Kit for the radioimmunological determination of free thyroxine (FT4) in serum

Kit is intended for professional use.

The kit comprises:

- 5 vials of $^{125}$I-FT4 tracer solution, < 150 kBq each, 105 mL, colour purple
- 10 x 50 test tubes coated with sheep anti T4 antibodies.
- 7 vials of FT4 calibrators, per 0.5 human serum and sodium azide, concentration in the nominal range of 0-70 FT4 pg/mL.
- 1 vial of FT4 control serum, 0.5 mL human serum and sodium azide, concentration stated.
- 5 vials of incubation buffer, 103 mL, colour green.
- 1 plastic bag
- 1 instruction for use.

* The values shown above are the target values. The actual values of each calibrator and control are shown on the reagents labels.

The dissolved reagents contain sodium azide as preservative. Avoid swallowing and contact with the skin or mucous membranes. Licensed under foreign equivalents of US Patent 4292 296.

1. Introduction

The iodine-containing amino acid thyroxine (T4), which is produced exclusively by the thyroid gland is a hormone whose serum concentration is regulated by a negative feedback mechanism involving thyrotropin (TSH). In healthy subjects the thyroid gland produces about 80 µg thyroxine per day, over 99.9 % of which is bound to plasma proteins in the blood. The most important carrier protein is the thyroxine-binding globulin (TBG) ; under normal conditions prealbumin (TBPA) and albumin (TBA) play a less important role. The biological half-life of T4 is about 8 days.

T4 is regarded as the hormone and precursor (or depot form) for triiodothyronine (T3), which has about 4 times the molar activity of T4, but is metabolized 10 times faster. T3 is produced mainly in the peripheral tissues from T4 by monodeiodases but is also secreted to a lesser extent by the thyroid gland itself. The physiological effect is attributed to the free hormones T3 and T4 which are not bound to the transport proteins. In T4 almost 0.03 % is present in the form of FT4. Their main function is to stimulate the metabolism. Variations from the normal level can affect all organs. In children abnormally low values can impair mental and physical development.

2. Clinical results and specific characteristics of the FT4 determination

2.1. Clinical significance of the FT4 determination

The main objective of the direct determination of FT4 is correctly to determine the thyroid metabolism even in the presence of changes in the binding proteins. Thus, for example, with simultaneous increases or reductions in the T4 and binding proteins the FT4 is in the normal range, consistent with a normal metabolic situation. A hypothyroid state, however, is always associated with a reduced, and a hyperthyroid state, always with an increased FT4 concentration.

Anomalies in binding protein can be caused both by changes in the concentration of TBG, TBPA or TBA or by changes in the binding capacity of these proteins. Thus the determination of free thyroxine gives a better indication of the metabolic situation than determining the total thyroxine alone.

Changes in binding can be due to physiological or pathological causes or to drug therapy, e.g. as a result of :

- Age, pregnancy
- Hereditary factors (e.g. familial dysalbuminaemic hyperthyroxinaemia)
- Fasting, serious diseases (NTI)
- Drugs (e.g. phenytoin, amiodarone, salicylates, heparin contraceptives).

During pregnancy or after taking oral contraceptives there is an oestrogen-induced increase in TBG. Fasting (anorexia nervosa) and serious diseases (sepsis, cardiac shock, pulmonary insufficiency, tumours, decompensated cirrhosis of the liver, terminal renal insufficiency) give rise to albumin deficiency. Drugs and free fatty acids can impair the capacity of the binding proteins.

2.2. Expected values

During a European multicentric study of RIA-gnost FT4 on 858 Euthyroid subjects, 95 % of them have been forward between 7-18 pg/ml = 9-23.2 pMol/L.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperthyroidism</td>
<td>&gt; 18 pg/mL</td>
</tr>
<tr>
<td></td>
<td>(&gt; 23.2 pMol/L)</td>
</tr>
<tr>
<td>Euthyroidism</td>
<td>7-18 pg/mL</td>
</tr>
<tr>
<td></td>
<td>(9 to 23.2 pMol/L)</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>&lt; 7 pg/mL</td>
</tr>
<tr>
<td></td>
<td>(&lt; 9 pMol/L)</td>
</tr>
</tbody>
</table>

Nevertheless, due to possible regional or laboratory-related variations it is advisable for the user of the test kit to determine his own ranges. In particular, values are decreasing in the elderly and/or in hospitalized patients (mainly those in intensive care units).
2.3. 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 0.5pg/mL (0.65 pMol/L).

2.4. Imprecision
This was evaluated with 3 samples assayed 20 times in the same series and in 20 different series.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mean (pg/mL)</th>
<th>CV (%)</th>
<th>Samples</th>
<th>Mean (pg/mL)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.18</td>
<td>8.15</td>
<td>4</td>
<td>4.30</td>
<td>14.90</td>
</tr>
<tr>
<td>2</td>
<td>14.55</td>
<td>5.10</td>
<td>5</td>
<td>14.35</td>
<td>5.06</td>
</tr>
<tr>
<td>3</td>
<td>33.30</td>
<td>3.30</td>
<td>6</td>
<td>32.75</td>
<td>6.60</td>
</tr>
</tbody>
</table>

3. Measuring principle and characteristic data of RIA-gnost® FT4
RIA-gnost FT4 is used for determining the concentration of free T4 by means of antibody-coated tubes and is carried out in two stages (2-stage assay). The serum sample is first incubated with a polyclonal solid-phase antibody and is then separated off again. In the second stage it is separated off and measured after incubation with the FT4 tracer.

In addition to the equilibria formed by T4 with natural binding proteins, there is an additional equilibrium with the antibody during the incubation of the serum.

The subsequent separation of the serum sample before the addition of the tracer prevents the natural binding proteins and other variables (see section 2.1) affecting the tracer reaction. The structure of the tracer was changed so that, compared with T4, it has higher immune reactivity to the antibody (reactive tracer). Thus an optimum curve with good accuracy can be obtained even with a low concentration of the solid-phase antibody.

4. Procedure
4.1. Equipment required
Precision micropipettes or similar with disposable plastic tips for 100 µL (0.1mL) and 1000 µL (1 mL) or 1 mL dispensor, horizontal shaker (200-350 rpm, preferably 300 rpm), gamma scintillation counter calibrated for 125 iodine measurement.

4.2. Preparation of the reagents
Do not mix reagents from different lots.

The determination of free thyroxine is performed under equilibrium conditions and is therefore temperature dependent. For this reason samples, controls, and calibrators have to be prepared at the same temperature. So, kit components stored at 2-8°C are brought to room temperature before use.

All the reagents which have not been used up should be stored at 2-8°C. Unused antibodies coated tubes after packaging opening must be stored in the plastic bag supplied with the kit.

If additional controls are used besides the kit control, care should be taken that these controls resemble the patient's samples with regards to the content of free hormones.

4.3. Preparation of the serum samples
After blood sampling, serum is obtained by the usual methods. It is assayed directly or stored for up to 24 hours at 2-8°C. Longer periods of storage should be at −20°C, preferably in aliquots, as repeated freezing and thawing should be avoided. After thawing, the serum samples must be carefully mixed.

4.4. Warning 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 for the determination of free thyroxine (FT4)

1. As shown in Table 1, a sufficient number of antibody-coated test tubes are numbered (calibrators, control serum, patient samples). All the test tubes in one batch should be treated in the same way. A new calibrator curve should be constructed for each new batch. It is recommended to perform the assays in duplicate for the calibrators, the control and the samples.
2. 100 µL of the calibrator (or patients' samples) are pipetted on to the base of the coated test tubes. A new pipette tip should be used for each sample.
3. 1000 µL incubation buffer are added to each tube (pipette or dispensor).
4. The test tubes are then shaken on a horizontal shaker for 30 min at 200-350 rpm (preferably 300 rpm) at room temperature (18-25°C).
5. They are then decanted, placed on filter paper and any traces of liquid adhering to the rim of the tube are removed by tapping the tube. Aspiration is also possible.
6. 1000 µL of iodine 125-FT4 tracer solution are then introduced into each test tube.
7. As given in 4, the tubes are shaken for 1 hour (± 10 min).
8. The solution is then removed by decanting and the tubes are placed upside down on an absorbent surface for 2 to 5 minutes. Any traces of liquid adhering to the rim of the test tube are removed by tapping briefly. Aspiration is also possible.
9. The radioactivity in the test tubes is measured for 1 minute in the 125I channel of a gamma scintillation counter.

General instructions
On occasions where large numbers of samples are to be assayed, reagents have to be pooled from more than one kit bearing the same lot number.
After having dispensed 50 single specimens into the tubes it is recommended to add the incubation buffer and then allow the mixture to stand. Likewise, the serum samples are then dealt with.
It is important to ensure that the batch is processed quickly under same conditions (temperature and shaker). There should not be more than 200 tubes in each batch. All the serum samples to be measured are then related to a calibrator curve. For the sake of simplicity, the 1000 µL of buffer and tracer solution required (stages 3 and 6) can be added with a multipipette.

4.6 Evaluation of the results
For each group of tubes, compute the mean counts. Calculate B/T value for the CAL0 and calculate B/Bo values for all the calibrators and control. Draw up the calibration curve by plotting the B/Bo of the calibrators against their concentrations. Read sample values directly from the calibration curve.
Conversion to pmol/L may be accomplished by using the following equation: FT4 (pmol/L) = FT4 (pg/mL) x 1.29.
The hyperbolic mathematical fitting model is recommended for calibration curve. Other data reduction functions may give slightly different results.

Example of a calibrator curve

<table>
<thead>
<tr>
<th>Tubes group</th>
<th>Mean cpm</th>
<th>B/T x 100</th>
<th>B/Bo x 100</th>
<th>Concentration pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>68231</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calibrator 0</td>
<td>37254</td>
<td>54.6</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Calibrator 1</td>
<td>30771</td>
<td>82.6</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>Calibrator 2</td>
<td>27062</td>
<td>72.3</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>Calibrator 3</td>
<td>21072</td>
<td>56.5</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td>Calibrator 4</td>
<td>18088</td>
<td>48.5</td>
<td>18.5</td>
<td></td>
</tr>
<tr>
<td>Calibrator 5</td>
<td>13489</td>
<td>36.2</td>
<td>39.0</td>
<td></td>
</tr>
<tr>
<td>Calibrator 6</td>
<td>11156</td>
<td>29.9</td>
<td>70.0</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>22040</td>
<td>59.1</td>
<td>10.8</td>
<td></td>
</tr>
</tbody>
</table>

4.7 Interference
No interference with bilirubin, haemoglobin and triglycerides, measured up to respective concentrations of equal to 250 mg/L, 10 g/L and 20 g/L, has been observed.
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.

ASSAY FLOW CHART

<table>
<thead>
<tr>
<th>Labelling of the test tubes</th>
<th>Calibrators (µL)</th>
<th>Control serum (µL)</th>
<th>Samples (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAL 0</td>
<td>CAL 1</td>
<td>CAL 2</td>
</tr>
<tr>
<td>Calibrators</td>
<td>100/100</td>
<td>100/100</td>
<td>100/100</td>
</tr>
<tr>
<td>Control serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients' samples</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incubation buffer (green)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shake for 30 min / 200-350 rpm at RT, preferably 300 rpm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shake and tap on to filter paper or aspirate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>125 I-FT4 tracer solution (violet)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shake for 60 min / 200-350 rpm at RT, preferably 300 rpm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shake and tap on to filter paper or aspirate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measure for 1 minute</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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BIBLIOGRAPHY:


