Luteinizing Hormone (LH) Product Code: 675-300

SUMMARY AND EXPLANATION OF THE TEST

Luteinizing hormone (LH) is a glycoprotein consisting of two subunits with a molecular mass of 30,000 daltons. The α-subunit is similar to other pituitary hormones (follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH) and chorionic gonadotrophin (CG)) while the β-subunit is unique. The β-subunit confers the biological activity to the molecule. The α-subunit consists of 89 amino acid residues while the β-subunit contains 129 amino acids. The carbohydrate content is between 15% and 30%.

The clinical usefulness of the measurement of luteinizing hormone (LH) in ascertaining the hormone status of fertility regulation via the hypothalamic-pituitary-gonadal axis has been well established (1,2). In addition, the advent of in vitro fertilization (IVF) technology to overcome infertility-associated problems has provided the impetus for rapid improvement in LH assay methodology from the technically demanding bioassay (3) to the procedurally simple and rapid immunometric or enzymatic assays.

In this method, LH calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled substrate (produced against distinct and different epitopes of LH) are added and the reactants mixed. Reaction between the various LH antibodies and native LH forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-luteinizing hormone antibody bound conjugate is separated from the unbound enzyme-luteinizing hormone conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a substrate. The results are expressed as IU/L.

The employment of several serum references of known luteinizing hormone levels permits 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 luteinizing hormone concentration.

PRINCIPLE

Immunoenzymometric assay:

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-LH antibody. Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

\[
E_{nA}B_{kA}B_{m} = \text{Biotinylated Monoclonal Antibody (Excess Quantity)}
\]

\[
A_{n} - \text{Native Antigen (Variable Quantity)}
\]

\[
E_{nA}B_{kA} = \text{Enzyme Labeled Antibody (Excess Quantity)}
\]

\[
A_{n}B_{m} = \text{Antigen-Antibodies Sandwich Complex}
\]

\[
k_{b} = \text{Rate Constant of Association}
\]

\[
\alpha = \text{Rate Constant of Dissociation}
\]

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

\[
E_{nA}B_{kA}B_{m} + \text{Streptavidin} \rightarrow \text{immobilized complex}
\]

\[
\text{Streptavidin} \rightarrow \text{immobilized complex} + \text{Antibodies-Antigen sandwich bound}
\]

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity, determined by reaction with a substrate that generates light, in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

REAGENTS

Materials Provided

A. LH Calibrators – 1ml/vial - Ions A-F

B. LH Tracer Reagent – 13 ml/vial - Ions C

C. Light Reaction Wells – 96 wells - Ions E

D. Wash Solution Concentrate – 20 ml

E. Signal Reagent A – 7ml/vial - Ions S

F. Signal Reagent B – 7ml/vial - Ions S

REAGENT PREPARATION

1. Wash Buffer

Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at room temperature 20-27°C.

2. Working Signal Reagent Solution - Store at 2 - 8°C

Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1 ml of B per two (8) well strips (A slight excess of solution is made). Discard the unused portion if not used within 36 hours after mixing. If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and high range for monitoring assay performance. The controls should be treated as unknowns and results 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 uncontrolled 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 Luteinizing Hormone in unknown specimens.

A dose response curve is used to ascertain the concentration of LH in unknown specimens.

1. Record the RLU’s (Relative Light Unit) obtained from the printout of the microplate reader as outlined in Example 1.

2. Plot the RLU’s for each duplicate serum reference versus the corresponding LH concentration in mIU/ml on linear graph paper.

3. Draw the best-fit curve through the plotted points.

4. To determine the concentration of LH for an unknown, locate the average RLU’s for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and determine the concentration (in mIU/ml) on the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLU’s (39442) of the unknown intersects the calibration curve at (57.5mIU/ml) LH concentration (See Figure 1).

Note 1: Computer data reduction software designed for chemiluminescence assays may also be used for the
Q.C. PARAMETERS

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

1. The Dose Response Curve should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

LIMITATIONS OF PROCEDURE

A. Assay Performance

1. It is important that the time of reaction in each well is held constant for reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
4. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
5. Use components from the same lot. No intermixing of reagents from different batches.
6. Multichannel pipettes are recommended for addition of reagents.
7. Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemic or hemolysed specimen(s) should not be used.

B. Clinical 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. LH is suppressed by estrogen but in woman taking oral contraceptives the level may be low or normal. Excessive dieting and weight loss may lead to low gonadotropin concentrations.
3. Luteinizing hormone is dependent upon diverse factors other than pituitary homeostasis. Thus, the determination alone is not sufficient to assess clinical status.

EXPECTED RANGES OF VALUES

A study of an apparent normal adult population was undertaken to determine expected values for the LH AccuLite™ CLIA method. The expected values are presented in Table 1.

PERFORMANCE CHARACTERISTICS

A. Precision

The within and between assay precision of the LH AccuLite™ CLIA were determined by analyses on three different levels of control sera. The number, mean value, standard deviation (σ) and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

B. Accuracy

The LH AccuLite™ CLIA was compared with a reference enzyme immunoassay. Biological specimens from normal and pregnant populations were assayed. The total number of such specimens was 80. The least square regression equation and the correlation coefficient were computed for this method in comparison with the reference method. The data obtained is displayed in Table 4.

C. Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the 0mIU/mL serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose. It was determined to be 0.8mIU/mL.

D. Specificity

The cross-reactivity of the LH AccuLite™ CLIA to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Luteinizing Hormone needed to produce the same light intensity.

REFERENCES


Revision: A                          Date: 09-18-06
Cat #: 675-300

For Orders and Inquiries, please contact

Monobind’s immunoassay products are designed to work in both manual and automated lab environments. AccuBind™ and AccuLite™ are compatible with any open-ended instrumentation, including chemistry analyzers, microplate readers and microplate washers. There may or may not be an application developed for your particular instrument, please visit the instrument section of our website, or contact techsupport@monobind.com

Monobind offers several instruments, including the Impulse 2 Luminometer CLIA Plate Reader designed hand-in-hand with our products and capable of 2-point calibration. Visit our website for more information.