The 125\textsuperscript{1}-human Luteinizing Hormone (hLH) IRMA system provides direct quantitative in vitro determination of human Luteinizing Hormone (hLH) in human serum. hLH can be assayed in the range of 0-150 mIU/ml using 100 µl serum samples.

**Introduction**

The human Luteinizing Hormone (Lutropin or LH) is a glycoprotein with a molecular weight of 30000, secreted by the adenohypophysis. Like other glycoprotein hormones (LH, TSH and HCG), hLH contains two different subunits, an α- and a β-chain, linked by noncovalent bonds. The primary structures of the α subunits of hLH and of those mentioned are virtually identical, whilst their β subunits are different. The β subunits are responsible for the immunological and biological specificity of these hormones.

The hLH synthesis and release is stimulated by the hypothalamic Gonadotrophin-Releasing Hormone (GnRH), whereas the lutein control further secretions of hLH by ovarian steroids secreted from the corpus luteum contains (S\textsubscript{2}-chain, linked by I-hLH IRMA system provides direct determination of human LH, TSH and HCG). hLH contains two different subunits, an α- and a β-chain, linked by noncovalent bonds. The primary structures of the α subunits of hLH and of those mentioned are virtually identical, whilst their β subunits are different. The β subunits are responsible for the immunological and biological specificity of these hormones.

The technology uses two high affinity monoclonal antibodies in an immunoradiometric assay (IRMA) system. The 125\textsuperscript{1} labelled signal-antibody binds to an epitope of the LH molecule spatially different from that recognized by the biotin-capture-antibody. The two antibodies react simultaneously with the antigen present in standards or samples, which leads to the formation of a capture antibody - antigen - signal antibody complex, also referred to as a “sandwich.”

During a 1-hour incubation period with shaking immuno-complex is immobilized to the reactive surface of streptavidin coated test tubes. Reaction mixture is then discarded, test tubes washed exhaustively, and the radioactivity is measured in a gamma counter. The concentration of antigen is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve plotting binding values against a series of calibrators containing known amount of hLH, the unknown concentration of hLH in patient samples can be determined.

**Contents of the kit**

1. 1 bottle of TRACER (21 ml), ready to use, containing about 740 kBq 125\textsuperscript{1}-anti-hLH and capture anti-hLH in buffer with red dye 0.1 % Na\textsubscript{2}SO\textsubscript{4}.
2. 6 vials of STANDARDS (6 x 1.0 ml), containing (S\textsubscript{1}-S\textsubscript{6}), 0.4, 2.0, 10, 40, 150 mIU/ml hLH (WHO 2\textsuperscript{nd} IS 80/552 Int.Std.) in bovine serum with 0.1% Na\textsubscript{2}SO\textsubscript{4}.
3. 1 vial of CONTROL SERUM. 1 ml of lyophilized human serum with 0.1% Na\textsubscript{2}SO\textsubscript{4}.
4. 2 boxes of COATED TUBES. Ready to use. 2 x 50 reactive test tubes, 1 x 275 mm, packed in plastic boxes.
5. 1 bottle of WASH BUFFER CONCENTRATE (20 ml), containing 0.2% Na\textsubscript{2}SO\textsubscript{4}. See Preparation of reagents.

**Materials, tools and equipment required**

Test tube rack, precision pipettes with disposables tips (100, 200 and 2000 µl), distilled water, vortex mixer, shaker, plastic foil, absorbent tissue, gamma counter.

**Recommended tools and equipment**

- Repeating pipettes (e.g. Eppendorf or else), required volumes in microlitres)
- Caution!
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- Homogenize all reagents and samples by gentle mixing to avoid foaming.
- Pipette 100 µl of standards, control and samples into the properly labelled tubes. Use rack to hold the tubes. Do not touch or scratch the inner bottom of the tubes with pipette tip.
- Pipette 200 µl of tracer into each tube.
- Seal all tubes with a plastic foil. Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube (min. 600 rpm recommended).
- Incubate tubes for 1 hour, shaking at room temperature.
- Add 2.0 ml of diluted wash buffer to each tube. Decant the supernatant from all tubes by the inversion of the rack. In the upside down position place the rack on an absorbent paper for 2 minutes.
- Return the tube rack to an upright position, and repeat step-8 one more time.
- Count each tube for at least 60 seconds in a gamma counter.

**Calculation of results**

The calculation is illustrated using representative data. The assay data collected should be similar to those shown in Table 2.

**Calculation of results**

Using semi-logarithmic graph paper plot B/T (%) for each standard versus the exponential (cpm) for each pair of assay tubes.

**Table 1. Assay Protocol, Pipetting Guide (all volumes in microlitres)**

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Total</th>
<th>Standard</th>
<th>Control</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Shake for 1 hour at room temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decant the fluid and blot on filter paper</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decant the fluid and blot on filter paper</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count radioactivity (60 sec/tube)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculate the results</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of hLH. The average per cent recovery for 9 serum pools spiked with hLH at 3 levels was: 98.6 ± 4.3 (mean ± SD).

Dilution test (linearity)

Three samples were measured in a series of dilution with zero-standard. The following equation obtained for measured (Y) versus expected (X) concentration demonstrates the good linearity:

\[ Y = 0.9688X + 0.1455 \]
\[ R = 0.9986 \quad n=12 \]

Expected Values

male: 1.9 - 9.4 mIU/ml
female:
- ovulatory peak: 25-94 mIU/ml
- pre- and postovulatory: 0.7-9.0 mIU/ml
- postmenopausal: 13-80 mIU/ml

It is recommended that each laboratory determine a reference range for its own patient population.

Limitations

- The reagents supplied in this kit are expected to have concentrations greater than the highest standard should be diluted with the S1 (0 mIU/ml) and reassayed.
- The results of this assay should be used in conjunction with other pertinent clinical information.

Procedural notes

Source of error!
Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.

Source of error!
To ensure the efficient rotation, tubes should be stored very tightly inside the test tube rack. Never use a rack type with open hole. An uneven or incomplete shaking may result in a poor assay performance.

Addition of wash buffer.
For the addition of wash buffer, the use of a common laboratory dispenser equipped with a 1-L glass bottle, and a flexible outlet tubing end is recommended. In lack of this tool a large-volume syringe attached to a repeating pipette can be used.

Additional information.
Components from various lots or from kits of different manufacturers should not be used.

Table 2. Typical assay data

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Count cpm</th>
<th>Mean cpm</th>
<th>B/T%</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>306942</td>
<td>306821</td>
<td>-</td>
</tr>
<tr>
<td>S1</td>
<td>87</td>
<td>104</td>
<td>96 0.03</td>
</tr>
<tr>
<td>S2</td>
<td>699</td>
<td>734</td>
<td>717 0.23</td>
</tr>
<tr>
<td>S3</td>
<td>2957</td>
<td>2901</td>
<td>2929 0.95</td>
</tr>
<tr>
<td>S4</td>
<td>14065</td>
<td>14110</td>
<td>14088 4.6</td>
</tr>
<tr>
<td>S5</td>
<td>54103</td>
<td>54115</td>
<td>54109 17.6</td>
</tr>
<tr>
<td>S6</td>
<td>17532</td>
<td>17543</td>
<td>175234 57.1</td>
</tr>
<tr>
<td>C</td>
<td>11538</td>
<td>11337</td>
<td>11438 3.7</td>
</tr>
</tbody>
</table>

Reproducibility

To determine inter-assay precision 4 patient samples were assayed in duplicates in 15 independent assays by 2 operators using different kit batches. Values obtained are shown below.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of replicates</th>
<th>Mean value</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>52.3</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>29.9</td>
<td>0.3</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>6.6</td>
<td>0.1</td>
<td>1.4</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>0.2</td>
<td>0.02</td>
<td>9.3</td>
</tr>
</tbody>
</table>

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