A small number of dwarfism cases have been documented in which both the basal level of HGH and the responses to challenge testing were normal. Such cases may involve tissue insensitivity to either growth hormone or the somatomedins, or immunoreactive but biologically inactive growth hormone. The GenWay Human Growth Hormone Enzyme Immunoassay provides a rapid, sensitive, and reliable test for HGH measurement. There is no cross-reactivity with hCG, TSH, LH, FSH and prolactin.

**PRINCIPLE OF THE ASSAY**

The GenWay Human Growth Hormone Enzyme Immunoassay (HGH ELISA) is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes a sheep anti-HGH antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti-HGH antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in HGH molecules being sandwiched between the solid phase and enzyme-linked antibodies.

After a 45 minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1N HCl, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of HGH is directly proportional to the color intensity of the test sample.

**REAGENTS AND MATERIALS PROVIDED**

- Sheep Anti-HGH-coated microtiter plate with 96 wells.
- Reference standard set, containing 0, 2.5, 5, 10, 25, and 50 ng/ml HGH, ready to use.
- Enzyme Conjugate Reagent, 13 ml.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

**MATERIALS REQUIRED BUT NOT PROVIDED**

- Distilled or deionized water
- Precision pipettes: 50 µl, 100 µl, and 1.0 ml.
- Disposable pipette tips
- Microtiter well reader capable of reading abs. at 450nm, with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater.
- Absorbent paper
- Graph paper
- Vortex mixer or equivalent
- Quality control material (e.g., BioRad Lyphochek Control sera)
**Storage of Test Kit and Instrumentation**

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

**Specimen Collection and Preparation**

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

**Reagent Preparation**

All reagents should be allowed to reach room temperature (18-25°C) before use.

**Assay Procedure**

1. Secure the desired number of coated wells in the holder.
2. Pipette 50μL of standards, specimens, and controls into appropriate wells.
3. Add 100 μL of Enzyme Conjugate Reagent into each well.
4. Mix thoroughly for 30 seconds.
5. Incubate at room temperature (18-25°C) for 45 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 5 times with distilled H₂O.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100μL of TMB Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature, in the dark, for 20 minutes.
11. Stop the reaction by adding 100μL of Stop Solution to each well.
12. Gently mix for 30 seconds. **Ensure that all of the blue color changes completely to yellow.**
13. Read absorbance at 450nm with a microtiter plate reader within 15 minutes.

**Calculation of Results**

1. Calculate the mean absorbance value (OD₄₅₀) from the duplicate set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/mL on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of HGH in ng/mL from the standard curve.

**Example of Standard Curve**

Results of a typical standard run with optical density readings at 450nm shown on the Y-axis against HGH (ng/mL) shown on the X-axis, are presented below. **NOTE:** the standard curve is for illustration only, and should not be used to calculate unknowns.

<table>
<thead>
<tr>
<th>HGH (ng/mL)</th>
<th>Absorbance (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.052</td>
</tr>
<tr>
<td>2.5</td>
<td>0.392</td>
</tr>
<tr>
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<td>25</td>
<td>1.946</td>
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<tr>
<td>50</td>
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</tbody>
</table>

**Expected Values**

Each laboratory must establish its own normal ranges based on patient population. A normal range for human growth hormone levels is difficult to define because of the normal physiological fluctuations in HGH concentration. In most adult subjects at rest, after an overnight fast, the HGH level in serum is 7 ng/ml or less. Changes in HGH levels in response to various stimuli gives a more accurate assessment of pituitary dysfunction. **The minimal sensitivity of the test is 0.5ng/ml.**

**Quality Control**

Good laboratory practice requires that low, medium and high quality control specimens (controls) be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges.
LIMITATIONS OF THE PROCEDURE

1. Serum samples demonstrating gross lipemia, gross hemolysis or turbidity should not be used with this test.
2. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

REFERENCES


062711SR12126