In this method, hCG/PRL/LH/FSH (referred to as antigens, in the Product Insert) can be added. A patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against different epitopes of the hormones) are added and the reaction is mixed. Reaction between the various antibodies (specific to the respective hormones) and native hormones forms a sandwich complex that binds with the streptavidin coated to the well.

In the PRL procedure, a sequential method of antibody addition is followed. That is, the biotinylated antibody is introduced first, and after an appropriate reaction period, the plate is washed. Then an enzyme labeled secondary antibody directed against a different epitope is added and the plate is processed as the other antigens.

After the completion of the required incubation period(s), the antigen-enzyme antibody enzyme bond complex is separated from the unreacted antigen and antibody by washing. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color. The development of several consecutive enzyme 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.

In women, FSH acts on the granulosa cells of the ovary, stimulating steroidogenesis. All ovulatory menstrual cycles have a characteristic stimulation of inhibin, which appears to specifically inhibit further folliculogenesis. While the follicle is growing, FSH concentration decreases. Near the time of ovulation a surge of the gonadotropins (LH and FSH) occurs, about midcycle, FSH peaks (lesser in magnitude than LH) to cause release of FSH, as well as LH, from the pituitary and is involved in the growth of the gland and in the induction and maintenance of milk production. There is evidence to suggest that prolactin may be involved in steroidogenesis in the gonad, acting synergistically with luteinizing hormone (LH). High levels of prolactin appear to inhibit steroidogenesis as well as inhibiting LH and follicle stimulating hormone (FSH) synthesis at the pituitary gland. 3,4 The clinical usefulness of the measurement of prolactin hormone (PRL) in ascertaining the diagnosis of hyperprolactinemia and for the subsequent evaluation of the effectiveness of the treatment has been well established.5,6

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 gonadotropin (CG)) while the β-subunit is unique. The β-subunit confers the biological activity to the molecule. The α-subunit consists of 98 amino acids while the β-subunit contains 129 amino acids. The carbohydrate content is between 15% and 30%.

Follicle stimulating hormone (FSH) is a glycoprotein consisting of two subunits with an approximate molecular mass of 15,000 daltons. The α-subunit is similar to other pituitary hormones (follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH) and chorionic gonadotropin (CG)) while the β-subunit is unique. The β-subunit confers the biological activity to the molecule. The α-subunit consists of 98 amino acids while the β-subunit contains 129 amino acids. The carbohydrate content is between 15% and 30%.

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, indirectly spermatogenesis is promoted.

In women, FSH acts on the granulosa cells of the ovary, stimulating steroidogenesis. All ovulatory menstrual cycles have a characteristic stimulation of inhibin, which appears to specifically inhibit further folliculogenesis.
9.0 TEST PROCEDURE (HCG, LH & FSH)

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20 - 25°C).

1. Format the microplates’ wells for each serum reference calibrator, 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.

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

3. Add 100µl of the PRL Biotin Reagent to each well.

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

5. Incubate 60 minutes at room temperature.

6. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times.

7. Add 0.100ml (100µl) of substrate solution to all wells. SHAKE THE PLATE AFTER SUBSTRATE ADDITION.

8. Add 0.050ml (50µl) of stop solution to each well and mix by aspiration. If decanting, tap and blot the plate dry with absorbent paper.

9. Read the absorbance in each well at 450nm (using a reference calibrator and controls to room temperature (20 - 25°C). Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20 - 25°C).

9.0 TEST PROCEDURE (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 100µl of the appropriate enzyme reagent to each well. It is very important to use the right ‘Enzyme Reagent’ for each assay.

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 (2) additional times for a total of three (3) washes.

8. Add 0.100ml (100µl) of substrate solution to all wells. DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION.

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

10. Add 0.050ml (50µl) of stop solution to each well and mix by rotation so that a uniform yellow color is obtained.

11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader as outlined in Example 1.

Note: It is very important to dispense all reagents in the center of the coated well. Always add reagents in the same order to minimize reaction time differences between wells.

9.0 TEST PROCEDURE (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 100µl of the PRL Biotin Reagent to each well. It is very important to use the right ‘Enzyme Reagent’ for each assay.

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 (2) additional times for a total of three (3) washes.

8. Add 0.100ml (100µl) of the Prolactin Enzyme Reagent to each well.

9. Incubate 30 minutes at room temperature.

10. Follow steps 6 through 10 as outlined in the procedure for HCG, LH & FSH above.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of each corresponding 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 antigen concentration in appropriate units 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.

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 ELISA assays may also be used for the data reduction.

*The data presented in the following Examples 2 and Figures is for illustration only and should not be used in lieu of a dose response curve prepared with each assay.

11.0 Q.C. PARAMETERS

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

1. The absorbance (OD) of the highest calibrator of any antigen should be 2.0 absorbance units.

2. Four out of five 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 Monobiol 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. 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 and standard curve.

5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution.

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 replication and spuriously high absorbance readings.

8. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed, are essential. Any deviation from Monobind’s IFU may yield inaccurate results.

9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed, are essential. Any deviation from Monobind’s IFU may yield inaccurate results.

10. All applicable national standards, regulations and laws, including but not limited to, good laboratory practices, 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. 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.

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 matrix interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be proteins for all kinds of immunomas (Boscato LM, Stuart MC. ‘Heterophilic interactions and have been known to be problems for all kinds of immunomas. Clin. Immunol. Immunopathol. 1988:3427-33).

4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are not in agreed upon ranges, the kit should be eliminated prior to diagnosing pregnancy.

5. 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 the 10% of the assigned concentrations.

6. False positive results may occur in the presence of a wide variety of trophoblastic and non-trophoblastic tumors that secrete hCG. Therefore, the possibility of hCG secreting neoplasia should be eliminated prior to diagnosing pregnancy.

7. Also, false positive results may be seen with assay specimens from individuals taking the drugs Pergonal® and Gonal®. Additionally Pergonal will often be followed with an injection of hCG.

8. Spontaneous microabortions and ectopic pregnancies will tend to have values which are lower than expected during a normal pregnancy while somewhat higher values are often seen in multiple pregnancies.4,5

9. Following therapeutic abortion, detectable hCG may persist for as long as three days. In postpartum patients, the hCG level after spontaneous abortion, will vary depending upon the quantity of viable residual trophoblast.

10. LH FSH is suppressed by estrogen but in woman taking oral contraceptives the level may be low or normal. Excessive drinking and weight loss may lead to low gonadotropin concentrations.

11. LH / FSH hormone(s) are dependent upon diverse factors other than pituitary gonadotropins and the determination alone is not sufficient to assess clinical status.

13. EXPECTED VALUES (hCG)

A study of non-pregnant females and adult males was undertaken to determine expected values for hCG in Fertility Panel AccuBind® ELISA Test System. The mean (X) values, standard deviations (σ) and expected ranges (±2 S.D.) are presented in Table 1.

<table>
<thead>
<tr>
<th>Number of Values</th>
<th>Mean (x)</th>
<th>Standard Deviation (σ)</th>
<th>Expected Ranges (±2σ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
<td>2.9</td>
<td>1.4</td>
<td>0.1 - 5.7</td>
</tr>
</tbody>
</table>

**TABLE 1**

Expected Values for hCG in Fertility Panel AccuBind® ELISA Test System were determined by analysis of 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 4 and Table 5.

15.0 PERFORMANCE CHARACTERISTICS

A. Precision

The within and between assay precision of LH in the Fertility Panel AccuBind® ELISA Test System were determined by analysis of 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 4 and Table 5.

15.1 Linearity

hCG in the Fertility Panel AccuBind® ELISA test system was compared with a reference method. 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 hCG ELISA in comparison with the reference method. The data obtained is displayed in Table 8.

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean (x)</th>
<th>Least Square Regression Analysis</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monobind</td>
<td>14.8</td>
<td>y = 0.81x + 9.34</td>
<td>0.989</td>
</tr>
</tbody>
</table>

The cross-reactivity of the PRL 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 doses of interfering substance to dose of Follitropin hCG needed to produce the same absorbance.
## PRL VAST®

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolactin Hormone (PRL)</td>
<td>&lt; 0.0001</td>
<td>1000 ng/ml</td>
</tr>
<tr>
<td>Luteinizing Hormone (LH)</td>
<td>&lt; 0.0001</td>
<td>1000 ng/ml</td>
</tr>
<tr>
<td>Follicropin (FSH)</td>
<td>&lt; 0.0001</td>
<td>1000 ng/ml</td>
</tr>
<tr>
<td>Chorionic gonadotropin (CG)</td>
<td>&lt; 0.0001</td>
<td>1000 ng/ml</td>
</tr>
<tr>
<td>Thrytropin (TSH)</td>
<td>&lt; 0.0001</td>
<td>1000 ng/ml</td>
</tr>
<tr>
<td>Growth Hormone (GH)</td>
<td>&lt; 0.0001</td>
<td>1000 ng/ml</td>
</tr>
</tbody>
</table>

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

### 16.0 REFERENCES