1. NAME AND INTENDED USE

hGH-RIACT is an immunoradiometric assay for a direct quantitative determination of human growth hormone (hGH) in serum.

2. INTRODUCTION

The human Growth Hormone (hGH) is a polypeptidic hormone consisting of 191 amino acids linked by two disulphide bonds, with a molecular weight of 22kD. It is synthesized by the somatotropic cells of the anterior pituitary in the form of a precursor or pre-hormone whose proteolytic cleavage gives rise to the active hormone. Its pulsatile secretion is controlled by the hypothalamus (neurotransmitters) and by physiological stimuli (stress, sleep, physical exercise, etc.). It circulates in the blood either in a free form or bound to proteins such as α2 macroglobulin and hGHBP (human growth hormone binding protein).

Human GH triggers the hepatic synthesis of insulin-like growth factor (IGF1), which is responsible for the growth of long bones (indirect effect). The hormone also has metabolic effects, such as protein synthesis, nitrogen retention, the increase of plasmatic fatty acid levels and of hyperglycemic properties (stimulation of neoglycogenesis and hepatic glycogenolysis).

It must be emphasized that hGH-N gene transcription gives rise to 2 proteins:
- GH 22 kD, which has a biological activity;
- GH 20 kD which only has a very weak biological activity (mainly a hyperglycemic effect).

The proportion of GH 20 kD compared to 22 kD in plasma is around 16%.

3. PRINCIPLE

hGH-RIACT is a solid phase two-site immunoradiometric assay. Two monoclonal antibodies were prepared against sterically remote antigenic sites on the hGH molecule, the first one is coated on the solid phase (coated tube), the second one radiolabelled with iodine 125 is used as a tracer.

The hGH molecules present in the standards or the samples to be tested are “sandwiched” between the two antibodies. Following the formation of the coated antibodies/antigen/iodinated antibody sandwich, the unbound tracer is easily removed by a washing step. The radioactivity bound to the tube is proportional to the concentration of hGH present in the sample.

4. REAGENTS

Each kit contains enough reagents for 100. 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 : ready for use.</td>
<td>2 packs of 50 tubes</td>
<td>2-8°C until the expiry date. Tubes removed from their packs must be stored in the bag supplied with the kit.</td>
</tr>
<tr>
<td>ANTI-hGH 125I : ready for use.</td>
<td>1 31 ml vial</td>
<td>2-8°C until the expiry date.</td>
</tr>
<tr>
<td>STANDARD 0 : lyophilized.</td>
<td>2 ml vial</td>
<td>2-8°C until the expiry date. After reconstitution, 2 days at 2-8°C.</td>
</tr>
<tr>
<td>STANDARDS : lyophilized.</td>
<td>0.5 ml vials</td>
<td>2-8°C until the expiry date. After reconstitution, 2 days at 2-8°C.</td>
</tr>
<tr>
<td>CONTROL : lyophilized.</td>
<td>0.5 ml vials</td>
<td>2-8°C until the expiry date. After reconstitution, 2 days at 2-8°C.</td>
</tr>
<tr>
<td>Tween 20 : concentrated solution.</td>
<td>1 10 ml vial</td>
<td>2-8°C until the expiry date. After dilution, store in a capped container for 15 days maximum.</td>
</tr>
</tbody>
</table>

(*) The values shown above are only target values: the true value of each standard or control is shown on its label. The standards are calibrated against the 2nd international standard WHO IS 98/574 (Somatropin – NIBSC – version dated 02 November 2000).

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.

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5.2. Basic 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 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 used for washing. Fully respect the incubation conditions and the washing instructions indicated.

6. SPECIMEN COLLECTION AND PREPARATION
The assay is performed directly on serum. If the assay is performed within 24 hours, the samples should be kept at 2-8°C. Otherwise, they should be divided into aliquots and stored deep frozen (-20°C).

Dilution
Should elevated hGH levels be suspected, the dilution is performed with the standard 0 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 50 µl, 300 µl, 500 µl and 2 ml (± 1 %). Their calibration should be checked regularly.
Equipment suitable for this assay is available from 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. Reconstitution and dispensing of the reagents into the tubes are also carried out at room temperature (18-25°C).
The assay requires the following groups of tubes:
Standard 0 group for the determination of non-specific binding,
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 triplicate for standard groups, in duplicate for samples.
Observe the order in which reagents are to be added:
Dispense 50 µl of standards, control or samples into the corresponding groups of tubes.
Add 300 µl of monoclonal anti-hGH tracer in each tube.
Incubate 2 hours ± 5 minutes at room temperature (18-25°C) under agitation (400 rpm).
Wash the coated tubes as follows:
Aspirate the content of the tubes as completely as possible.
Add 3.0 ml of washing solution (diluted tween 20) to each tube, wait 5 minutes before emptying them again.
Repeat the process one more.
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. As much as possible of the incubation and washing solutions must be removed. If the washing is carried out manually, the tip of the aspirating device must be placed right at the bottom of the tube.
Measure the radioactivity bound to the coated tubes with a gamma scintillation counter.

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
For each group of tubes, calculate the mean counts after subtracting the background.
Draw up the standard curve by plotting the standard’s cpm against their concentrations.
Read the sample values directly from the curve, correcting the read value for the dilution factor, if necessary.
Typical standard curve (example only) : these data must under no circumstances be substituted for results obtained in the laboratory.

<table>
<thead>
<tr>
<th>Tube groups</th>
<th>Mean cpm</th>
<th>Concentration (µIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 0</td>
<td>36</td>
<td>0</td>
</tr>
<tr>
<td>Standard 1</td>
<td>373</td>
<td>0.21</td>
</tr>
<tr>
<td>Standard 2</td>
<td>2957</td>
<td>2.3</td>
</tr>
<tr>
<td>Standard 3</td>
<td>14213</td>
<td>12.2</td>
</tr>
<tr>
<td>Standard 4</td>
<td>31811</td>
<td>33</td>
</tr>
<tr>
<td>Standard 5</td>
<td>50108</td>
<td>65.6</td>
</tr>
<tr>
<td>Control</td>
<td>10622</td>
<td>8.9</td>
</tr>
<tr>
<td>A</td>
<td>5310</td>
<td>4.4</td>
</tr>
<tr>
<td>B</td>
<td>20321</td>
<td>18.3</td>
</tr>
<tr>
<td>C</td>
<td>35243</td>
<td>38.3</td>
</tr>
</tbody>
</table>

It is recommended to smooth the curve with a spline model.

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 samples concerned and re-assay.

11. EXPECTED VALUES
The values given below are only indicative and it is recommended for each laboratory to establish its own normal range.

Distribution of normal values
These values have been obtained from presumed healthy adult subjects from both sexes (n = 109). All values are in the 0 – 28.5 µIU/ml range and 93 % are below 15 µIU/ml.

12. SPECIFIC CHARACTERISTICS OF THE ASSAY
12.1. Imprecision
This has been assessed either using 2 samples with different concentrations 30 times in the same serie of assay or with 4 samples in triplicate in 24 different series.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mean (µIU/ml)</th>
<th>Within-run (C.V. %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.1</td>
<td>2.1</td>
</tr>
<tr>
<td>2</td>
<td>63</td>
<td>1.3</td>
</tr>
</tbody>
</table>

12.2. Recovery test
Known quantities of hGH were added to human sera. The recovery percentages of hGH in the samples ranged from 95 to 110 %.

12.3. Dilution test
Samples with high level were diluted. Recovery percentages obtained were between 100 and 122 %.

12.4. Specifity
The antibodies used in this assay do not present any cross reaction with the structurally similar substances : hPL and prolactin. Furthermore, the cross reaction with the 20kD hGH is less than 5 % for concentration up to 3 750 µIU/ml (22kD hGH proportional concentration above 24 000 µIU/ml).

12.5. Detection limit.
The detection limit is defined as being the smallest detectable concentration different from zero with a probability of 95 %. It has been assessed as being 0.03 µIU/ml.

ASSAY FLOW CHART

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Standards µl</th>
<th>Samples µl</th>
<th>[125I]anti-hGH µl</th>
<th>Incubate for 2 hours ± 5mn under agitation at 18-25°C</th>
<th>Wash 2 times with the washing solution</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standards</td>
<td>50</td>
<td>-</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>50</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samples</td>
<td>-</td>
<td>50</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
12.6. Measuring range: 0.03 NIU/ml – 65.6 NIU/ml