Monobind Inc.
Lake Forest, CA 92630, USA

VAST™
Versatile Analyte Selection Technology
4 Analytes 1 Kit
CLIA Microwells

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 endocrinologies.

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 placental tissue, beginning with the primitive trophoblast, almost from the time of implantation, and serves to support the corpus luteum during the early weeks of pregnancy. hCG or hCG similar glycoproteins can also be produced by a wide variety of trophoblastic and nontrophoblastic tumors. The measurement of hCG by assay systems with suitable sensitivity and specificity has proven great value in the detection of 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 peak of 50,000 to 100,000mIU/ml is attained by the third month, then a gradual decline is observed.[2, 3,18]

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 supports spermatogenesis.[9,10]

In women, FSH acts on the granulosa cells of the ovary, stimulating steroidogensis. 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). As the follicular phase progresses, FSH concentration decreases. Near the time ovulation occur, about midcycle, FSH peaks (lesser in magnitude than LH) to its highest level.[8]

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 [luteinizing stimulating 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. Stimulation by gonadotropin-releasing hormone (GnRH) 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.

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 89 amino acid residues while the β-subunit contains 129 amino acids. The carbohydrate content is between 15 and 30%.[6,8,9]

The clinical usefulness of the measurement of luteinizing hormone (LH) in ascertaining the homeostasis of fertility regulation via the hypothalamic - pituitary - gonadal axis has been well established.[4,6] 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 to the procedurally simple and rapid immunoenzymometric assays.

Prolactin hormone (PRL), secreted from the lactotrophs of the anterior pituitary, is a protein consisting of a single polypeptide chain containing approximately 200 amino acids. The primary biological action of the hormone is on the mammary gland where it 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.[4, 24, 25]

The clinical usefulness of the measurement of prolactin hormone (PRL) in ascertaining the diagnosis of hyperprolactinemia and for the subsequent monitoring the effectiveness of the treatment has been well established.[26]

In this method, hCG/PRL//LH/FSH (referred to as antigens, in the Product Insert) combination calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of the hormones) are added and the reactants 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. Than an enzyme liked 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 antibody enzyme bound conjugate is separated from the unbound enzyme antigen conjugate by aspiration or decantation. 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.

3.0 PRINCIPLE

Immunoenzymometric assay (Types 3 and 4):
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 specific monoclonal 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:

$$^{Enz}Ab + Ag_{(Antigen)} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab - Ag_{(Antigen)} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
$Ag_{(Antigen)}$ = Native Antigen (Variable Quantity)
^{Enz}Ab = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab - Ag_{(Antigen)} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = 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:
$^{Enz}Ab - Ag_{(Antigen)} - {}^{Btn}Ab_{(m)} + Streptavidin_{C.W.} \Rightarrow$ immobilized complex
$Streptavidin_{C.W.}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

For PRL assay, the sequence of addition is separated into two steps. That is, the biotinylated antibody binds to the prolactin antigen and is simultaneously deposited to the well surface. The second incubation initiates the binding of the enzyme linked antibody to the antigen bound through the biotinylated antibody on the well.

After equilibrium is attained, the antibody-bound fraction 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: Reagents for 2 X 96 well Microplate

A. Combi-Cal™ FSH/LH/hCG/sPRL Calibrators - 1ml/vial (lyophilized*) - Icons A-F
Six (6) vials of references for antigens at levels indicated below. A preservative has been added. The calibrators, human serum based, were calibrated using a reference preparations indicated in the chart.
**Reconstitute each vial with 1ml of distilled or deionized water. The reconstituted calibrators are stable for one (1) year at 2-8°C.*

Analyte	hCG (mIU/ml)	hLH (mIU/ml)	PRL (ng/ml)	FSH (mIU/ml)
A	0	0	0	0
B	25	5	10	5
C	100	25	25	10
D	250	50	50	25
E	500	100	100	50
F	1000	200	250	100
Ref #	3rd IS (75/537)	1st IRP (68/40)	3rd IS (84/500)	2nd IRP (78/549)

B. hCG Tracer Reagent - 13ml/vial - Icon Ⓔ
One (1) vial containing enzyme labeled antibody and biotinylated monoclonal mouse IgG specific for hCG in buffer, blue dye, and preservative. Store at 2-8°C.

C. PRL Biotin Reagent - 13ml/vial – Icon ▽
One (1) vial containing biotin labeled antibody specific for PRL in buffer, green dye, and preservative. Store at 2-8°C.

D. PRL Tracer Reagent - 13ml/vial - Icon Ⓔ
One (1) vial containing enzyme labeled antibody specific for PRL in buffer, red dye, and preservative. Store at 2-8°C.

E. LH Tracer Reagent - 13ml/vial - Icon Ⓔ
One (1) vial containing enzyme labeled antibody and biotinylated monoclonal mouse IgG specific for hLH in buffer, yellow dye, and preservative. Store at 2-8°C.

F. FSH Tracer Reagent - 13ml/vial - Icon Ⓔ
One (1) vial containing enzyme labeled antibody and biotinylated monoclonal mouse IgG specific for FSH in buffer, green dye, and preservative. Store at 2-8°C.

G. Wash Solution Concentrate – 20 ml/vial – Icon 💧
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-30°C.

H. Light Reaction Wells – 2 x 96
Two 96-well white microplates coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

I. Signal Reagent A – 2 x 7 ml/vial - Icon C^A
Two vials containing Luminol in a buffer. Store at 2-8°C.

J. Signal Reagent B – 2 x 7 ml/vial – Icon C^B
Two vials containing hydrogen peroxide in buffer. Store at 2-8°C.

K. Product Insert

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for 2 x 96 well microplate.

4.1 Required But Not Provided:
1. Pipette(s) capable of delivering 0.025ml (25µl) and 0.050ml (50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%
3. Microplate washers or a squeeze bottle (optional).
4. Microplate luminometer.
5. Container(s) for mixing of reagents (see below).
6. Absorbent Paper for blotting the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
9. Timer.
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 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.

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 cells.

In patients receiving therapy with high biotin doses (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.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) can not be assayed within this time, the sample(s) may 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, 0.100ml (100µl) of the specimen is required for LH and FSH. For prolactin and hCG, 0.050ml (50µl) of sample is needed.

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 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.

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

2B. **For LH and FSH:** Pipette 0.050ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.

3. Add 0.100ml (100 µl) of the appropriate tracer reagent 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 **LH** and/or **FSH** or 20 minutes for **hCG.**
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 samder 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 (100µl) 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 0.100ml (100µl) of the sequential Prolactin Tracer 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.

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.

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.

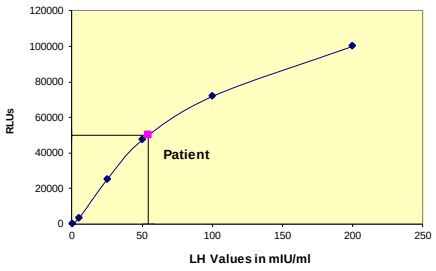
Note: Computer data reduction software designed for CLIA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

Example 1 (hLH)

Sample I.D.	Well Number	RLU (A)	Mean RLU (B)	Value (mIU/ml)
Cal A	A1	23	29	0
	B1	36		
Cal B	C1	3171	3257	5
	D1	3344		
Cal C	E1	24812	25038	25
	F1	25265		
Cal D	G1	46654	47213	50
	H1	47773		
Cal E	A2	71993	71800	100
	B2	71609		
Cal F	C2	99504	100000	200
	D2	100496		
Ctrl 1	E2	960	961	**2.2**
	F2	963		
Ctrl 2	G2	63515	64252	**80.2**
	H2	64989		
Patient	A3	49976	50005	**54.5**
	B3	50033		

* The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay. In addition, the RLUs of the calibrators have been normalized to 100,000 RLUs for the F calibrator (greatest light output). This conversion minimizes differences caused by efficiency of the various instruments that can be used to measure light output.

Figure 1

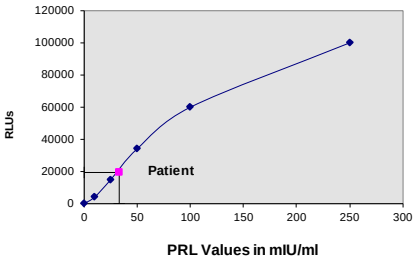


Example 2 (PRL)

Sample I.D.	Well Number	RLU (A)	Mean RLU (B)	Value (ng/ml)
Cal A	A1	88	86	0
	B1	84		
Cal B	C1	3367	3498	10
	D1	3629		
Cal C	E1	12707	12793	25
	F1	12878		
Cal D	G1	31366	34224	50
	H1	37083		
Cal E	A2	63012	60033	100
	B2	57054		
Cal F	C2	102583	100000	250
	D2	97417		
Ctrl 1	E2	1928	1992	**6.6**
	F2	2056		
Ctrl 2	G2	6428	6870	**16.1**
	H2	7311		
Patient	A3	19496	19505	**33.1**
	B3	19513		

* The data presented in Example 2 and Figure 2 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay. In addition, the RLUs of the calibrators have been normalized to 100,000 RLUs for the F calibrator (greatest light output). This conversion minimizes differences caused by efficiency of the various instruments that can be used to measure light output.

Figure 2

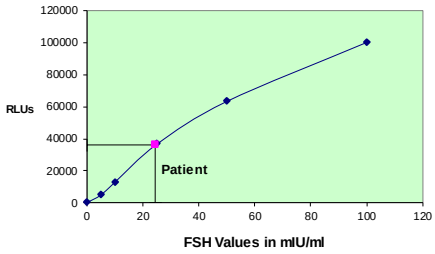


Example 3 (FSH)

Sample I.D.	Well Number	RLU (A)	Mean RLU (B)	Value (mIU/ml)
Cal A	A1	177	183	0
	B1	189		
Cal B	C1	4799	4958	5
	D1	5117		
Cal C	E1	12847	12899	10
	F1	12951		
Cal D	G1	35014	36585	25
	H1	38146		
Cal E	A2	62903	63245	50
	B2	63587		
Cal F	C2	100056	100000	100
	D2	99944		
Ctrl 1	E2	5183	5221	**5.19**
	F2	5258		
Ctrl 2	G2	68278	69108	**56.14**
	H2	69488		
Patient	A3	35110	35990	**24.5**
	B3	36869		

* The data presented in Example 3 and Figure 3 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay. In addition, the RLUs of the calibrators have been normalized to 100,000 RLUs for the F calibrator (greatest light output). This conversion minimizes differences caused by efficiency of the various instruments that can be used to measure light output.

Figure 3

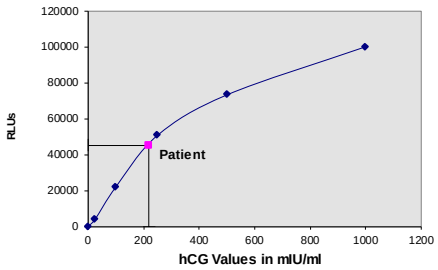


Example 4 (HCG)

Sample I.D.	Well Number	RLU (A)	Mean RLU (B)	Value (mIU/ml)
Cal A	A1	32	26	0
	B1	21		
Cal B	C1	3977	4042	25
	D1	4107		
Cal C	E1	22030	22060	100
	F1	22090		
Cal D	G1	50307	50760	250
	H1	51212		
Cal E	A2	71765	73231	500
	B2	74697		
Cal F	C2	100181	100000	1000
	D2	99819		
Ctrl 1	E2	416	448	**4.3**
	F2	479		
Ctrl 2	G2	29298	29566	**135.0**
	H2	29833		
Patient	A3	45539	45335	**220.0**
	B3	45131		

* The data presented in Example 4 and Figure 4 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay. In addition, the RLUs of the calibrators have been normalized to 100,000 RLUs for the F calibrator (greatest light output). This conversion minimizes differences caused by efficiency of the various instruments

Figure 4



11.0 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.

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. 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 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 or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
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. 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.
10. 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.
11. 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. Patient specimens with hormone concentrations above the high (F) calibrator should be diluted with zero calibrator and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor.
13. Use multichannel pipets for reagent dispensing in order to avoid drift.

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 maximal 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 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 in combination with clinical examination, patient history and all other clinical findings.
4. 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.
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 10% of the assigned concentrations.
6. False positive results may occur in the presence of a wide variety of trophoblastic and nontrophoblastic tumors that secrete hCG. Eliminate the possibility of hCG secreting neoplasia prior to diagnosing pregnancy.
7. Also, false positive results may be seen when assaying specimens from individuals taking the drugs Pergonal* and Clomid**.
8. Spontaneous micro abortions and ectopic pregnancies tend to have values lower than expected during a normal pregnancy while somewhat higher values are often seen in multiple pregnancies.[4,5,6]
9. Following therapeutic abortion, detectable hCG may persist for as long as three to four weeks. The disappearance rate of hCG, after spontaneous abortion, will vary depending upon the quantity of viable residual trophoblast.[4,5,6,7]
10. LH /FSH are suppressed by estrogen but in woman taking oral contraceptives the level may be low or normal.
11. LH / FSH hormone(s) are 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

13.1 hCG

A study of non-pregnant females and adult males was undertaken to determine expected values for the VAST® AccuLite® CLIA hCG assay. The mean (X) values, standard deviations (σ) and expected ranges (±2 S.D.) are presented in Table 1.

TABLE I
Expected Values for the hCG

Number	125
Mean (X)	2.9
Standard Deviation (σ)	1.4
Expected Ranges (±2σ)	0.1 - 5.7

Expected levels for hCG during normal pregnancy[3] are listed in Table 2.

TABLE 2
Expected Values for hCG levels (3rd IS 75/537) during normal pregnancy (in mIU/ml)

1st week	10 – 30
2nd week	30 – 100
3rd week	100 – 1000
4th week	1,000 -10,000
2nd & 3rd month	30,000 - 100,000
2nd trimester	10,000 - 30,000
3rd trimester	5,000 - 15,000

13.2 LH, FSH and PRL

A study of an apparent normal adult population was undertaken to determine expected values for the LH, FSH and PRL VAST® AccuLite® CLIA assay(s). The expected values are presented in Table 3.

TABLE 3
Expected Values for the LH, FSH & PRL

	LH	PRL	FSH
Women		Adult	
Follicular Phase	0.8-10.5	1.2-19.2	3.0-12.0
Midcycle	18.4-61.3		8.0-22.0
Luteal Phase	0.8-10.5		2.0-12.0
Postmenopausal	8.2 -40.8		35 - 151
Men	0.7-7.4	1.8- 17.0	1.0-14.0

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the Fertility Panel AccuLite® CLIA Test System were determined by analyses on three different levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 4 and Table 5.

TABLE 4
Within Assay Precision - Values in mIU/ml hCG-LH-FSH Values in ng/ml PRL (20 Replicates/level)

Level 1	hCG	LH	PRL	FSH
Mean	6.4	1.4	4.9	5.0
σ	0.1	0.1	0.1	0.3
CV	1.4%	7.4%	2.5%	6.0%

Level 2	hCG	LH	PRL	FSH
Mean	22.3	20.5	11.6	24.4
σ	0.6	0.5	0.4	0.9
CV	2.5%	2.4%	3.3%	3.7%

Level 3	hCG	LH	PRL	FSH
Mean	225.6	41.2	23.7	41.8
σ	5.4	1.7	1.3	1.5
CV	2.4%	4.2%	5.4%	3.6%

TABLE 5
Between Assay Precision - Values in mIU/ml hCG-LH-FSH Values in ng/ml PRL

Level 1	hCG	LH	PRL	FSH
Mean	4.9	1.7	5.1	5.9
σ	0.7	0.12	0.5	0.6
CV	13.2%	6.8%	9.8%	10.4%

Level 2	hCG	LH	PRL	FSH
Mean	21.7	23.7	15.5	28.5
σ	2.1	2.3	1.4	3.0
CV	9.5%	9.7%	8.9%	10.6%

Level 3	hCG	LH	PRL	FSH
Mean	232.4	50.8	28.8	50.9
σ	19.9	5.3	2.2	5.5
CV	8.5%	10.5%	7.5 %	10.7%

14.2 Sensitivity

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

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

The VAST® hCG CLIA procedure has a sensitivity of 0.25 mIU. This is equivalent to a sample containing 5mIU/ml 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 mIU/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The VAST® LH 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 6.

TABLE 6 (LH)

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	14.8	y=0.081+0.93(x)	0.989
Reference	15.1		

Only slight amounts of bias between the VAST® CLIA LH procedure and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

The VAST® FSH CLIA assay was compared with a reference immunochemiluminescence assay. Biological specimens from low and elevated populations were used (the values ranged from 0.1mIU/ml – 133mIU/ml). Values outside the highest calibrator were measured by dilution with 0 calibrator and multiplication by the dilution factor. The total number of such specimens was 128. 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 7.

TABLE 7 (FSH)

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	18.0	y=0.93(x)+1.5	0.994
Reference	21.0		

Only slight amounts of bias between the VAST® FSH CLIA procedure and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

This VAST® hCG CLIA procedure 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 8.

TABLE 8 (hCG)

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	14.8	y=0.081+0.93(x)	0.989
Reference	15.1		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement

The VAST® Prolactin CLIA test was compared with a reference chemiluminometric (ICMA) method. Biological specimens from normal and pregnant populations were assayed. The total number of such specimens was 86. 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 9.

TABLE 9 (PRL)

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	19.0	y=1.63+1.01(x)	0.973
Reference	17.3		

Only slight amounts of bias between the VAST® PRL CLIA procedure and the reference method are indicated by the closeness of mean values. The least square regression equation and the correlation coefficient indicates excellent method agreement

14.4 Specificity

The cross-reactivity of the VAST® LH CLIA assay 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.

VAST® LH

Substance	Cross Reactivity	Concentration
LH	1.0000	-
β-LH subunit	< 0.0001	1000ng/ml
FSH	< 0.0001	1000ng/ml
hCG	< 0.0001	1000ng/ml
TSH	< 0.0001	1000ng/ml

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 doses of interfering substance to dose of Follicle Stimulating Hormone needed to produce the same light intensity.

VAST® FSH

Substance	Cross Reactivity	Concentration
FSH	1.0000	-
LH	< 0.0001	1000ng/ml
hCG	< 0.0001	1000ng/ml
TSH	< 0.0001	1000ng/ml

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 interfering substance to dose of Chorionic Gonadotropin needed to produce the same light intensity.

VAST® hCG

Substance	Cross Reactivity	Concentration
Chorionic Gonadotropin (hCG)	1.0000	----
β-hCG subunit	< 0.0001	1000ng/ml
Follitropin (FSH)	< 0.0001	1000ng/ml
Lutropin Hormone (LH)	< 0.0001	1000ng/ml
Thyrotropin (TSH)	< 0.0001	1000ng/ml

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 interfering substance to dose of prolactin hormone needed to produce the same absorbance.

VAST® PRL

Substance	Cross Reactivity	Concentration
Prolactin Hormone (PRL)	1.0000	--
Luteinizing Hormone (LH)	< 0.0001	1000ng/ml
Follitropin (FSH)	< 0.0001	1000ng/ml
Chorionic gonadotropin (hCG)	< 0.0001	1000ng/ml
Thyrotropin (TSH)	< 0.0001	1000ng/ml
Growth Hormone (GH)	< 0.0001	1000ng/ml

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 96.1.0 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)
hLH	25,000 (mIU/ml)
hFSH	40,000 (mIU/ml)
hPRL	None Detected

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