SUMMARY AND EXPLANATION OF THE TEST

Follicle Stimulating Hormone (FSH) is a glycoprotein consisting of two subunits with an approximate molecular mass of 35,500 daltons. The α-subunit is similar to other pituitary hormones that stimulate hormone (LH), thyroid stimulating hormone (TSH) and chorionic gonadotropin (CG) while the β-subunit is unique. The β-subunit confers the biological activity to the molecule. Simultaneous release of the growth hormone (GH) and FSH causes release of FSH, as well as LH, from the pituitary, and is transported by the blood to their sites of action, the testes or ovary.

In men, FSH acts on the Sertoli cells of the testis, stimulating the synthesis of inhibin, which appears to specifically inhibit further FSH secretion, and androgen-binding protein. Thus, it indirectly suppresses spermatogenesis.

In women, FSH acts on the granulosa cells of the ovary, stimulating steriodogenesis. All ovulatory menstrual cycles have a characteristic pattern of FSH, as well as LH, secretion. The menstrual cycle is divided into a follicular phase and a luteal phase by the midcycle surge of the gonadotropins (LH and FSH). After equilibrium is attained, the antibody-bound fraction is separated from unbound antibody 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.

PRINCIPLE

Immunoenzymometric assay (TYPE 3):

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 surface with the biotinylated antigen. 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:

\[ \text{Enzyme} \times \text{Biotinylated Monoclonal Antibody} \times \text{Native Antigen} \times \text{Biotinylated Antibody} \times \text{Streptavidin} \]

In women, FSH acts on the granulosa cells of the ovary, stimulating the synthesis of inhibin, which appears to specifically inhibit further FSH secretion, and androgen-binding protein. Thus, it indirectly suppresses spermatogenesis.

In men, FSH acts on the Sertoli cells of the testis, stimulating the synthesis of inhibin, which appears to specifically inhibit further FSH secretion, and androgen-binding protein. Thus, it indirectly suppresses spermatogenesis.

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 "CDC Guidelines for the Prevention of Transmission of HIV, hepatitis B virus, and other bloodborne pathogens in health-care settings, 2001." Good laboratory procedures for handling blood products can be found in the "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells. 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 time, the sample(s) may be stored at temperatures of 20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100 ml of the specimen is required.

REAGENT PREPARATION:

A. FSH Calibrators --1 ml/vial - Icons A-F

Six (6) vials of references for FSH Antigen at levels of 0(A), 10(B), 100(C), 1000(D), 5000(E) and 100,000(F) mIU/mL. Store at 2-8°C. A preservative has been added. Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 2nd IRP (78/549).

B. FSH Enzyme Reactant ---13 ml/vial - Icon

One (1) vial containing enzyme labeled biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coat Plate -- 96 wells - Icon

One (1) vial containing streptavidin coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at -20°C.

D. Wash Solution -- 20 ml - Icon

One (1) vial containing enzyme labeled biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

E. Substrate A ++7.0ml/vial - Icon A

One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B ++7.0ml/vial - Icon B

One (1) bottle containing hydrogen peroxide (H2O2) in buffer. Store at 2-8°C.

TEST PROCEDURE

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

1. Format the microplate wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.

2. Pipette 0.050ml of the appropriate serum reference, control or specimen into the assigned well.

3. Add 0.010 ml (100μl) of FSH-Enzyme Reagent solution to all wells.

4. Swirl the microplate gently for 20-30 seconds to mix and cover.

5. Incubate 60 minutes at room temperature.

6. Discard the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

7. Add 300μl of wash buffer (see Reagent Preparation Section) decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.

8. Add 0.010 ml (100μl) of working substrate solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells.

9. Incubate at room temperature for fifteen (15) minutes.

10. Add 0.050ml (50μl) of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.

11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

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.
CALCULATION OF RESULTS
A dose response curve is used to ascertain the concentration of follicle stimulating 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 FSH concentration in mIU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.

To determine the concentration of FSH for an unknown, locate the absorbance (OD) of calibrator F should be > 0.579 (1968).

REFERENCES:

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

A. Precision
The within and between assay precisions of the FSH Accubind™ ELISA test system were determined by analyses on three different levels of control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V) for each of these control sera are presented in Table 2 and Table 3.

LEAST SQUARE REGRESSION EQUATION AND CORRELATION COEFFICIENT INDICATES EXCELLENT METHOD AGREEMENT.

B. Interpretation
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.

C. Sensitivity
The Follicle Stimulating Hormone procedure has a sensitivity of 0.04 mIU. This is equivalent to a sample containing 0.8 mIU/ml FSH concentration.

D. Specificity
The cross-reactivity of the FSH 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 FSH.

EXPECTED RANGES OF VALUES
A study of an apparent normal adult population was undertaken to determine expected values for the FSH Accubind™ ELISA Test System. The expected values are presented in Table 1.

EXPECTED VALUES FOR THE FSH ACCUBIND™ ELISA TEST SYSTEM (mIU/ml)

MEN

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicotropin (FSH)</td>
<td>1.0000</td>
<td></td>
</tr>
<tr>
<td>Lutein Hormone (LH)</td>
<td>&lt;0.0001</td>
<td>1000 ng/ml</td>
</tr>
<tr>
<td>Chorionic Gonadotropin (HCG)</td>
<td>&lt;0.0001</td>
<td>1000 ng/ml</td>
</tr>
<tr>
<td>Thyrotropin (TSH)</td>
<td>&lt;0.0001</td>
<td>1000 ng/ml</td>
</tr>
</tbody>
</table>

This FSH Accubind™ ELISA method was compared with a reference radioimmunoassay. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 106. The least square regression equation and the correlation coefficient were computed for the FSH Accubind™ ELISA test method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 1

Table 1 presents the least square regression equation and correlation coefficient for the FSH Accubind™ ELISA test system.

TABLE 2

Table 2 presents the within and between assay precisions of the FSH Accubind™ ELISA test system.

TABLE 3

Table 3 presents the between assay precision of the FSH Accubind™ ELISA test system.

REFERENCES:


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