Monobind Inc.
Lake Forest, CA 92630, USA

VAST™
Versatile Analyte System Technology
3 Analytes 1 Kit
ELISA Microwells

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

Product Code: 8525-300

1.0 INTRODUCTION

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

2.0 SUMMARY AND EXPLANATION OF THE TEST

Monitoring of hCG, AFP and uE3 concentrations, at regular intervals, is considered to be very important to determine 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 0.125ml (125µl) of patient serum (0.050ml (50µl) for AFP, 0.050ml (50µl) for uE3 and 0.025ml (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 25ng/ml after one year of postpartum. Thereafter, the levels reduce further to less than 10ng/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 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.

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 200,000mIU/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® AccuBind® ELISA test system measures not only AFP, but hCG and 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 (Enzyme) 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 color, In HCG and AFP the intensity of the color 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.

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

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ab = Enzyme 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:

$^{Enz}Ab - Ag - {}^{Btn}Ab_{(m)}$ + $Streptavidin_{C.W.} \Rightarrow$ Immobilized complex
$Streptavidin_{C.W.}$ = 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 = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{Btn}$ = Antigen-Antibody Complex
$^{Enz}Ag\ Ab_{Btn}$ = Enzyme-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.

$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin_{CW}} \Rightarrow \underline{\text{immobilized complex}}$
$\underline{Streptavidin_{CW}}$ = Streptavidin immobilized on well
$\underline{\text{Immobilized complex}}$ = 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. Triple Screen Calibrators - 1ml/vial (Lyophilized) - Icons A-F
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 Enzyme Reagent – 13ml/vial - Icon E
One (1) vial contains enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, yellow dye, and preservative. Store at 2-8°C.

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

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

F. Streptavidin Coated Microplate – 2 x 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

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

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

I. Substrate A – 2 x 7ml/vial - Icon S^A
One (1) vial contains tetramethylbenzidine (TMB) in buffer. Store at 2-8°C. See "Reagent Preparation."

J. Substrate B – 2 x 7ml/vial - Icon S^B
One (1) vial contains hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C. See "Reagent Preparation."

K. Stop Solution – 2 x 8ml/vial - Icon STOP
One (1) vial contains a strong acid (1N HCl). Store at 2-8°C.

L. Product Instructions

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 kit.

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 Reader with 450nm and 620nm wavelength absorbance capability.
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.

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 all 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 0.5ml (500µl) 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 Substrate Solution –** Stable for one (1) year
   Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2-8°C for up to 60 days.

Note: Do not use the working substrate if it looks blue.
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*****

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, control and specimens (diluted for hCG) into the assigned well.

(For AFP and hCG):

3a. Add 0.100ml (100µl) of the AFP Enzyme Reagent or hCG Enzyme 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 Enzyme 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 Antibody biotin reagent to all the wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 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. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

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 gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of assayed analytes 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 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 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 (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 absorbance of an unknown sample 0.256 intersects the AFP DRC at a concentration of 22.9ng/ml (Figure 1 & Example 1).

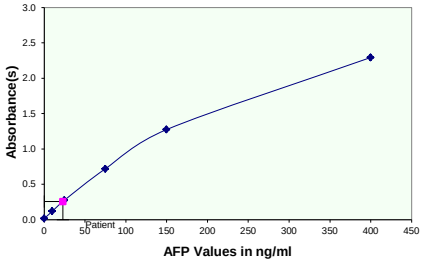
* While regular monitoring of pregnancy hCG levels rise exponentially and thus exceed the upper limits of the *Dose Response Curve* (DRC). It is essential to dilute these samples to obtain valid results. (Please see ***'Patient Sample Preparation'*** under section 'Reagent Preparation). Also see the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

EXAMPLE 1 (AFP)

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.015	0.015	0
	B1	0.015		
Cal B	C1	0.115	0.120	10
	D1	0.126		
Cal C	E1	0.256	0.277	25
	F1	0.298		
Cal D	G1	0.697	0.720	75
	H1	0.743		
Cal E	A2	1.221	1.274	150
	B2	1.326		
Cal F	C2	2.200	2.293	400
	D2	2.386		
Patient	E2	0.253	0.256	22.9
	F2	0.258		

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

FIGURE 1
AFP

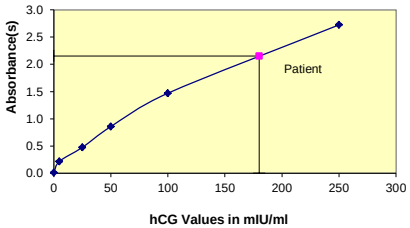


EXAMPLE 2 (HCG)

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (mIU/ml)
Cal A	A1	0.014	0.015	0
	B1	0.015		
Cal B	C1	0.222	0.220	10
	D1	0.217		
Cal C	E1	0.474	0.475	25
	F1	0.477		
Cal D	G1	0.855	0.857	50
	H1	0.860		
Cal E	A2	1.488	1.470	100
	B2	1.452		
Cal F	C2	2.707	2.724	250
	D2	2.741		
Diluted*Patient (1:1071)	E2	0.483	0.476	25.7*
	F2	0.468		

**** Patient Sample Concentration = 25.7x861=22,128mIU/ml***

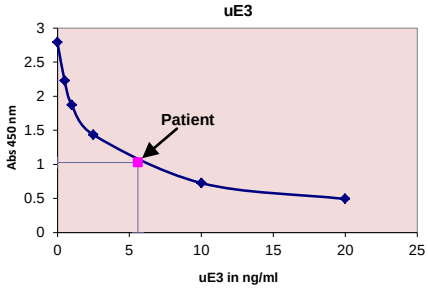
FIGURE 2
hCG



EXAMPLE 3 (uE3)

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	2.765	2.790	0
	B1	2.815		
Cal B	C1	2.219	2.226	0.5
	D1	2.235		
Cal C	E1	1.872	1.870	1.0
	F1	1.868		
Cal D	G1	1.416	1.434	2.5
	H1	1.451		
Cal E	A2	0.711	0.727	10.0
	B2	0.743		
Cal F	C2	0.482	0.493	20.0
	D2	0.503		
Patient	E2	1.037	1.028	5.2
	F2	1.019		

FIGURE 3
uE3



11.0 Q.C. PARAMETERS

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

1. For AFP & hCG the absorbance (OD) of calibrator 'F' should be ≥ 1.3.
2. For uE3 the absorbance of calibrator 'A' should be ≥ 1.3.
3. 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 substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
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 spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
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 procedures 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. ***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 AccuBind® ELISA 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 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.
7. 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 cancer** 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.
8. 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 & 2. The values depicted below represent limited in house studies in concordance with published literature.[11,15,16]

TABLE 1
(Normal Values HCG during pregnancy)

HCG	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® AccuBind® ELISA 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 for AFP (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	20	33.1	1.85	5.6%
Level 2	20	140.5	7.45	5.3%
Level 3	20	230.5	10.45	4.5%

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

Sample	N	X	σ	C.V.
Level 1	10	31.5	1.75	5.6%
Level 2	10	135.8	8.54	6.3%
Level 3	10	244.5	9.58	3.9%

*As measured in ten experiments in duplicate.

14.1.2. Precision (hCG)

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

Sample	N	X	σ	C.V.
Level 1	20	2.8	0.15	5.4%
Level 2	20	15.2	0.65	4.2%
Level 3	20	178.0	10.50	5.9%

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

Sample	N	X	σ	C.V.
Level 1	10	3.1	0.17	5.5%
Level 2	10	15.4	0.81	5.3%
Level 3	10	185.6	11.10	6.0%

*As measured in ten experiments in duplicate.

14.1.3. Precision (uE3):

TABLE 7
Within Assay Precision for uE3 (Values in ng/ml)

Sample	N	X	σ	C.V.
Low	24	1.58	0.13	8.3%
Normal	24	5.17	0.37	7.1%
High	24	9.06	0.59	6.5%

TABLE 8
Between Assay Precision for uE3 (Values in ng/ml)

Sample	N	X	σ	C.V.
Low	10	1.47	0.14	9.5%
Normal	10	4.93	0.39	7.9%
High	10	8.99	0.54	6.0%

*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 (ng/ml)	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® AccuBind® ELISA 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 301. The least square regression equation and the correlation coefficient were computed for the AFP ELISA 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
This Method (Y)	6.60	y = -0.7514 + 0.9639x	0.978
Reference (X)	6.43		

The Triple Screen Panel VAST® AccuBind® ELISA 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 110. The least square regression equation and the correlation coefficient were computed for the hCG ELISA in comparison with the reference method. The data obtained is displayed below.

TABLE 11 (hCG)

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

The Triple Screen Panel VAST® AccuBind® ELISA Test System for uE3 was compared with a reference method. Biological specimens from low, normal and high uE3 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 uE3 ELISA 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
This Method (Y)	3.84	y = -0.1744 + 0.9794x	0.952
Reference (X)	3.74		

Only slight amounts of bias between the Triple Screen Panel VAST® AccuBind® ELISA 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® AccuBind® ELISA 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® AccuBind® ELISA system. The results are tabulated below in Table 13.

TABLE 13

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: 2013-APR-15** **DCO: 0838**
Product Code: 8525-300

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)
	K)	1 (8ml)	2 (8ml)

For Orders and Inquiries, please contact

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

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

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

IVD 2° 8°

EC REP **CEpartner4U,** Esdoornlaan 13, 3951DB Maarn, The Netherlands www.cepartner4u.eu