Kit for the radioimmunological determination of human follicle-stimulating hormone

The kit comprises:

1. vial of $^{125}$I-hFSH, < 300 kBq, 11 ml buffer with bovine albumin, mouse monoclonal antibodies, sodium azide and red dye.
2. 2 x 50 test tubes coated with anti-hFSH antibodies (monoclonal, mouse).
3. 1 vial of hFSH standard, 2 ml buffer, human serum, bovine albumin and sodium azide, 0 mIU hFSH / ml.
4. 6 vials of hFSH standards, per 0.5 ml human serum, bovine albumin and sodium azide, concentration in the nominal range of 1-200 mIU hFSH/ml.
5. 1 vial of hFSH control serum, 0.5 ml human serum, bovine albumin and sodium azide, concentration stated.
6. 1 tube of wash reagent, 3 buffer tablets.
7. 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 follicle-stimulating hormone (folitropin, hFSH) is a glycoprotein which is formed in the anterior lobe of the pituitary. The biological half-life of hFSH in the circulation is 5-7 minutes.

hFSH consists of two polypeptide chains which are known as $\alpha$ and $\beta$ subunits. The $\beta$ subunit determines the specific biological action and the immunological behaviour of the hormone. The $\alpha$ subunit is identical to the $\alpha$ subunits of other glycoprotein hormones such as leuteinizing hormone (hLH), chorionic gonadotropin (hCG) and thyrotropic hormone (hTSH).

The primary action of hFSH is the regulation of gonadal function. Together with hLH, hFSH controls the synthesis of sex steroids and maturation in the ovum in women. In men spermatogenesis is stimulated by hFSH via the androgen-binding protein.

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.

2. Clinical results obtained with RIA-gnost® hFSH

2.1. Clinical significance of the quantitative hFSH 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 hFSH in the serum may be either raised (primary hypogonadism) or lowered (secondary hypogonadism) as a result of the interaction between sex hormones and gonadotropins.

More frequently, however, the disorder between the pituitary and the gonads, in which basal values of hFSH are normal or in the upper range of normal, is only detectable from the lack of (secondary hypogonadism) or overshooting (primary hypogonadism) stimulation of the anterior lobe of the pituitary when LH-releasing hormone is administered intravenously.

Like LH secretion, the release of hFSH is relatively constant and low in fertile men, whereas in fertile women there is a cyclical release of the hormone.

In women an hFSH peak is observed at the same time as the rise in LH, but it is not so pronounced. Whereas the LH level remains low, apart from this peak, the hFSH values are also slightly raised in the follicular phase. During the menopause there is a marked rise in hFSH concentration but it falls again in the post-menopause period.

The determination of hFSH facilitates differential diagnosis of disorders of human fertility, i.e. : distinguishing between the primary (ovarian) and secondary (hypothalamic) cause of disorders of the female cycle and the corresponding classification of hypogonadism in men. In children determination of hFSH helps in the diagnosis of pubertal disorders (delayed or precocious puberty).

2.2. Normal values

2.2.1 Basal values

During the course of clinical trials with RIA-gnost® hFSH, the normal range of basal hFSH 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 hFSH (WHO 2nd IRP 78/549/ml (Tab.1).

The normal range in healthy adults (1-9 mIU/ml) usually differs from the raised basal values in patients with primary hypogonadism (9-100 mIU/ml) and in women in the post-menopause period (19-130 mIU/ml). In secondary hypogonadism the basal values up to 4 mIU/ml are within the range of normal values for healthy subjects.

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

<table>
<thead>
<tr>
<th></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>91</td>
<td>4.7</td>
<td>3 - 8</td>
</tr>
<tr>
<td>Middle of cycle</td>
<td>32</td>
<td>6.9</td>
<td>4 - 18</td>
</tr>
<tr>
<td>Luteal phase</td>
<td>89</td>
<td>3.6</td>
<td>2 - 8</td>
</tr>
<tr>
<td>Post-menopause &gt; 50 years</td>
<td>92</td>
<td>67</td>
<td>19 - 130</td>
</tr>
<tr>
<td>Men</td>
<td>147</td>
<td>3.6</td>
<td>1 - 9</td>
</tr>
<tr>
<td>Children</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 10 years</td>
<td>31</td>
<td>1.1</td>
<td>0.2 - 3.4</td>
</tr>
<tr>
<td>&gt; 12 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypogonadism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>primary</td>
<td>30</td>
<td>43</td>
<td>9 - 100</td>
</tr>
<tr>
<td>secondary</td>
<td>10</td>
<td>1.2</td>
<td>&lt; 4</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 hFSH increases in healthy adults of reproductive age 30 to 60 minutes after i.v. administration of 100µg LH-RH to about twice the basal values. 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® hFSH

3.1. Principle

RIA-gnost® hFSH enables the in vitro determination of follicle-stimulation hormone in human serum (or plasma) using the principle of a 1-stage sandwich assay. A complex of anti-hFSH antibodies (monoclonal, mouse) which are bound to the tube wall, hFSH in the sample and $^{125}$I-labelled anti-hFSH 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 evaluation of the unknowns is carried out by reading off from a standard curve constructed under identical conditions.

The monoclonal antibodies used in the kit are very specific for hFSH. There is virtually no risk of cross-reactivity with hLH, hTSH and hCG in the physiologically relevant concentration ranges. Samples outside the measuring range are diluted with S. The standards are calibrated against hFSH WHO 2nd IRP 78/549.
3.2. Specific characteristics of the assay

3.2.1 Imprecision

This was evaluated with 3 samples assayed 10 times in the same series and in 30 different series.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mean (mIU/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-run</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.4</td>
<td>3.4</td>
</tr>
<tr>
<td>2</td>
<td>10.7</td>
<td>2.9</td>
</tr>
<tr>
<td>3</td>
<td>13.5</td>
<td>2.6</td>
</tr>
<tr>
<td>Between-run</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.3</td>
<td>3.0</td>
</tr>
<tr>
<td>2</td>
<td>10.6</td>
<td>2.5</td>
</tr>
<tr>
<td>3</td>
<td>13.7</td>
<td>2.6</td>
</tr>
</tbody>
</table>

3.3. Detection limit

The detection limit of the method is defined as being the smallest concentration different from 0 with a confidence interval of 95%. It has been determined as being 0.10 mIU/ml.

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 that the unbound tracer fraction is completely removed (decantation/aspiration). During aspiration the capillary tubes must not become blocked; after decantation tap them well on to cellulose.

c) Check the measuring device and any associated equipment that may be used to ensure that the background effect is kept constant, and if necessary decontaminate.

d) Exclude the adverse effect of external sources of radiation.

4. Working procedure

4.1. Equipment required

Microtitre pipettes with removable plastic tips: 100 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 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 serum samples should be carefully mixed after thawing and 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 1)

1. Number sufficient coated tubes as stated in Table 2 (standards, control serum, serum samples).

2. Pipette 100µl standard or patient samples on to the bottom of the coated tubes. Use a new pipette tip for each sample.

3. Dispense 100µl [125I] anti-hFSH into each test tube.

4. Shake the test tubes on a horizontal shaker for 2 hours at 17-27°C.

5. Then introduce 1 ml wash 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 than one kit bearing the same lot number have to be pooled.
- 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® hFSH (coated tube) is shown in figure 1. The measured values of the control serum and the patients’ samples are marked on the graph and the desired hFSH-content per millilitre is read off from the standard curve.

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.

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Tab 2: hFSH assay procedure

<table>
<thead>
<tr>
<th>Standards (µl)</th>
<th>Control serum (µl)</th>
<th>Samples (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₀</td>
<td>S₁</td>
<td>C 1 2 Etc.</td>
</tr>
<tr>
<td>S₂</td>
<td>100/100</td>
<td></td>
</tr>
<tr>
<td>S₃</td>
<td>100/100</td>
<td></td>
</tr>
<tr>
<td>S₄</td>
<td>100/100</td>
<td></td>
</tr>
<tr>
<td>S₅</td>
<td>100/100</td>
<td></td>
</tr>
<tr>
<td>S₆</td>
<td>100/100</td>
<td></td>
</tr>
<tr>
<td>Control serum C</td>
<td>100/100</td>
<td></td>
</tr>
<tr>
<td>Patients’ samples</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>¹²⁵I anti-hFSH tracer</td>
<td>100 µl =--------------------------►</td>
<td></td>
</tr>
</tbody>
</table>

incubate for 2 hours (horizontal shaker)

Decant (aspirate); wash with 1 ml

Measure for 1 minute

Fig 1. Example of a standard curve

* --- Normal scale

--- 10-fold enlargement

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