**β-Human Chorionic Gonadotropin, Follicle Stimulating Hormone, Luteinizing Hormone, Prolactin Hormone Sequential (hCG/FSH/LH/PRLs VAST) Fertility Panel Test System Product Code: 8375-300**

1.0 INTRODUCTION

**Intended Use:** The Quantitative Determination of HCG, FSH, LH, and PRLs Concentration in Human Serum and Plasma by a Microplate Enzyme Immunoassay, Chemiluminescence.

Measurements of these hormones are used as an aid in the determination and therapeutic monitoring of reproductive endocrinopathies.

2.0 SUMMARY AND EXPLANATION OF THE TEST

**Human chorionic gonadotropin (hCG) concentration increases dramatically in blood and urine during normal pregnancy.** hCG is secreted by placentas, beginning with the primitive trophoblast, almost simultaneously with implantation, and serves to support the corpus luteum during the early weeks of pregnancy. hCG or hCG-like glycoproteins can also be produced by a wide variety of neoplastic and nonneoplastic lesions. The measurement of hCG by assay systems with suitable sensitivity and specificity has proved to be a valuable detection pregnancy and the diagnosis of early pregnancy disorders. hCG is detectable as early as 10 days after ovulation, reaching 100 mIU/mL by the first missed period. A pregnancy test performed at 11-12 days after the last menstrual period is 95-99% effective.2,11

**In men, FSH acts on the Sertoli cells of the testis, stimulating the production of spermatozoa.** Follicle stimulating hormone (FSH) stimulates spermatogenesis. In women, FSH acts on the granulosa cells of the ovary, stimulating follicle development and the secretion of estrogen. In men, FSH acts on the Sertoli cells of the testis, stimulating the production of spermatozoa. FSH secretion, and androgen-binding protein. Thus, it indirectly supports spermatogenesis.9,10

**FSH secretion, and androgen-binding protein.** Thus, it indirectly supports spermatogenesis.

**2.0 SUMMARY AND EXPLANATION OF THE TEST**

**3.0 PRINCIPLE**

**Immunoenzymometric assay (Types 3 and 4):** The essential reagents required for an immunoenzymometric assay include an antibody (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization of the antibody on the surface of a microplate was performed through the interaction of streptavidin coated with the antibody and added biotinylated monoclonal antibody. On mixing mononuclear cell-bound antibody, the enzyme-labeled antibody and a serum containing native reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. 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 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 the specific hormone concentration.

**K. Product Insert**

**4.1 Required But Not Provided:**

1. Pipettes capable of delivering 0.025ml (25µl) and 0.050ml (50µl) volumes with a precision of better than 1.5%.
2. Dispensers for repetitive deliveries of 0.100ml (100µl) and 0.300ml (300µl) volumes with a precision of better than 1.5%.
3. Distilled or deionized water.
4. Microplate luminometer.
5. Container(s) for mixing of reagents (see below).
6. Absorbent Paper for blotsing the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
10. Storage container for storage of wash buffer.
11. Distilled or deionized water.

**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 required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be regarded as hazardous and incapable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control, "Guidelines for the Management of Biomedical Laboratories," 2nd edition, 1986, HHS.

**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 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 redcap venipuncture tube without additives. Allow the blood to clot. Centrifuge the specimen to separate the serum from the clot.

**In patients receiving therapy with high bioitin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last bioitin administration, preferably overnight to allow for substantial bioitin removal from the body.**

**Materials Provided: Reagents for 2 x 96 well Microplate A fastacting enzyme and antibody:**

Six (6) vials of references for antigens at levels indicated below. The samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) is not used within this time, the samples may be stored at temperatures of -20°C for up to 30 days. The concentration of contaminants is low, and the freezing and thawing. When assayed in duplicate, 0.100ml (100µl) of the specimen is required for LH and FSH. For prolactin and hCG, 0.050ml (50µl) of sample is needed.
8.0 REAGENT PREPARATION

1. Wash Buffer
   Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature until expiration date printed on concentrate label.

2. Working Signal Reagent - 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 1ml of B per two (2) eight 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.

Note: Do not use reagents that are contaminated or have bacteria growth.

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**

9.1 HCG, LH & FSH

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

2. For HCG: Pipette 0.025ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.

3. Add 0.100ml (100 µl) of the sequential PRL Biotin Reagent to each well. As there is an excess amount of reagent added to each well, it is very important to use the right Tracer Reagent for each assay for correct results.

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

5. Incubate 45 minutes at room temperature for HCG and 20 minutes for LH & FSH above.

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

7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (4) additional times for a total of five (5) washes.

8. Add 100 µl of ‘Working Signal Reagent’ to all wells. Always add reagents in the sample to minimize reaction time differences between wells.

9. Incubate in the dark for five (5) minutes.

10. Read the ‘Relative Light Units’ (RLU) in each well using microplate luminometer. The results should be read within thirty (30) minutes of adding the working signal reagent.

9.2 PROLACTIN

1. Format the microplates’ 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 0.025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.

3. Add 0.100ml (100 µl) of the sequential PRL Biotin Reagent to each well. It is very important to use the right Tracer Reagent for each assay for accurate results.

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

5. Incubate 30 minutes at room temperature.

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

7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (4) additional times for a total of five (5) 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 four (4) additional times.

8. Add 100 µl of Working Signal Reagent to all wells. Always add reagents in the sample to minimize reaction time differences between wells.

9. Incubate in the dark for five (5) minutes.

10. Read the ‘Relative Light Units’ (RLU) in each well using microplate luminometer. The results should be read within thirty (30) minutes of adding the working signal reagent.

10.0 CALCULATION OF RESULTS

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

1. Record the RLU obtained from the printout of the microplate luminometer as outlined in Example 1.

2. Plot the light intensity for each duplicate serum reference versus the corresponding hormone concentration in respective units on linear graph paper.

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

4. To determine the concentration of corresponding hormone for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in relative units) from the horizontal axis of the graph.

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

### Example 1 (LH)

<table>
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<tr>
<th>Sample L.D.</th>
<th>Well</th>
<th>RLU (A)</th>
<th>Mean RLU (B)</th>
<th>Value (mIU/ml)</th>
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</table>
13. Use multichannel pipets for reagent dispensing in order to avoid cross-contamination.

5. If computer controlled data reduction is used to interpret the result, it is important to know that the error in the calculation of the result by the dilution factor.

1. Dose Response Curve should be within established parameters.

2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind, Inc.

12.1 Assay Performance

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. Heterophilic, hemolyzed or grossly contaminated specimen(s) should not be used.

4. If more than one plate is used, it is recommended to repeat the assay with different plates.

5. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.

6. Failure to remove adhering solution adequately in the aspiration/dilution/flow step(s) may result in poor replication and spurious results.

7. Use components from the same lot. No intermingling of reagents or pipettes should be used.

8. Accurate and precise pipetting, as well as following the exact protocol, is necessary to eliminate any time-deviation during reaction.

12.2 Interpretation

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

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

3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophile antibodies often cause these problems and are known to be problems for all kinds of immunassays.

4. 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.

5. It is important to calibrate all the equipment e.g. Pipetters, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

6. No intermingling of reagents or pipettes should be used.

7. Accurate and precise pipetting, as well as following the exact protocol, is necessary to eliminate any time-deviation during reaction.

8. Use components from the same lot. No intermingling of reagents or pipettes should be used.

12.3 Performance Characteristics

14.0 Precision

The within and between assay precision of the Fertility Panel Assay (FPA) Test System were determined by analyses over three different levels of control sera. The mean, number standard deviation and coefficient of variation for each of these control sera are presented in Table 3 and Table 4.

14.1 Accuracy

The VAST® hCG CLIA assay was compared with a reference radioimmunoassay. Biological specimens from normal and pregnant populations were assayed. The total number of such specimens was 110. 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 5.

14.2 Sensitivity

The VAST® LH CLIA procedure has a sensitivity of 0.01 mIU. This is equivalent to a sample containing 0.2mIU/L LH concentration.

The VAST® FSH CLIA procedure has a sensitivity of 0.01mIU/L FSH concentration.

The VAST® hCG CLIA procedure has a sensitivity of 0.25 mIU/L hCG concentration.

The VAST® PRL CLIA procedure has a sensitivity of 0.02ng. This is equivalent to a sample containing 0.8 ng/mL PRL concentration.

The sensitivity was ascertained by determining the variability of the 0.1mIU/L serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Specificity

The cross-reactivity of the VAST® LH CLIA assay to select 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 dosages of interfering substance.
to dose of Luteinizing Hormone needed to produce the same light intensity.

The cross-reactivity of the VAST® FSH CLIA method 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 Luteinizing Hormone needed to produce the same light intensity.

The cross-reactivity of the VAST® Chorionic Gonadotropin CLIA test 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 Chorionic Gonadotropin needed to produce the same light intensity.

The cross-reactivity of the VAST® Prolactin CLIA method 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 prolactin hormone needed to produce the same light intensity.

The low cross-reactivity of the antibodies employed in this system permits the use of VAST® calibrators due to essentially zero cross reaction (ZCR).

14.5 Linearity and Hook Effect
Three different lots of reagent preparations of the Fertility Panel VAST® AccuLite® CLIA procedures were used to assess the linearity and hook effect.

The test showed a good dose recovery of 96.10 to 105.4% when linear dilutions of very high concentrations, in pooled sera, were assayed with VAST® CLIA procedures.

Massive concentrations were used for spiking in pooled human patient sera. VAST® CLIA procedures did not show any high dose hook effect with following concentrations of respective analytes.

Analyte  |  High Dose
--- | ---
HCG | 100,000 (mIU/ml)
LH | 25,000 (mIU/ml)
FSH | 40,000 (mIU/ml)
PRL | None Detected

15.0 REFERENCES

5. Lenton E., Neal L. and Sulaiman R., "Plasma Concentrations of LH subunit."