Monobind Inc.
Lake Forest, CA 92630, USA

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

Alpha-Fetoprotein, β-Human Chorionic Gonadotropin, Unconjugated Estriol (AFP/hCG/uE3 VAST®) Triple Screen Panel Test System Product Code: 8575-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Alpha-Fetoprotein (AFP), β-Human Chorionic Gonadotropin (hCG) and Unconjugated Estriol (uE3) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Chemiluminescence

2.0 SUMMARY AND EXPLANATION OF THE TEST

Monitoring of hCG, AFP and uE3 concentrations, at regular intervals, is considered to be very important in determining the fetal well-being. The collective information provided by these three assays (*Triple Screen)* provides the clinician with the comprehensive picture of the development of a healthy fetus and the health of the mother. Any anomaly seen during the first trimester can be corrected unless it is caused by some genetic abnormality. Monobind provides the clinician with a single tool to monitor all three analytes, using 125 µl of patient serum (50µl for AFP, 50µl for uE3 and 25µl for hCG), in a single 75 minutes combination assay.

Alpha-Fetoprotein (AFP) is a glycoprotein with a molecular weight of 70 kDA. AFP is normally produced during fetal development by the hepatocytes, yolk sac and to a lesser extent by the gastrointestinal tract. Serum concentrations reach the highest level at twelve weeks of gestation. This peak level gradually decreases to less than 25 ng/ml after one year of postpartum. Thereafter, the levels reduce further to less than 10 ng/ml. The presence of abnormally high AFP concentrations in pregnant women is considered a risk marker for open neural tube defects (ONTDs).

Elevated levels of AFP are found in patients with primary hepatoma and yolk sac-derived germ tumors. AFP is the most useful marker for the diagnosis and management of hepatocellular carcinoma.

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 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.

According to the literature, serum and urine concentrations of biologically active (non-nicked) hCG is detectable as early as 10 days after ovulation, reaching 100mIU/ml by the first missed period. It rises exponentially in the first trimester, doubling almost every 48 hours to a peak (50,000 to 300,000mIU/ml) by the end of the first trimester. Then a gradual decline is observed reaching approximately one fifth of the peak and remains at this level until term.

Unconjugated estriol in the serum of pregnant women originates almost exclusively from precursors in the fetus, via the placenta.[3] The clinical evidence shows that in uncomplicated pregnancies, the production of estriol increases steadily throughout the last trimester; however, in pregnancies complicated by placental insufficiency the synthesis of estriol decreases rapidly. For many years the most commonly used method for monitoring estriol synthesis (as an index to fetal stress) has been to measure estriol and estriol conjugates in a 24 hour urine sample.[4] However, changes in renal clearance and diurnal variations can make the results of these determinations suspect. In recent years, investigators have found the determinations of unconjugated estriol in plasma during pregnancy as an alternative to the urinary assay to be a better marker of fetal stress.[6] Abnormally low levels of estriol in a pregnant woman may indicate a problem with the development in the child. Levels of estriol in non-pregnant women do not change much after menopause, and levels are not significantly different from levels in men.[7]

The Triple Screen Panel VAST® AccuLite® CLIA test system measures not only AFP, but beta-hCG and unconjugated Estriol (uE3) as well. The test is more accurate and screens for additional genetic disorders. Generally speaking, the combination test will identify ≥ 60% of the babies with Down syndrome and 80-90% of the babies with neural tube defects. This option had not been available, especially in developing countries, with conventional testing like ultrasound alone.[11]

In this method, the combination calibrator (containing different levels of AFP, hCG and E3), 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 AFP and hCG) are added and the reactants mixed. Reaction between the various analyte specific antibodies and native analyte forms a sandwich complex that binds with the streptavidin coated to the well. In the case of uE3 an E3 analog coupled with HRP (Tracer) is added followed by specific biotinylated E3 antibody. A competition occurs between labeled E3 and the native E3 for a limited number of sites on the antibody.

After the completion of the required incubation period, the excess enzyme labeled antibody or analog is washed off via a wash step. Addition of a suitable substrate produces light. In hCG and AFP the intensity of the light is directly proportional to the concentration while in E3 it is inversely proportional to the concentration of the analyte.

The employment of several serum references of known levels of hCG, AFP and E3 permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's concentration can be interpolated.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3 for hCG - AFP):
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, biotinylated (AFP/hCG) antibody.

After adding 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 for AFP and hCG.

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

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ab = Tracer labeled Antibody (Excess Quantity)
$^{Enz}Ab - Ag - {}^{Btn}Ab_{(m)}$ = 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:
$^{Enz}Ab - Ag - {}^{Btn}Ab_{(m)} + Streptavidin_{CW} \Rightarrow$ Immobilized complex
$Streptavidin_{CW}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to 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 for AFP and hCG. 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.

Competitive Enzyme Immunoassay (TYPE 7) for uE3:
The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen.
Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the followed equation:

$$^{Enz}Ag + Ag + Ab_{Btn} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{Btn} + {}^{Enz}AgAb_{Btn}$$

Ab_{Btn} = Biotinylated Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ag = Tracer-antigen Conjugate (Constant Quantity)
$AgAb_{Btn}$ = Antigen-Antibody Complex
$^{Enz}AgAb_{Btn}$ = Tracer-antigen Conjugate -Antibody Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.
$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin}_{CW} \Rightarrow \underline{immobilized\ complex}$
$\underline{Streptavidin}_{CW}$ = Streptavidin immobilized on well
$\underline{Immobilized\ complex}$ = sandwich complex bound to the solid surface

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, 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 2x96 well Microplate

A. Combi-Cal AFP/hCG/uE3 Calibrators - 1ml/vial (lyophilized*) - Icons A-F
Six (6) vials of serum references at levels indicated below. A preservative has been added.
**Reconstitute each vial with 1ml of distilled or deionized water. The reconstituted calibrators are stable for one (1) year at 2-8°C.*

Cal	AFP[1]	hCG[2]	uE3[3]
A	0	0	0
B	10	10	0.5
C	25	25	1.0
D	75	50	2.5
E	150	100	10
F	400	250	20
Units	ng/ml	mIU/ml	ng/ml

[1]AFP calibrated against WHO 1st IRP 72/225
[2] hCG calibrated against WHO 3rd IS 75/537
[3]uE3 prepared gravimetrically from 99+% pure preparations
uE3 calibrators can be expressed in molar concentrations (nM/L) by multiplying by 3.45.
For example: 1ng/ml x 3.45 = 3.45 nM/L

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

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

D. uE3 Tracer Reagent – 6 ml/vial Icon Ⓔ
One (1) vial contains Estriol (Analog)-horseradish peroxides (HRP) conjugate in a protein stabilizing matrix with red dye. Store at 2-8°C.

E. uE3 Biotin Reagent – 6 ml/vial Icon ▽
One (1) vial contains biotin labeled specific biotinylated affinity purified rabbit IgG in buffer, dye, and preservative. Store at 2-8°C.

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

G. Wash Solution Concentrate – 20 ml/vial - Icon
One (1) vial contains surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

H. Sample Diluent – 75 ml/vial
One (1) vial contains normal human serum free of hCG stabilized with preservatives.

I. Signal Reagent A – 2 x 7 ml/vial - Icon C[A]
Two (2) vials contain luminol in buffer. Store at 2-8°C. See "Reagent Preparation."

J. Signal Reagent B -- 2 x 7 ml/vial - Icon C[B]
Two (2) vials containing hydrogen peroxide in buffer. Store at 2-8°C. See "Reagent Preparation."

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 a 192 well kit see table on last page for 96 well kits.

4.1 Required But Not Provided:
1. Pipette(s) capable of delivering 0.025, 0.050, 0.100ml (25, 50 & 100µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Luminometer.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum 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 or heparanised plasma in type, and taken with the usual precautions in the collection of venipuncture samples. The blood should be collected in a redtop (with or without gel additives) venipuncture tube or, for plasma use, evacuated tube(s) containing heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma 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) cannot be assayed within this time, the sample(s) may be stored at -20°C or cooler for up to 30 days, in smaller aliquots. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required for each of the three (3) parameters.

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 solution to 1000ml with distilled or deionized water in a suitable storage container. The diluted wash buffer can be stored at 2-30°C for up to 60 days.
2. **Patient Sample Preparation: For hCG patient samples* (first trimester), dilutions should be made as follows:**
   Place 0.5 ml of Sample Diluent into a test tube and add 0.025ml (25µl) of patient sample. Vortex to mix. (Dilution 1:21). Remove 0.025ml (25µl) of (1:21) dilution and dispense into another test tube containing 1.0ml (1000µl) of Sample Diluent (1/41) (Final Dilution 1:861). Assay the 1:861 dilutions and multiply the results by the dilution factor 861.
   *If hCG from normal populations is to be run, no dilutions are required unless the patient's hCG is suspected to be greater than 250mIU/ml.
3. **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 bacterial 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*

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen (as is and dilutions) to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025ml (25µl) of the appropriate serum reference calibrator, control and specimens (diluted for hCG) into the assigned well.

(For AFP and hCG):

3a. Add 0.100ml (100µl) of the AFP Tracer Reagent or hCG Tracer Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**

(For uE3):

3b. Add 0.050ml (50µl) of the U-Estriol Tracer Reagent to all wells. Swirl the plate gently for 20-30 seconds to mix the contents.
3c. Add 0.050ml (50µl) of the U-Estriol Biotin reagent to all the wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 45 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 four (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 working signal solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE PLATE AFTER SIGNAL ADDITION

9. Incubate at room temperature for five (5) minutes.
10. Read the RLUs (Relative Light Units) in each well in a microplate luminometer for at least 0.2 seconds/ well. **The results can be read within 30 minutes of adding the signal solution.**

10.0 CALCULATION OF RESULTS

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

1. Record the RLUs obtained from the printout of the luminometer as outlined in Example 1.
2. Plot the RLUs for each duplicate serum reference calibrator versus the corresponding analyte concentration in corresponding 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 analyte for an unknown, locate the average RLUs 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 (ng/ml for AFP and uE3 and mIU/ml for hCG*) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the mean of duplicate RLUs of an unknown sample 32787 intersects the AFP DRC at a concentration of 109.9 ng/ml (Figure 1 & Example 1).

Note: During regular monitoring of pregnancy, hCG levels rise exponentially and thus exceed the upper limits of the *Dose Response Curve* (DRC). It is essential to dilute these samples to obtain valid results. (Please see ***'Patient Sample Preparation'*** under section "Reagent Preparation"). Also see the bottom of data table '**Example 2'** for calculations of patient sample concentrations.

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

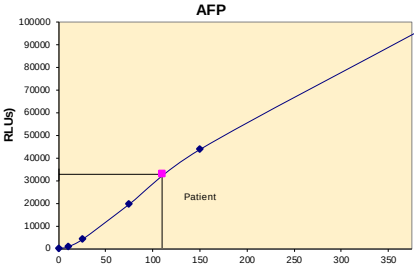
EXAMPLE 1 (AFP)

Sample I.D.	Well Number	RLUs	Mean RLU	Value (ng/ml)
Cal A	A1	12	11	0
	B1	10		
Cal B	C1	955	888	10
	D1	821		
Cal C	E1	3903	4067	25
	F1	4230		
Cal D	G1	18397	19691	75
	H1	20984		
Cal E	A2	45330	43823	150
	B2	42315		
Cal F	C2	100923	100000	400
	D2	99077		
Patient	E2	32619	32787	109.9
	F2	32954		

*The Figures and Examples are for example only. Do not use it for calculating your results

FIGURE 2

AFP



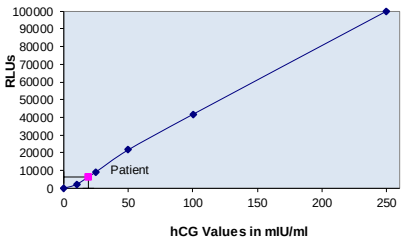
EXAMPLE 2 (hCG)

Sample I.D.	Well Number	RLUs	Mean RLU	Value (mIU/ml)
Cal A	A1	5	0.015	0
	B1	4		
Cal B	C1	2254	0.220	10
	D1	2311		
Cal C	E1	9401	0.475	25
	F1	9091		
Cal D	G1	21184	0.857	50
	H1	22211		
Cal E	A2	42244	1.470	100
	B2	41206		
Cal F	C2	99763	2.724	250
	D2	100237		
Diluted* Patient (1:861)	E2	6320	0.476	19.2*
	F2	6153		

**** Patient Sample Concentration = 19.2x861=16,531mIU/ml***

FIGURE 2

hCG



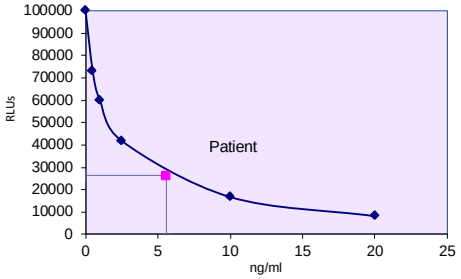
U

EXAMPLE 3 (uE3)

Sample I.D.	Well Number	RLUs	Mean RLU	Value (ng/ml)
Cal A	A1	100344	100000	0
	B1	99656		
Cal B	C1	74311	72854	0.5
	D1	71397		
Cal C	E1	59258	59840	1.0
	F1	60421		
Cal D	G1	40365	41824	2.5
	H1	43260		
Cal E	A2	17147	16696	10.0
	B2	16248		
Cal F	C2	8520	8146	20.0
	D2	7771		
Patient	E2	25903	26219	5.6
	F2	26534		

FIGURE 3

uE3



The data presented in Example 1-3 and Figure 1-3 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 calibrator with the greatest light output. This conversion minimizes differences cause by efficiency of the various instruments that can be used to measure light.

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.

12.2 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 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 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.

AFP has a low clinical sensitivity and specificity as a tumor marker. Clinically, an elevated **AFP value alone is not of diagnostic value as a test for cance**r and should only be used in conjunction with other clinical manifestations (observations) and diagnostic parameters. AFP levels are known to be elevated in a number of benign diseases and conditions including pregnancy and non-malignant liver diseases such as hepatitis and cirrhosis.

Patient's complete history and clinical information available from all related sources should be considered before making any differential diagnosis. No single test or technique is enough to guarantee the validity of an important clinical decision.

13.0 EXPECTED RANGES OF VALUES

Values for AFP, hCG and uE3 for a normal, healthy population and pregnant women, during gestation cycle, are given in Table 1 and 2. The values depicted below represent limited in house studies in concordance with published literature.[1,1,15,16]

TABLE 1 (Normal Values hCG during pregnancy)

Normal Male/Female	≤ 5.7 mIU/ml
During Normal gestation (mIU/ml)	
1st Week	10 – 30
2nd Week	30 – 100
3rd Week	100 – 1000
4th Week	1,000 – 10,000
2nd & 3 Month	30,000 – 350,000
2nd Trimester	10,000 – 30,000
3rd Trimester	5,000 – 15,000

TABLE 2
Median Values during Gestation.

Gestation (Week)	AFP (ng/ml)	hCG (IU/ml)	uE3 (ng/ml)
15	40.14	40.88	0.68
16	42.91	33.87	0.87
17	52.34	28.71	1.17
18	61.50	26.74	1.51
19	75.57	18.76	1.91
20	83.31	19.24	2.02
21	90.46	23.46	2.78

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

14.1.1. Precision (AFP)

The within and between assay precision of the Triple Screen Panel VAST® 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 3 to 8.

TABLE 3
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	20	11.9	0.6	5.0%
Level 2	20	101.4	4.7	4.6%
Level 3	20	203.5	8.5	4.2%

TABLE 4
Between Assay Precision* (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	10	11.6	0.7	6.0%
Level 2	10	98.9	5.3	5.4%
Level 3	10	209.3	10.6	5.1%

*As measured over ten experiments in duplicate

14.1.2. Precision (hCG)

TABLE 5
Within Assay Precision (Values in mIU/ml)

Sample	N	X	σ	C.V.
Level 1	20	6.1	0.45	7.5%
Level 2	20	22.1	1.18	5.3%
Level 3	20	154.3	8.87	5.8%

TABLE 6
Between Assay Precision* (Values in mIU/ml)

Sample	N	X	σ	C.V.
Level 1	10	6.4	0.52	8.1%
Level 2	10	21.0	1.34	6.4%
Level 3	10	149.6	9.87	6.6%

*As measured in ten experiments in duplicate

14.1.3. Precision (uE3):

TABLE 7
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Low	24	1.23	0.12	9.8%
Normal	24	5.05	0.39	7.7%
High	24	9.23	0.51	5.5%

TABLE 8
Between Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Low	10	1.32	0.12	9.1%
Normal	10	4.91	0.41	8.4%
High	10	8.78	0.57	6.5%

*As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

Sensitivity of the Triple Screen Panel VAST® AccuLite® CLIA Test System was determined by running 20 replicates of '0' calibrator. 2SD's of the mean was calculated from the dose response curve.

TABLE 9

Analyte	Sensitivity/Sample	Sensitivity/ml
AFP	0.025 ng/T	1.0 ng/ml
hCG	0.02 mIU/T	0.8 mIU/ml
uE3	2.9 pg/T	0.115 ng/ml

14.3 Accuracy

The Triple Screen Panel VAST® AccuLite® CLIA Test System for AFP was compared with a reference method. Biological specimens ranging from 2.5 to 601 ng/ml concentrations were assayed. The total number of such specimens was 75. The least square regression equation and the correlation coefficient were computed for the AFP procedure in comparison with the reference method. The data obtained is displayed in Table 10.

TABLE 10 (AFP)

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
Monobind (Y)	7.54	y = -0.623+1.01x	0.923
Reference (X)	8.23		

The Triple Screen Panel VAST® AccuLite® CLIA Test System for hCG was compared with a reference method. Biological specimens from normal and pregnant populations were assayed. The total number of such specimens was 88. The least square regression equation and the correlation coefficient were computed for the hCG procedure in comparison with the reference method. The data obtained is displayed below.

TABLE 11 (hCG)

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
Monobind (y)	28.5	y = 1.020 + 0.98x	0.954
Reference(x)	30.2		

The Triple Screen Panel VAST® AccuLite® CLIA Test System for uE3 was compared with a reference method. Biological specimens from low, normal and high unconjugated Estriol level populations were used (The values ranged from 0.15 – 29.1 ng/ml. The total number of such specimens was 58. The least square regression equation and the correlation coefficient were computed for this unconjugated Estriol procedure in comparison with the reference method. The data obtained is displayed in Table 12.

TABLE 12 (uE3)

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
Monobind (Y)	2.78	y= -0.174+0.957x	0.938
Reference(x)	3.05		

Only slight amounts of bias between the Triple Screen Panel VAST® AccuLite® CLIA Test System and the reference methods are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity:

No interference was detected with the performance of the Triple Screen Panel VAST® AccuLite® CLIA test system upon addition of massive amounts of the following substances to a human serum pool. If cross reaction occurred, the % cross reaction is noted.

Cross Reactant	AFP	hCG	uE3
AFP	100%	10 μg/ml	10 μg/ml
hCG	10 IU/ml	100%	NT*
uE3	NT*	NT*	100%-
ASA**	100μg/ml	100μg/ml	100μg/ml
Ascorbic Acid	100μg/ml	100 μg/ml	100μg/ml
CEA	10 μg/ml	10 μg/ml	NT*
PSA	1.0μg/ml	1.0μg/ml	NT*
HLH	10 IU/ml	10 IU/ml	NT*
TSH	100mIU/ml	100mIU/ml	NT*
PRL	100μg/ml	100μg/ml	NT*
Estriol	NT*	NT*	100%
Androstenedione	NT*	NT*	10μg/ml
Cortisol	NT*	NT*	1.0 mg/ml
Cortisone	NT*	NT*	10 μg/ml
Corticosterone	NT*	NT*	10 μg/ml
DHEA-S	NT*	NT*	100 μg/ml
DHT	NT*	NT*	100 μg/ml
Estradiol	NT*	NT*	10 ng/ml
E-3 Sulfate	NT*	NT**	0.62%
Prednisone	10 μg/ml	10 μg/ml	10 μg/ml
Progesterone	10 μg/ml	10 μg/ml	10 μg/ml
Spirolactone	10 μg/ml	10 μg/ml	10 μg/ml
Testosterone	NT*	NT*	10 μg/ml

****ASA = Acetylsalicylic Acid.** ***NT= Not Tested**

14.5 Linearity & Hook Effect:

Massive amounts of related analytes were diluted in pooled human serum and tested, in linear dilutions to check the hook effect of the antibody system used in the Triple Screen Panel VAST® AccuLite® CLIA test system. The results are tabulated below in Table 14.

Table 14

Analyte	Maximum Dose
AFP	100,000 ng/ml
hCG	100,000 mIU/ml
uE3	1000 ng/ml

15.0 REFERENCES

1. Wild D, *The Immunoassay Handbook*, Stockton Press, 445 (1994).
2. Henry JB, *Clinical Diagnosis and Management by Laboratory Methods,* WB Saunders Company, 1075 (1996).
3. Wild D, The Immunoassay Handbook, Stockton Press p400-02 (1994).
4. Li D, Mallory T, Satomura S, "AFP; a new generation of tumor marker for hepatocellulor carcinoma", *Clin Chem Acta,* 313, 15-9 (2001).
5. Kohn J, Weaver PC, 'Serum alpha-fetoprotein in hepatocellular carcinoma. *Lancet*, ii: 334-337 (1974).
6. Cuckle HS, Wald NJ, "Maternal serum alpha-fetoprotein measurement: a screening test for Down Syndrome", *Lancet*, I: 926-929 (1984).
7. Rhys J, Henley R, Shankland D. 'Evaluation of an enhanced luminescence assay for α-fetoprotein.' *Clin Chem*, 32, 2066-2069 (1986).
8. Mizejewski GJ, 'Alfa-fetoprotein structure and function; relevant to isoforms, epitopes and conformational variants' *Exp Biol Med*, 226, 337-408 (2001).
9. Johnson OJ, Williams R, 'Cirrhosis and etiology of hepatocellular carcinoma', *J Hepatology,* 4, 140-147 (1987).
10. Javadpour N, 'The role of biologic tumor markers in testicular cancer', *Cancer*, 45, 1755-61 (1980).
11. Canick JA, Rish S. 'The accuracy of assigned risks in maternal serum screening', *Prenatal Diagnosis*; 18:413-415 (1998).
12. Kosasa TS," Measurement of human chorionic gonadotropin". *Journal of Reproductive Medicine,* 26, 201-06 (1981).
13. Batzer F, 'Hormonal Evaluation of Early Pregnancy', *Fertility and Sterility*, 34, 1-12 (1980).
14. Goeblesman, U. Katagiri, H. Stanczyk et al., "Estriol assays in obstetrics". *J. Steroid Biochemistr, .6, 703-709 (1975).*
15. NIH State-of-the Science Conference Statement on Management of Menopause-Related Symptoms. NIH Consensus State Sci Statements. Mar 21-23; 22(1), 1-38 (2005).
16. Tietz NW, ED: *Clinical Guide to Laboratory Tests* 3rd Ed, Philadelphia, WA Saunders Co (1995).

Revision: 3 **Date: 2019-Jul-16** **DCO: 1353**
MP8575 Product Code: 8575-300

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	1 (13ml)
	C)	1 (13ml)	1 (13ml)
	D)	1 (6ml)	1 (6ml)
	E)	1 (6ml)	1 (6ml)
	F)	1 plate	2 plates
	G)	1 (20ml)	1 (20ml)
	H)	1 (40ml)	1 (75ml)
	I)	1 (7ml)	2 (7ml)
	J)	1 (7ml)	2 (7ml)

For Orders and Inquires, please contact

Monobind Inc.
100 North Pointe Drive
Lake Forest, CA 92630 USA

Tel: +1 949.951.2665 Mail: info@monobind.com
Fax: +1 949.951.3539 Fax: www.monobind.com

IVD 2° 8° CE

EC REP **CEpartner4U,** Esdoornlaan 13
3951 DBMaarn, The Neatherlands
www.cepartner4u.eu

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD In Vitro - Diagnostic Medical Device	2° 8° Temperature Limitation Storage Condition (2-8° C)	Consult Instructions for Use
REF Catalogue Number	Σ Contains Sufficient Test for Σ	LOT Batch Code
Used By (Expiration Day)	Date of Manufacturer	Manufacturer
EC REP Authorized Rep in European Country	CE European Conformity	