3.0 PRINCIPLE

Immunoenzymometric assay (Type 3): The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place after the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-
Tropinin-I antibody.

Upon mixing biotin labeled monoclonal antibody, the enzyme-
labeled antibody and a serum containing the native antigen reaction results in antigen antibody with, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

\[ \text{EnzAb(x-GH)-AgGH-BtnAb(m)} = \text{Sandwich Complex} \]

After sufficient time for reaction, the antibody bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody bound fraction is directly proportional to the native antigen concentration. By utilizing several different antibodies such as bones and muscles and indirectly through the release of somatomedins, a family of insulin-like growth factor (IGF) hormones, produced in the liver (2). In particular, somatotropin C (IGF-1) is essential for bone growth in children.

The clinical usefulness of the measurement of growth hormone (GH) in children has been well established in ascertainning linear bone growth along the epiphyseal plate. Abnormal elevated levels lead to gigantism while complete absence slows the rate of growth to one-third to one-half of normal. In adults, the epiphyseal growth plates have fused; GH excess gradually produces acromegaly, a coarse thickening of the bones of the skull, hands and feet.

In this method, GH calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of GH) are added and the reactants mixed. Reaction between the various affinity are allowed to separate from the unbound enzyme-growth hormone conjugate is separated by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce light.

The employment of several serum references of known growth hormone levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen’s activity can be correlated with growth hormone concentration.

3.1 IMMUNOENZYMOOMETRIC ASSAY

1. Preparation of working signal reagent, in which the working signal reagent is diluted 1: 5 with wash buffer.
2. Format the microplate wells for each serum reference, control and patient specimen to be assayed in duplicate.
3. Add 0.100 ml of working signal reagent to all wells (see Reagent Preparation Section).
4. Add 0.100 ml (100µl) of hGH Tracer Reagent to all wells.
5. Pipette 0.050 ml (50µl) of hGH Tracer Reagent to all wells.
6. After sufficient time for reaction, the antibody bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody bound fraction is directly proportional to the native antigen concentration. By utilizing several different antibodies such as bones and muscles and indirectly through the release of somatomedins, a family of insulin-like growth factor (IGF) hormones, produced in the liver (2). In particular, somatotropin C (IGF-1) is essential for bone growth in children.

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The employment of several serum references of known growth hormone levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen’s activity can be correlated with growth hormone concentration.
6. Failure to remove adhering solution adequately in the aspiration or decantation each step may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents is allowed. Investigators should measure light output. The conversion varies between instruments that may interfere in the assay and cause falsely low values. Genetic variants or degradation products may alter antibody-binding characteristics. Interference is not necessarily caused by efficiency of the various instruments that may interfere in the assay and cause falsely low values. Genetic variants or degradation products may alter antibody-binding characteristics. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.2 Interpretation
1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.

2. Laboratory reporting results for the assay should be limited to good laboratories. Such results should be strictly followed to ensure proper device usage.
3. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

14.3 Accuracy
The method was compared with a reference microplate enzyme immunoassay (ELISA). Biological specimens from normal and elevated samples were assayed. The total number of such samples was 65. The least square regression equation and the correlation coefficient were computed for this method in comparison with the reference method. The data obtained is presented in Table 3.

14.4 Sensitivity
This method was compared with a reference microplate enzyme immunoassay (ELISA). Biological specimens from normal and elevated samples were assayed. The total number of such samples was 65. The least square regression equation and the correlation coefficient were computed for this method in comparison with the reference method. The data obtained is presented in Table 3.

In order for the assay results to be considered valid the following criteria should be met:

1. Patients on hGH replacement may develop antibodies to hGH that may interfere in the assay and cause falsely low values. Genetic variants or degradation products may alter antibody-binding characteristics. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

5. The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a dose response curve prepared with each assay. In addition, the RLUs of the calibrators have been normalized to 100,000 RLU's for the F calibrator prepared with each assay. In addition, the RLU's of the calibrators have been normalized to 100,000 RLU's for the F calibrator prepared with each assay. In addition, the RLU's of the calibrators have been normalized to 100,000 RLU's for the F calibrator prepared with each assay.

**14.0 PERFORMANCE CHARACTERISTICS**

14.1 Precision
The within and between assay precision of the Growth Hormone (hGH) assay was determined by testing several of the key samples with levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Tables 2 and 3.

| TABLE 2 | Within Assay Precision (Values in µU/ml) |
| Sample | N | X | σ | C.V. |
| Level 1 | 24 | 12.57 | 0.30 | 2.4 |
| Level 2 | 24 | 28.73 | 0.78 | 2.7 |
| Level 3 | 24 | 51.55 | 2.55 | 5.5 |

**TABLE 3**

| TABLE 3 | Between Assay Precision* (Values in µU/ml) |
| Sample | N | X | σ | C.V. |
| Level 1 | 14 | 10.54 | 1.10 | 10.5 |
| Level 2 | 14 | 23.99 | 1.20 | 5.0 |
| Level 3 | 14 | 55.04 | 3.23 | 5.9 |

*As measured in several experiments in duplicate.

**14.5 Specificity**

The cross reactivity of the hGH AccuLite™ CLIA method 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.

**15.0 REFERENCES**


**TABLE 4**

| TABLE 4 | DCO:0641 |
| Cat #: | 1775-300 |

For Orders and Inquiries, please contact...