1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Luteinizing Hormone Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 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 identical to other pituitary hormones follicle stimulating hormone (FSH), thyroid-stimulating hormone (TSH) and gonadotropin (CG) while the β-subunit is unique. The β-subunit contains the biological activity to the molecule. The α-subunit consists of 89 amino acids while the β-subunit comprises 129 amino acids. The carbohydrate content is between 15% and 30%.

The clinical usefulness of the measurement of luteinizing hormone (LH) in ascertainment of the homeostasis of fertility regulation via the hypothalamic - pituitary - gonadal axis has been well established. 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 biosay to the procedurally simple and rapid immunoenzymometric assays.

In this method, LH calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal antibody (directed against different and distinct 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 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 serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided

A. LH Calibrators – 1ml/Bag - Icons A-F
   Six (6) vials of references for LH Antigen at levels of 0(A), 5(B), 25(C), 50(D), 100(E) and 200(F) mIU/mL. Store at 2-8°C. A preservative has been added.
   Note: The calibrators, human serum based, were calibrated relative to the WHO 2 nd International Reference Preparation, which was assessed by the WHO 2nd IS 80/552.
B. LH Enzyme Reagent – 13 ml/Bag - Icons
   One (1) vial containing enzyme labeled purified antibody, biotinylated immobilized mouse IgG in buffer, dye, and preservative. Store at 2-8°C.
C. Streptavidin Coated Plate – 96 wells - Icons
   One (1) 96-well streptavidin coated microplate in an aluminum bag with a drying agent. Store at 2-8°C.
D. Wash Solution Concentrate – 20 ml/Bag - Icons
   One (1) vial containing tetramethylbenzine (TMB) in buffer. Store at 2-8°C.
E. Streptavidin – 7ml/Bag - Icons
   One (1) vial containing hydrogen peroxide (H2O2) in buffer. Store at 2-8°C.
F. Stop Solution – 8ml/Bag - Icons
   One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.
G. Microplate Reader with 450nm and 620nm wavelength absorbance capability.

4.1 Required But Not Provided:

1. Pipette capable of delivering 0.050ml (50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 and 0.350ml (100µl and 350µl)
3. Microplate washer or a squeeze bottle (optional)
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Paper or plastic aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

**For In Vitro Diagnostic Use**

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1 & 2 and HCV. Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures 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.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum in type and the usual precautions in the collection of venipuncture samples shall be taken. Assay accuracy is compromised when normal values are in a fasting morning sample should be obtained. The blood should be collected in a plain red-top venipuncture tube without additives or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biodoses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Note: Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this period, it should be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100 ml (100 µl) of the specimen is required.

7.0 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 unbiological samples determined in every test procedure performed. Quality control charts should be plotted and compared with the trends of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate uncontrolled changes in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Wash Buffer
   Dilute contents of wash concentrate to 1000ml with distilled or deionised water in a sterile storage container.
   Store at 2-8°C for up to 60 days.
2. Working Substance Solution – Stable for one year
   Prepare 1ml of the amber vial labeled Solution ‘A’ into the clear vial labeled Solution ‘B’. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).

**Test Procedure should be performed by a skilled individual or trained professional**

1. Format the microplate wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the vials.
2. Pipette 0.050 ml (50µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of LH-Enzyme Reagent to all wells.
4. Swirl the mixture gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Place the microplate in the microplate reader for 30 minutes of adding the stop solution.

Note 1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of luteinizing hormone (LH) 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 LH concentration in mIU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw a smooth line curve through the plotted points.
4. To determine the concentration of LH for an unknown, locate the average absorbance of the duplicates for each unknown on the x-axis of the graph, find the corresponding point on the curve, and read the concentration (in mIU/mL) from the y-axis. Any results obtained are considered as potentially infectious (the unknown may be averaged as indicated). In the following example, the average absorbance (1.005) intersects the dose response curve at 42.7 mIU/mL LH concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

*The data presented in Example 1 and Figure 1 is for illustration only and may not be used in lieu of a dose response curve prepared with each assay.

**Test Procedure should be performed by a skilled individual or trained professional**
1. It is important that the time of reaction in each well is held constant to achieve reproducible results.

2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.

3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.

4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.

5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.

6. Plate readers measure vertically. Do not touch the bottom of the wells.

7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor reproducibility and spurious results.

8. Use components from the same lot. No intermixing of reagents from different batches.

9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential.

10. All applicable national standards, regulations and laws, including but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.

11. 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.

12. Risk Analysis - as required by CE Mark IVD Directive 98/79/EC - for this and other devices made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

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

2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.

3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interference between specimen and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (Boscato LM Stuart MC. ‘Heterophilic antibodies: a problem for all immunoassays’ Clin.Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.

4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.

5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.

6. 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.

7. 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.

8. Luteinizing hormone is dependent upon diverse factors other than pituitary homeostasis. Thus, the determination alone is not sufficient to assess clinical status.

13.0 EXPECTED RANGES OF VALUES

A study of an apparent normal adult population was undertaken to determine expected values for the LH AccuBind® ELISA Test System. The expected values are presented in Table 1.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the LH AccuBind® ELISA Test System were determined by analyses on three different levels of control sera. The number (N), mean value (X), standard deviation (o) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

14.2 Sensitivity

The LH AccuBind® ELISA Test System has a sensitivity of 0.003mIU/L. This is equivalent to a sample containing 0.054 mIU/L LH concentration. The analytical sensitivity (detection limit) was ascertained by determining the variability of the 0.0 mIU/L calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

This LH AccuBind® ELISA Test System was compared with a reference radioimmunoassay. Biological specimens from normal, and pregnant populations were assayed. The total number of such specimens were 21. The least square regression equation and the correlation coefficient were computed for the LH ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

*As measured in ten experiments in duplicate.

14.4 Specificity

The cross-reactivity of the LH AccuBind® ELISA Test System 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 absorbance.

15.0 REFERENCES


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