Kit for the radioimmunological determination of human luteinizing hormone (hLH)

The kit comprises:

1. 1 vial of 125I-anti-hLH antibody (monoclonal, mouse), < 300 kBq, 11 ml buffer with mouse immunoglobulins, bovine albumin, sodium azide and a red dye.
2. 2 x 50 test tubes coated with anti-hLH antibodies (monoclonal, mouse).
3. 1 vial of hLH standard, solution per 2 ml human serum and sodium azide, 0 mIU/mL.
4. 6 vials of hLH standards, per 0.5 ml lyophilised human serum and sodium azide, concentration in the nominal range of 1-200 mIU hLH/mL (standards are calibrated against certified reference preparations hLH WHO 2nd IRP 80/552).
5. 1 vial of hLH control serum, 0.5 ml lyophilised human serum and sodium azide, concentration stated.
6. 1 tube of wash reagent, 3 buffer tablets.
7. 1 plastic bag
8. 1 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

Human luteinizing hormone (lutropin, hLH) is a glycoprotein which is formed in the anterior lobe of the pituitary. The biological half-life of hLH in the circulation is 20 to 25 minutes.

hLH consists of two polypeptide chains which are known as α and β subunits. The β subunit determines the specific biological action and the immunological behaviour of the hormone. The α subunit is identical to the α subunits of other human glycoprotein hormones such as follicle-stimulating hormone (hFSH), chorionic gonadotropin (hCG) and thyrotropic hormone (hTSH).

The primary action of hLH is the regulation of gonadal function. hLH controls the synthesis of sex steroids in women the synthesis of progesterone in the ovary and in men that of testosterone in the testes. Together with hFSH it is involved in those mechanisms which trigger ovulation.

Synthesis of the gonadotropins hLH and hFSH in the pituitary and their release are stimulated by the luteinizing hormone-releasing hormone (LH-RH) formed in the hypothalamus. The sex steroids regulate gonadotropin secretion by means of a multiple feedback mechanism (negative inhibition-by-effects and positive-stimulation).

2. Clinical results obtained with RIA-gnost® hLH

2.1. Clinical significance of the quantitative hLH assay

Synthesis of the gonadotropins in the anterior lobe of the pituitary and their concentration in the serum is controlled by the regulatory mechanism mentioned above, involving the hypothalamus, pituitary and gonads. If one part of the regulatory mechanism between the pituitary gland and gonads is impaired, the basal concentration of hormones and gonadotropins in the serum may be either raised (primary hypogonadism) or in the lower range of normal (secondary hypogonadism) as a result of the interaction between sex hormones and gonadotropins.

More frequently, however, a pathological condition in which basal values are normal or in the upper range of normal, is only detectable from the lack of secondary hLH in the serum may be either raised (primary hypogonadism) or overshooting (primary hypogonadism) stimulation of the anterior lobe of the pituitary when LH-releasing hormone is administered intravenously. Release of hLH is relatively constant and low in fertile men whereas in fertile women there is a cyclical release of the hormone. Typical of a normal menstrual cycle is a 1:2 day peak in the middle of the cycle which plays an important role in triggering ovulation. Before and after ovulation the hLH concentration is about the same as in healthy men. During the menopause the basal hLH concentration rises, then falls again in the post-menopause period.

The determination of hLH therefore facilitates differential diagnosis of disorders of human fertility, i.e.: distinguishing between the primary (ovarian) and secondary (hypophysial) cause of disorders of the female cycle and the corresponding classification of hypogonadism in men. In children determination of hLH helps in the diagnosis of pubertal disorders (delayed or precocious puberty). Another indication is the determination of ovulation in the treatment of infertility.

2.2. Normal values

2.2.1. Basal values

During the course of clinical trials with RIA-gnost® hLH, the normal range of basal hLH concentrations was calculated for women with a normal menstrual cycle (i.e. not taking oral contraceptives), for men and for children in the prepubertal phase.

The 5th and 95th percentiles were selected as the lower and upper limits for the determination of the normal range. The values are given in mIU hLH (WHO 2nd IRP 80/552/mIU) (tab.1).

The hLH level in healthy adults (1-10 mIU/ml) usually differs markedly from the raised serum values in patients with primary hypogonadism (5-22 mIU/ml) and in women in the luteal phase of the menstrual cycle (85-125 mIU/ml). In secondary hypogonadism the basal values up to 1 mIU/ml are within the lower range of normal values for healthy subjects.

Table 1: hLH basal values (mIU/ml in healthy subjects and patients with infertility disorders)

<table>
<thead>
<tr>
<th>Gender</th>
<th>n</th>
<th>Median</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular phase</td>
<td>10</td>
<td>3.1</td>
<td>1 - 7</td>
</tr>
<tr>
<td>Midcycle</td>
<td>41</td>
<td>18.7</td>
<td>6 - 73</td>
</tr>
<tr>
<td>Luteal phase</td>
<td>65</td>
<td>3.1</td>
<td>0.5 - 10</td>
</tr>
<tr>
<td>Post-menopause &gt; 50 years</td>
<td>89</td>
<td>26.3</td>
<td>12 - 58</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 10 years</td>
<td>147</td>
<td>2.2</td>
<td>1 - 5</td>
</tr>
<tr>
<td>&lt; 12 years</td>
<td>30</td>
<td>-</td>
<td>0 - 1</td>
</tr>
<tr>
<td>Hypogonadism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>26</td>
<td>15.1</td>
<td>5 - 22</td>
</tr>
<tr>
<td>Secondary</td>
<td>10</td>
<td>-</td>
<td>&lt; 1</td>
</tr>
</tbody>
</table>

2.2.2 Stimulation test with LH-RH

In disorders of the regulatory mechanism stimulation with LH-releasing hormone and subsequent determination of hLH (and hFSH) gives an indication of the function and reserve capacity of the anterior pituitary in terms of these two gonadotropins.

Basal values and stimulation values indicate whether and where disorders have occurred in the regulatory mechanism, i.e. the reproductive glands (testes, ovary) or in the central regulatory gland (pituitary).

The serum concentration of hLH increases in healthy adults of reproductive age 30 minutes after i.v. administration of 100μg LH-RH to, on average, 4-5 times (2-8 times) the basal value. In secondary hypogonadism there is only very little or no increase whereas the values in primary hypogonadism rise significantly, usually with a marked increase in basal values.

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

RIA-gnost® hLH permits the in vitro determination of luteinizing hormone in human serum (or plasma) using the principle of a 1-step sandwich assay. A complex of anti-hLH antibodies (monoclonal, mouse) which are bound to the tube wall, hLH in the sample and 125I-labelled anti-hLH antibodies (monoclonal, mouse) is formed during this process. At the end of the reaction the amount of free tracer is removed by decantation (or aspiration) and subsequent washing.

The amount of tracer specifically bound to the coated test tubes is measured with a gamma scintillation counter.

The determination of the unknowns is carried out by reading off from a standard curve constructed under identical conditions.

When carrying out duplicate determinations it is possible to measure a maximum of 42 patient samples by constructing a standard curve.

The monoclonal antibodies used in the kit are very specific for hLH. There is virtually no risk of cross-reactivity with hTSH, hFSH and hCG in the physiologically relevant concentration ranges. Samples outside the measuring range are diluted with S₀.
Tab 2 : hLH assay procedure

<table>
<thead>
<tr>
<th>Labelling of the test tubes</th>
<th>Standards (µl)</th>
<th>Control serum (µl)</th>
<th>Patients samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S0</td>
<td>S1</td>
<td>S2</td>
</tr>
<tr>
<td>Standard</td>
<td>100/100</td>
<td>100/100</td>
<td>100/100</td>
</tr>
<tr>
<td>Control serum C</td>
<td>100/100</td>
<td>100/100</td>
<td>100/100</td>
</tr>
</tbody>
</table>

**Anti-hLH tracer**

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Incubate for 2 hours (horizontal shaker)

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1 ml

Decant (aspirate) : wash with 1 ml

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Measure for 1 minute

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### 4. Working procedure

#### 4.1. Equipment required

Microtiter pipettes with removable plastic tips : 500 µl, measuring cylinders, horizontal shakers, dispensers : 1 ml, gamma scintillation counter.

#### 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). The standards (apart from S0) and the control serum are dissolved in 500 µl twice-distilled water. The washing buffer is prepared by dissolving three buffer tablets in 300 ml distilled water.

All unused reagents 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.

#### 4.3. Preparation of the serum samples

When blood samples have been taken, 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 stored for a longer period, the temperature should be –20°C. The samples should be carefully refrigerated after thawing or after they have been taken from the refrigerator.

#### 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. Assay procedure (see Table 2)

1. Number sufficient coated tubes as stated in Table 2 (standards, control serum, serum samples).
2. Pipette 100 µl standard (or patient samples) on to the bottom of the coated tubes. Use a new pipette tip for each sample.
3. Dispense 100 µl 125I anti-hLH into each test tube.
4. Shake the test tubes on a horizontal shaker for 2 hours at 17-27°C.
5. Then pipette 1 ml washing buffer into each test tube, decant (aspirate) and wash again with 1 ml.
6. Measure the tubes for 1 minute in the gamma scintillation counter.

#### General notes

- On occasions where large numbers of samples are to be assayed, reagents from more that one kit bearing the same lot number have to be pooled. Under these circumstances all unknowns must be referred to a single standard curve.
- Further information and recommendations concerning alternative test methods, e.g. determination in the urine, are available on request.

#### 4.6. Evaluation of the results

A typical standard curve for RIA-gnost® hLH (coated tube) is shown in Fig 1. The mean is calculated from the measured values of the control serum and the patients’ sera and the hLH content per millilitre of serum is read off from the standard curve.

**Fig 1. Example of a standard curve**

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### 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 receptions 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 other 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.