Human Growth Hormone

ELISA

Intended Use:
The Quantitative determination of Growth Hormone concentration in Human serum by a Microplate Immunoenzymometric assay.

Summary and Explanation Of The Test:
Growth Hormone (hGH, Somatotropin) secreted from the pituitary gland is a polypeptide consisting of 191 amino acids. hGH is a major growth factor and stimulates the growth of almost all tissues in the body. The biological actions of the hormone are in direct proportion to the concentration of its target organs. In the absence of hGH, growth and development of the body are very slow. Excessive amounts of hGH in the body for any reason can lead to gigantism in children or acromegaly in adults. The presence of elevated hGH levels can be assessed by measurement of hGH in the blood. hGH concentration in the blood changes with time, so the time of sampling is very important. The concentration of hGH in the blood is influenced by sleep, exercise, feeding, growth, stress, and many hormones. Moreover, it is also influenced by endocrine glands. Therefore, the hGH concentration in the blood can indicate the presence of disease. For example, the concentration of hGH in the blood can be increased in Cushing's disease, and decreased in hyperprolactinemia and primary hypothyroidism.

Test Procedure:
1. Five hundred units of streptavidin is added to each well of a 96-well microtiter plate. The sample is added, and after incubation, the complex is washed off. The plate is dried and then incubated with an enzyme conjugate. After incubation, the complex is washed off. The plate is dried and then incubated with a suitable substrate to produce color. The color intensity is measured spectrophotometrically. The amount of hGH in the sample can be calculated from a standard curve.

Principle:
First, the antigen is prepared by adding an excess of hGH to a streptavidin-coated well. Then, the antibody is added to the well, and the mixture is washed to remove unbound antibody. The complex of antibody-bound hGH is then washed, and the plate is dried. The plate is then incubated with an enzyme conjugate labeled with hGH. After washing, the plate is incubated with a suitable substrate to produce color. The amount of hGH in the sample can be calculated from a standard curve.

Reagents:
- Enzyme-labeled antibody (hGH) 1x6ml
- Wash Buffer
- Wash Concentrate
- Working Substrate Solution
- Stop Solution
- Substrate
- Immobilized Complex

Materials Provided:
- Growth Hormone Kit Contents (Volume)
- Growth Hormone Calibration, hGH Antigen
- hGH Enzyme Reagent
- Wash Solution Concentrate
- Substrate A
- Substrate B
- Streptavidin coated Microplate
- Stop Solution

Note:
- Do not use reagents beyond the expiration date.
- Do not mix different lot numbers.
- Use within 60 days.

Precautions:
- Store reagents at room temperature (20-22°C).
- Do not thaw reagents before use.
- Do not use reagents beyond the expiration date.
- For accurate results, the sample should be fresh.

Quality control materials:
- hGH Standards, hGH Control

Equipment:
- Microplate Reader with 450nm and 620nm wavelength
- Dispenser(s) for repetitive deliveries of 0.100ml and 0.300ml volumes with a precision of better than 1.5%.
- Microplate washers or a squeeze bottle (optional).
- Plastic wrap or microplate cover for incubation steps.
- Absorbent Paper for blotting the microplate wells.
- Microscope

Technique:
1. Format the microplates' wells for each serum reference, control or specimen into the assigned wells.
2. Pipette 0.050 ml (50μl) of the appropriate serum, control or specimen into the assigned well.
3. Pipette 0.050 ml (50μl) of the appropriate sample into the assigned well.
4. Incubate for 60 minutes at room temperature (20-22°C).
5. Wash 3 times.
6. Add 0.050 ml (50μl) of the appropriate Stop Solution to the assigned well.
7. Stop the reaction by incubation for 30 minutes at room temperature (20-22°C).
8. Discard the contents of the microplate.
9. Wash 3 times.
10. Read the plate dry and calculate the absorbance.

Required But Not Provided:
- Microplate Reader
- Dispenser(s) for repetitive deliveries of 0.100ml and 0.300ml volumes with a precision of better than 1.5%.
- Microplate washers or a squeeze bottle.
- Plastic wrap or microplate cover for incubation steps.
- Vacuum aspirator.
- Meter.

Note:
- Do not use reagents beyond the expiration date.
- Do not mix different lot numbers.
- Use within 60 days.

For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals


Good laboratory practices for handling blood products can be found in the Center for Disease Control / National Institute of Health, ‘Biosafety in Microbiological and Biomedical Laboratories’ 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50μl) of stop solution to each well and mix gently for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.

Quality Control
Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

Results:
A dose response curve is used to ascertain the concentration of Growth Hormone in unknown specimens. 1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1. 2. Plot the absorbance for each duplicate serum reference versus the corresponding HGH concentration in μIU/ml on linear graph paper (do not average the duplicates of the serum references before plotting). Draw the best-fit curve through the plotted points. 4. To determine the concentration of HGH for an unknown, locate the average absorbance of the duplicate samples on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration in μIU/ml from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.672) intersects the dose response curve at (20.5 μIU/ml) HGH concentration (See Figure 1).

Table 1. Expected Values for the Growth Hormone ELISA Test System (in μIU/ml)

<table>
<thead>
<tr>
<th>Specimens</th>
<th>N</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>175</td>
<td>9.1</td>
</tr>
</tbody>
</table>

A. Interpretation
1. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
2. Growth hormone secretion follows a circadian rhythm characterized by discontinuous pulsatile discharge bursts with intervening periods during the day when GH levels are undetectable. The highest levels, in two major bursts, are usually attained within one to two hours after the onset of sleep. Other physiological stimuli of growth hormone are stress, exercise, high protein meals and hypoglycemia.
3. Hypoglycemia inhibits growth hormone concentrations. Age is an important factor in growth hormone concentrations. At birth, GH is high and generally declines with age with the exception of a burst during the growth phase of adolescence. Women typically have a 50% higher level than their age-matched males.
4. Since growth hormone concentration is pulsatile and sporadic during the course of the day (coupled with its short half-life) single serum random levels do not yield clinically useful information. To overcome this problem, provocative tests are utilized that employ physiological or pharmacological stimuli to induce the secretion or inhibition of GH. For these reasons, the determination of growth hormone alone is not sufficient to assess a clinical status.

Expected Ranges Of Values
Because of the pulsatile and sporadic nature of growth hormone secretion, reference intervals for basal levels are without meaning. However, normal levels rarely have been reported above 150 μIU/ml. The well rested, fasting (12 hour) subjects should have GH levels of 60 μIU/ml or less.

Provocative tests for GH response are normally used to correct for an inherited factor or pituitary deficit. Simultaneous procedures measure the secretion ability of the anterior pituitary to release GH. Children suspected of growth retardation are common subjects for stimulatory testing. Several dynamic tests are available to induce GH release: exercise, L dopa administration, insulin tolerance test and aramine infusion. Each laboratory should assess the normal response, but a peak GH release in excess of 24 μIU/ml is probably normal in all cases. Inhibitory testing measure the suppression of GH release from the anterior pituitary. Inhibitory tests are useful in ascertaining growth hormone excess and the resulting conditions of gigantism and Acronegaly. The glucose tolerance test is a dynamic test to measure growth hormone excess. The failure of GH levels to fall below 1 μIU/ml within 60-120 minutes suggests excess GH secretion.

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of normal persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of the expected values established by the manufacturer only until an in house range can be determined by the analysts. For best results, tests should be performed within two room temperatures of the area in which the laboratory is located.

Performance Characteristics:
A. Precision
The within and between assay precision of the hGH ELISA Microplate Procedure were determined by analyses on three different levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>24</td>
<td>10.38</td>
<td>0.33</td>
<td>3.13%</td>
</tr>
<tr>
<td>Level 2</td>
<td>24</td>
<td>26.23</td>
<td>1.17</td>
<td>4.45%</td>
</tr>
<tr>
<td>Level 3</td>
<td>24</td>
<td>61.80</td>
<td>3.40</td>
<td>5.50%</td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>39</td>
<td>10.48</td>
<td>0.48</td>
<td>4.58%</td>
</tr>
<tr>
<td>Level 2</td>
<td>39</td>
<td>26.08</td>
<td>1.77</td>
<td>6.78%</td>
</tr>
<tr>
<td>Level 3</td>
<td>39</td>
<td>64.61</td>
<td>4.56</td>
<td>7.09%</td>
</tr>
</tbody>
</table>

B. Accuracy
The Fortress hGH Elisa Microplate Procedure was compared with a reference immunoradiometric method. Biological specimens from normal and elevated samples were assayed. The total number of such specimens was 80. The least square regression equation and the correlation coefficient were computed for the GH in comparison with the reference method. The data obtained is displayed in Table 4.

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean(x)</th>
<th>Least Square Regression Analysis</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>This Method</td>
<td>15.2</td>
<td>Y = 0.031 - 0.96(x)</td>
<td>0.985</td>
</tr>
<tr>
<td>Reference</td>
<td>15.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Only slight amounts of bias between the hGH Elisa method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

C. Sensitivity
The hGH Elisa method has a sensitivity of 0.025 uIU and the reference method has a sensitivity of 0.5 uIU/ml hGH concentration.

D. Specificity:
The cross reactivity of the hGH Elisa test system to selected substances was evaluated by adding the interfering substances to a serum matrix at various concentrations. The cross reactivity was calculated by deriving a ratio between dose of interfering substance to dose of growth hormone needed to produce the same absorbance.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Hormone (GH)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Luteinizing Hormone (LH)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Follicle Stimulating Hormone (FSH)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Chorionic Gonadotropin (CG)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Thyroid Stimulating Hormone (TSH)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Prolactin Hormone (PRL)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Reference: