Kit for the radioimmunological determination of human thyrotropin (hTSH)

The kit comprises:

1. Vial of $^{125}$I-hTSH antibody (monoclonal, mouse), < 300 kBq, 11.5 ml buffer with bovine albumin, monoclonal antibodies, sodium azide and a red dye.
2. 2 x 50 test tubes coated with anti-hTSH antibodies (monoclonal, mouse).
3. 7 vials of hTSH standards, per 1 ml human serum, bovine albumine and sodium azide, concentration in the nominal range of 0 - 50 µIU hTSH/ml.
4. Vial of hTSH control serum, 1 ml human serum, bovine albumine and sodium azide, concentration stated.
5. Vial of wash reagent, 3 buffer tablets.
7. Instruction for use.

The dissolved reagents contain sodium azide as preservative. Avoid swallowing and contact with the skin or mucous membranes. 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.

1. Introduction

Thyrotropin (TSH), a hormone produced by the anterior lobe of the pituitary, is a glycoprotein with a molecular weight of ~ 30,000. It consists of two chemically different sub-units, an alpha and a beta chain. Only the complete intact molecule (hTSH) is biologically effective. The other glycoprotein hormones hLH, hFSH, hCG also consist of two sub-units. The $\alpha$ sub-units of this group of hormones are virtually identical, whereas the $\beta$ sub-units are hormone-specific and differ in their structure.

2. Clinical results with RIA-gnost® hTSH

2.1. Clinical significance of the quantitative hTSH determination

A feedback mechanism exists between the thyroid, the pituitary, the hypothalamus and the thyroid hormones triiodothyronine (T3) and thyroxine (T4) which circulate in the blood (Fig. 1).

Thyrotropin releasing hormone (TRH) is formed as a neurosecretion of the hypothalamus. TRH promotes the secretion of thyrotropin (TSH) from the pituitary and also stimulates its synthesis. TSH in turn acts to enhance the synthesis in the thyroid and the release of T4 and T3. The release of TSH is regulated by the circulating free fraction of the thyroid hormones in the blood. TSH levels are depressed when peripheral concentrations of the free fraction of thyroid hormones are high; conversely, TSH levels are elevated when peripheral concentrations of thyroid hormones are low.

This regulatory mechanism forms the basis for the TRH test. Administration of synthetic TRH to subjects with normal pituitary function gives rise to the secretion of immunoreactive TSH from the pituitary. In hyperthyroidism TSH synthesis is interrupted because of the feedback mechanism mentioned at the outset so no TSH can be secreted. In hypothyroidism on the other hand the feedback mechanism results in a higher TSH content in the pituitary and in a disproportionately large rise in the already raised basal TSH level in response to the TRH stimulus.

2.2. Normal values

The normal range of the hTSH concentration in the serum of men and women was determined as part of the clinical testing of RIA-gnost®hTSH:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Range</th>
<th>Units/ml</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal thyroid function</td>
<td>0.25 – 4</td>
<td>µIU/ml</td>
<td>1966</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td>&lt; 0.25</td>
<td>µIU/ml</td>
<td>182, predominantly &lt; 0.1 µIU/ml</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>≥ 5</td>
<td>µIU/ml</td>
<td>50</td>
</tr>
</tbody>
</table>
After TRH stimulation the TSH level in normal subjects rises to 2-30 µIU/ml \( (n = 417) \). In patients with hyperthyroidism the TSH level is below 0.5 µIU/ml \( (n = 59) \), whereas in hypothyroidism the values rise over 30 µIU/ml \( (n = 8) \). In severe, persistent general disorders the TSH serum concentration may be affected, at least the TSH response to TRH stimulation is reduced. Drugs can stimulate TSH secretion (e.g. dopamine antagonists, hypocalcaemia, iodide) or inhibit it (e.g. L-dopa, corticosteroids). A slight fall in the TSH level and a decrease in the TRH-stimulated TSH secretion is possible with increasing age.

3. Principle of measurement and characteristic data of the RIA-gnost® hTSH

3.1. Principle
RIA-gnost® hTSH permits the in vitro determination of thyrotropin in human serum (or plasma) by the principle of a 1-step sandwich assay. During this process a complex of solid-phase anti-hTSH antibodies (monoclonal, mouse), hTSH in the sample and \(^{125}\text{I}\) labelled anti-hTSH antibodies (monoclonal, mouse) is formed. At the end of the reaction the free amount of tracer is removed by decanting (or aspiration) and subsequent washing.

The amount of tracer bound specifically to the coated test tubes is measured with a gamma counter. Evaluation of the unknown samples is carried out by reading off from a standard curve constructed under identical conditions. When carrying out duplicate determinations a maximum of 42 patients samples can be measured together with a standard curve.

The monoclonal antibodies used in the kit are highly specific for hTSH. The possibility of a cross-reaction to hLH, hFSH and hCG is virtually excluded in the concentration ranges that are physiologically relevant.

Samples outside the measuring range are diluted with wash buffer.

The standards are calibrated against hTSH WHO 80/558.

Note: The extremely high sensitivity of the assay can only be achieved if the following points are borne in mind:

a) Avoid external contamination of the test tubes.

b) Ensure complete removal (by decantation/aspiration) of the unbound tracer fraction. During aspiration, blockage of the capillaries must be prevented; after decantation, tap the tubes thoroughly onto cellulose material.

c) Check the measuring equipment and any additional material that may be used to keep the zero effect constant and if necessary decontaminate.

d) Exclude interference from external sources of radiation.

3.2. Specific characteristics of the assay

3.2.1. Imprecision
This has been evaluated with 3 samples assayed 10 times in the same series and in 10 different series.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mean (µIU/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.30</td>
<td>5.7</td>
</tr>
<tr>
<td>2</td>
<td>3.95</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>28.7</td>
<td>1.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mean (µIU/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1.55</td>
<td>5.4</td>
</tr>
<tr>
<td>5</td>
<td>3.61</td>
<td>4.8</td>
</tr>
<tr>
<td>6</td>
<td>9.81</td>
<td>2.4</td>
</tr>
</tbody>
</table>

3.2.2 Detection limit
The detection limit is defined as being the smallest concentration different from 0 with a confidence interval of 95%. It has been determined as being 0.03 µIU/ml.

4. Working instructions

4.1. Equipment required
Microlitre pipettes with disposable plastic tip 100 µl, 200 µl, measuring cylinder, horizontal shaker (300 ± 50) rpm, gamma scintillation counter calibrated for 125 iodine measurement.

4.2. Preparation of the reagents
The kit components which have been stored at 2-8°C are brought up to room temperature (17-27°C) before use. The wash buffer is prepared by dissolving the three buffer tablets in 300 ml distilled water. All the unused reagents should be stored at 2-8°C. Unused antibody coated tubes after packaging opening must be stored in the plastic bag supplied with the kit.

4.3. Preparation of the serum samples
After taking blood samples, serum or plasma is obtained by the usual methods. The serum or plasma is used directly in the assay or stored for up to 3 days at 2-8°C. If they are to be stored for a longer period, a temperature of –20°C is recommended. The serum samples should be mixed carefully after thawing.

4.4. Warnings and precautions
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.
4.5. Procedure
1. A sufficient number of coated test tubes is numbered (standards, control serum, serum samples) as is given in Table 1.
2. 200 µl standard (or patient's samples) are pipetted into the coated test tubes. A new pipette tip should be used for each sample.
3. 100 µl ¹²⁵I-anti-hTSH is dispensed into each test tube.
4. The test tubes are then shaken on a horizontal shaker (300 ± 50 rpm) for 2 hours at 17-27°C.
5. 1 ml wash buffer is then placed into each tube, decanted (aspirated) and washed with 1 ml.
6. The test tubes are then measured for 1 minute in a gamma scintillation counter.

4.6. Evaluation of the results
A typical standard curve for RIA-gnost®-hTSH (coated tube) is shown in Figure 2.
The counts per minute of the individual standards S₀ – S₆ are plotted against the appropriate hTSH concentration (µIU/ml) on graph paper that has been prepared accordingly. The “best fit” standard curve is constructed through these points.
The measure values of the control serum and the patient's samples are marked on the graph and the desired hTSH content per millilitre serum is read off from the standard curve.

Tab 1 : hTSH assay procedure

<table>
<thead>
<tr>
<th>Labelling of test tubes</th>
<th>Standards (µl)</th>
<th>Control serum (µl)</th>
<th>Samples (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standards</td>
<td>S₀ 200/200</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S₁ 200/200</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S₂ 200/200</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S₃ 200/200</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S₄ 200/200</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S₅ 200/200</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S₆ 200/200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control serum</td>
<td></td>
<td>200/200</td>
<td></td>
</tr>
<tr>
<td>Samples</td>
<td></td>
<td>200/200</td>
<td>200/200</td>
</tr>
<tr>
<td>Anti-hTSH-Tracer</td>
<td>←-------------------------- 100 µl --------------------------→</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wash buffer</td>
<td>←-------------------------- 1 ml --------------------------→</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shake for 2 hours (300 ± 50 rpm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Decant (aspirate) ; wash with 1 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Measure</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Fig. 2) Example of a standard curve

* — * Normal scale
· — · 10-fold enlargement
5. Radioprotection rules

This radioactive product may only be received, purchased, stored or used by persons 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 safety.

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.