Kit for the radioimmunological determination of human prolactin.

The kit contains:

1 vial of $^{125}$I-prolactin antibodies (monoclonal, mouse), < 300 kBq, 11 ml buffer with bovine albumin, mouse monoclonal antibodies, sodium azide and red dye.

2 x 50 test tubes coated with anti-prolactin antibodies (monoclonal, mouse).

5 vials of prolactin standards, 0.3 ml freeze dried, buffer, human serum, bovine albumin and sodium azide, concentration in the nominal range of 70 - 6000 µIU human prolactin/ml.

1 vial of prolactin control serum, 0.3 ml freeze dried, human serum, bovine albumin and sodium azide, concentration stated.

1 vial of diluent/zero standard, 20 ml, buffer, bovine albumin and sodium azide, blue color.

1 tube of wash reagent, 5 buffer tablets.

1 plastic bag.

1 User’s instructions

The dissolved reagents contain sodium azide as a 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

Prolactin is a polypeptide hormone comprising 198 amino acids with 3 disulphide bridges; its molecular weight is approximately 22 000. It is secreted by the anterior lobe of the pituitary and, unlike the other anterior pituitary lobe hormones, is regulated by an inhibitor substance known as prolactin inhibiting factor (PIF)* of the hypothalamus. The biological half-life of prolactin in plasma is 14-20 min.

The physiological significance of prolactin is largely unclear, particularly in men. Its role in initiating and maintaining lactation has been firmly established. Basal serum levels are elevated during pregnancy and for 6-12 weeks after parturition. The stimulus of suckling during breast-feeding produces a reflex release of prolactin. Certain metabolic effects are known, some of which have been linked with the structural similarity between prolactin and growth hormone and human placental lactogen.

2. Clinical results obtained with RIA-gnost® Prolactin

2.1. Clinical significance of the quantitative prolactin assay

Increased prolactin secretion (hyperprolactinaemia) is relatively common (the incidence is approximately 20% in secondary amenorrhoea) and may be caused by a number of factors:
- prolactin-secreting micro and macroadenomas of the anterior lobe of the pituitary (prolactinomas),
- suprasellar disturbances of PIF transport in the pituitary stalk (suprasellar tumors or cysts),
- drugs (a few psychotropic agents, dopamine antagonists, high-dose oestrogens),
- functional predominance of factors which promote prolactin secretion (elevated TRH secretion in primary hypothyroidism).

Pathologically raised serum prolactin levels result in clinically important disease states including disturbances of sexual function. In women, increases in prolactin to supranormal levels produce menstrual disturbances (anovulation, amenorrhoea), sometimes associated with galactorrhoea. Disturbed libido and potency are encountered in men with raised prolactin levels.

2.2. Indications for prolactin assay:
- women with menstrual disturbances (primary and secondary amenorrhoea),
- men with disturbed libido and potency,
- suspected prolactin-secreting pituitary adenoma,
- disturbances of sexual function after medication with drugs possessing prolactin-releasing activity.

When taking blood samples remember that physical activity, stress and gynaecological examinations may produce significant increases in prolactin concentrations. Blood samples should not be taken within 2 hours of the patient waking up; it is also essential that blood be sampled before any medical examination.

2.3. Normal values

During clinical trials with RIA-gnost® Prolactin, the normal range of basal prolactin concentrations was calculated for women with a normal menstrual cycle (i.e. not taking oral contraceptives), for women during the menopause and for men.

The upper limit of the normal range was calculated from 177 serum samples from healthy women of child-bearing age and from 178 serum samples from healthy men (95th percentile). The upper limit for women is 700 µIU prolactin/ml (median = 254 µIU/ml), and that for men is 370 µIU/ml (median = 140 µIU/ml). Prolactin levels fall markedly during the menopause (median = 186 µIU/ml, upper limit 490 µIU/ml). All values are stated in µIU prolactin (WHO IRP 84/500)/ml (Tab.1).

<table>
<thead>
<tr>
<th>Table1 : Basal prolactin concentrations in healthy men and women</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td>Women</td>
</tr>
<tr>
<td>Women (post-menopause)</td>
</tr>
<tr>
<td>Men</td>
</tr>
</tbody>
</table>

* = presumably dopamine.
2.4. TRH stimulation in normal patients
Stimulation with an i.v. dose of 200 µg TRH may be used to evaluate the reserve capacity of the anterior pituitary. Peak serum prolactin concentrations are attained after about 30 minutes. Mean concentrations rise to 1120 µIU/ml in women with a normal menstrual cycle, and to 560 µIU/ml in men. On average, these values correspond to a 5- to 6-fold increase in serum prolactin levels in the subjects tested. However, prolactin levels after TRH stimulation are subject to extreme scattering, and 2- to 10-fold increases over basal concentrations may be measured.

2.5. Pathologically raised serum prolactin levels
The increase in the basal prolactin level depends on the nature and severity of the patient's clinical condition. All values over 700 µIU/ml in women and over 370 µIU/ml in men, as measured with RIA-gnost® Prolactin, may indicate hyperprolactinaemia.

3. RIA-gnost® Prolactin principle and characteristics

3.1. Principle
RIA-gnost® Prolactin permits the in vitro determination of human prolactin in human serum or plasma using the principle of a 2-step sandwich assay. A complex of antiprolactin antibodies (monoclonal, mouse) which are bound to the tube wall, prolactin in the sample and 125I-labelled anti-prolactin antibodies (monoclonal, mouse) is formed during this process.

The amount of tracer specifically bound to the coated tubes is measured with a gamma scintillation counter. Evaluation of the unknowns is carried out by reading from a standard curve constructed under identical conditions.

When carrying out duplicate determinations, it is possible to measure a maximum of 43 patient samples by constructing one standard curve.

The monoclonal antibodies used in the kit are highly specific for prolactin. Samples outside the measuring range are diluted with diluent.

The standards are calibrated against prolactin WHO IRP 84/500.

Note:
The extremely high sensitivity of the assay can only be achieved if the following points are respected:
a. Avoid external contamination of the test tubes.
b. Ensure that the unbound tracer fraction is completely removed (decanting/aspiration). During aspiration the capillary tubes must not become blocked; after decanting tap them well onto an absorbent.
c. Regularly 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 any external sources of radiation.

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 12 different series.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mean (µIU/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>136</td>
<td>8.3</td>
</tr>
<tr>
<td>2</td>
<td>448</td>
<td>6.9</td>
</tr>
<tr>
<td>3</td>
<td>3075</td>
<td>5.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Samples</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>136</td>
<td>10.8</td>
</tr>
<tr>
<td>2</td>
<td>433</td>
<td>7.8</td>
</tr>
<tr>
<td>3</td>
<td>2914</td>
<td>7.5</td>
</tr>
</tbody>
</table>

3.2.2. Specificity
The RIA-gnost kit is specific for total prolactin: monomeric, “big” and “big-big” forms.

3.2.3. 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 2 µIU/ml.

4. Assay procedure

4.1. Equipment required
Microlitre pipettes with disposable plastic tips 50, 300 µl, measuring cylinders, horizontal shakers, 1 ml dispensers, gamma scintillation counter calibrated for 125 iodine measurement.

4.2. Reagent preparation
The kit components, which have been stored at 2-8°C, are brought up to room temperature (18-25°C). The standards and the control serum are dissolved in 300 µl distilled water. The wash buffer is prepared by dissolving five buffer tablets in 500 ml distilled water. Unused antibody coated tubes must be stored in the plastic bag supplied with the kit.

4.3. Sample preparation
Serum or plasma is obtained from blood samples by the usual methods. The serum or plasma is used directly in the assay or stored for up to 24 hours at 2-8°C. If stored for a longer period, the temperature should be −20°C. The serum samples must be carefully mixed after thawing or after they have been taken from the refrigerator. All the samples outside the measuring range are diluted 1:10 with the diluent supplied with the kit.
4.4. SAFETY 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. PROTOCOL (SEE TABLE 2)

1. Number sufficient coated tubes as stated in Table 2 (1 diluent D = S₀, 5 standards S₁-S₅, 1 control serum C, serum samples).
2. Pipette 50 µl standard or patients’ samples into the bottom of the coated tubes. Use a new pipette tip for each sample.
3. Place 100 µl diluent D in each test tube.
4. Shake the test tubes on a horizontal shaker for 30 minutes at 18-25°C.
5. Then pipette 1 ml wash buffer into each test tube, decant or aspirate and wash again with 1 ml.
6. Dispense 100 µl ¹²⁵I-anti-prolactin solution into the bottom of each tube.
7. Shake for 30 minutes, as described in 4.
8. Wash, as described in 5.
9. Measure the tubes for 1 minute in the gamma scintillation counter.

GENERAL NOTES:

On occasions when large numbers of samples are to be assayed, reagents from more than one kit with the identical lot number have to be pooled. The total batch assayed should not exceed 200 tubes.

4.6. RESULTS

The radioactivity measured in counts per minute of all individual tubes of the standards S₀-S₅ (diluent D = standard S₀) is plotted against the appropriate prolactin concentration on a prepared graph (see example of a standard curve). The “best fit” standard curve is drawn through these points.

The mean is calculated from the measured values of the control serum and the patients’ sera and the prolactin concentration in µIU per ml serum is read from the standard curve.

Table 2: Prolactin assay procedure

<table>
<thead>
<tr>
<th>Standards (µl)</th>
<th>Control serum (µl)</th>
<th>Samples (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labelling of the test tubes</td>
<td>S₀</td>
<td>S₁</td>
</tr>
<tr>
<td>Standards</td>
<td>S₀</td>
<td>D</td>
</tr>
<tr>
<td>Control serum</td>
<td>50</td>
<td>[\text{Labelling of the test tubes: Incubate for 30 minutes (horizontal shaker)}]</td>
</tr>
<tr>
<td>Patients’ samples</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Diluent D (blue)</td>
<td>100 µl</td>
<td>[\text{Wash buffer: Decant or aspirate; wash again with 1 ml}]</td>
</tr>
<tr>
<td>¹²⁵I-anti-prolactin antibodies</td>
<td>100 µl</td>
<td>[\text{Incubate for 30 minutes (horizontal shaker)}]</td>
</tr>
<tr>
<td>Wash buffer</td>
<td>1 ml</td>
<td>[\text{Measure for 1 minute}]</td>
</tr>
</tbody>
</table>

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.