th>Concentration Range</th>
<th>Number of Patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 5 ng/ml</td>
<td>11</td>
<td>7.4</td>
</tr>
<tr>
<td>5 - 10 ng/ml</td>
<td>37</td>
<td>25.0</td>
</tr>
<tr>
<td>10 - 20 ng/ml</td>
<td>61</td>
<td>41.0</td>
</tr>
<tr>
<td>20 - 30 ng/ml</td>
<td>20</td>
<td>13.0</td>
</tr>
<tr>
<td>30 - 50 ng/ml</td>
<td>15</td>
<td>10.0</td>
</tr>
<tr>
<td>&gt; 50 ng/ml</td>
<td>5</td>
<td>3.4</td>
</tr>
</tbody>
</table>
12. SPECIFIC CHARACTERISTICS OF THE ASSAY

12.1. Precision of the test
The precision of the test is indicated by the intra-assay and inter-assay reproducibility.

<table>
<thead>
<tr>
<th>SERUM</th>
<th>X (ng/ml)</th>
<th>CV (%)</th>
<th>SERUM</th>
<th>X (ng/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.22</td>
<td>7.7</td>
<td>F</td>
<td>0.8</td>
<td>16.7</td>
</tr>
<tr>
<td>B</td>
<td>8.2</td>
<td>3.1</td>
<td>G</td>
<td>7.9</td>
<td>7.0</td>
</tr>
<tr>
<td>C</td>
<td>43.8</td>
<td>2.6</td>
<td>H</td>
<td>43.0</td>
<td>3.1</td>
</tr>
<tr>
<td>D</td>
<td>116.0</td>
<td>1.4</td>
<td>I</td>
<td>111.0</td>
<td>2.0</td>
</tr>
<tr>
<td>E</td>
<td>373.0</td>
<td>1.8</td>
<td>J</td>
<td>299.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

12.2. Accuracy of the test
The accuracy of the assay can be determined by a spiked test and a dilution test.

a) Spiked test
Five normal sera (without autoantibodies) and samples of each of the most frequent disease have been spiked with 33 ng/ml of thyroglobulin (table VI).

The spiked test has been done in the following way:

Test = 20 µl of standard 6 (200 ng/mL) are added to 100 µl of the test sample.
Control = 20 µl of buffer solution are added to 100 µl the test sample.

The percentage recovery is calculated by means of the formula:

\[
\text{Recovery} = \frac{(\text{Test} - \text{Control}) \times 100}{\text{Theoretical spiked value}}
\]

b) Dilution test
Three samples with different level of autoantibodies have been diluted with the buffer solution.

<table>
<thead>
<tr>
<th>AUTO ANTIBODIES (U/ml)</th>
<th>DILUTION FACTOR</th>
<th>THEORICAL VALUE (ng/ml)</th>
<th>OBSERVED VALUE (ng/ml)</th>
<th>RECOVERY (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>Neat</td>
<td>-</td>
<td>73.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1/2</td>
<td>36.5</td>
<td>34.9</td>
<td>96.0</td>
</tr>
<tr>
<td></td>
<td>1/4</td>
<td>18.3</td>
<td>17.2</td>
<td>96.0</td>
</tr>
<tr>
<td></td>
<td>1/8</td>
<td>9.1</td>
<td>8.8</td>
<td>97.0</td>
</tr>
<tr>
<td>-</td>
<td>Neat</td>
<td>-</td>
<td>182.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1/2</td>
<td>91.0</td>
<td>94.6</td>
<td>104.0</td>
</tr>
<tr>
<td></td>
<td>1/4</td>
<td>45.5</td>
<td>51.2</td>
<td>113.0</td>
</tr>
<tr>
<td></td>
<td>1/8</td>
<td>22.8</td>
<td>23.9</td>
<td>105.0</td>
</tr>
<tr>
<td>+</td>
<td>Neat</td>
<td>-</td>
<td>155.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1/2</td>
<td>77.5</td>
<td>82.9</td>
<td>107.0</td>
</tr>
<tr>
<td></td>
<td>1/4</td>
<td>38.8</td>
<td>44.3</td>
<td>114.0</td>
</tr>
<tr>
<td></td>
<td>1/8</td>
<td>19.4</td>
<td>22.4</td>
<td>115.0</td>
</tr>
</tbody>
</table>
c) Hook-effect
Thyroglobulin concentrations up to 28 000 ng/ml give a higher signal than the last standard, using the rapid procedure (one-step). However few samples with levels >8,000 ng/ml are usually encountered. With the standard procedure (two-steps) the hook effect is increased to greater than 800,000 ng/ml.

12.3. Specificity of the test
The antibodies used in this assay have been selected in order to avoid any interference with anti-thyroglobulin autoantibodies, making a routine spiked test unnecessary.
A spiked test has been done on 611 samples (351 with autoantibodies and 260 without autoantibodies). The recovery percents obtained follow a Gauss’ distribution around the 100% value. Similar results are obtained with samples including autoantibodies or not.

This validates the non-interference of the autoantibodies, due to the selection of the monoclonal antibodies used in the assay.

**SPIKED TEST**

- samples with autoantibodies (n = 351)
- samples without autoantibodies (n = 260)

12.4. Detection limit
The detection limit measured by analytical method is defined as being the smallest detectable concentration different from zero with a probability of 95 %. It has been assessed as being 0.2 ng/ml. The functional sensitivity is defined as being the measured concentration by imprecision profile for a CV equal to 20 %. It has been assessed as being 0.7 ng/ml.

12.5 Measuring range
0.7 – 500 ng/ml