

11.0 Q.C. PARAMETERS

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

- For AFP & hCG, the absorbance (OD) of calibrator 'F' should be ≥ 1.3
- For uE3, the absorbance of calibrator 'A' should be ≥ 1.3
- 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

- It is important that the time of reaction in each well is held constant to achieve reproducible results.
- Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
- Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
- If more than one (1) plate is used, it is recommended to repeat the dose response curve.
- 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.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
- Use components from the same lot. No intermixing of reagents from different batches.
- 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.
- 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.
- 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.
- 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

- Measurements and interpretation of results must be performed by a skilled individual or trained professional.**
- 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.
- 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;34:27-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
- For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
- 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.**
- 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 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.
- 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)
		1 st Week
		2 nd Week
		3 rd Week
		4 th Week
		2 nd & 3 Month
		2 nd Trimester
		3 rd Trimester

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*
Estradiol	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
Cross Reactant	AFP	hCG	uE3
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

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TABLE 14

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)

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Glossary of Symbols

(EN 980/ISO 15223)

