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

Alpha-Fetoprotein, Carcinoembryonic Antigen, Total Prostatic Specific Antigen (AFP/CEA/tPSA VAST®) Cancer Panel Test System

Product Code: 8475-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of AFP, CEA and PSA Concentration in Human Serum and Plasma by a Microplate Enzyme Immunoassay, Chemiluminescence. Measurements of these tumor markers are used as an aid in the diagnosis and monitoring of various oncological disorders.

2.0 SUMMARY AND EXPLANATION OF THE TEST

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 a peak level of up to 10 mg/ml at twelve weeks of gestation.[1] 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.

Elevated levels of AFP are found in patients with primary heptatoma and yolk sac-derived germ tumors. AFP is the most useful marker for the diagnosis and management of hepatocellular carcinoma.[2]

AFP is also elevated in pregnant women. Presence of abnormally high AFP concentrations in pregnant women provides a risk marker for Down syndrome.[3]

Carcinoembryonic antigen (CEA) is a glycoprotein with a molecular weight of 180 kDA. CEA is the first of the so-called carcinoembryonic proteins that was discovered in 1965 by Gold and Freeman.[5] CEA is the most widely used marker for gastrointestinal cancer.

Although CEA is primarily associated with colorectal cancers, other malignancies that can cause elevated levels of CEA include breast, lung, stomach, pancreas, ovary and other organs. Benign conditions that cause significantly higher than normal levels include inflammation of lung and gastrointestinal (GI) tract and benign liver cancer.[6,8] Heavy smokers, as a group, have higher than normal baseline concentration of CEA.

Prostate Specific antigen (PSA) is a serine protease with chymotrypsin-like activity.[15,17,19] The protein is a single chain glycoprotein with a molecular weight of 28.4 kDA.[16] PSA derives its name from the observation that it is a normal antigen of the prostrate but is not found in any other normal or malignant tissue.

PSA is found in benign, malignant and metastatic prostrate cancer. Since prostate cancer is the second most prevalent form of male malignancy, the detection of elevated PSA levels plays an important role in the early diagnosis.

3.0 PRINCIPLE

Immunoenzymometric assay (Type 3):
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 monoclonal anti-marker specific 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:

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

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
Ag_{CM} = Cancer Marker Antigen (Variable Quantity)
^{Enz}Ab = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab - Ag_{CM} - {}^{Btn}Ab_{(m)}$ = 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:
$^{Enz}Ab - Ag_{CM} - {}^{Btn}Ab_{(m)} + Streptavidin_{C.W.} \Rightarrow$ Immobilized complex
$Streptavidin_{C.W.}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

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 MATERIALS

Reagents for 2 X 96 -well microplate, provided

A. Combi-Cal CEA/AFP/PSA Calibrators - 1ml/vial - Icons A-F
Six (6) vials of references for markers at levels indicated below. A preservative has been added. The calibrators, human serum based, were calibrated using a reference preparations indicated in the chart.

Analyte	AFP (ng/ml)	CEA (ng/ml)	tPSA (ng/ml)
A	0	0	0
B	5	5	2
C	25	10	5
D	100	25	10
E	250	100	25
F	500	250	50
Ref #	1st IRP AFP	IRP 73/601	1st IS 96/670

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

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

D. VAST tPSA Tracer Reagent – 13 ml/vial - Icon
One (1) vial containing enzyme labeled antibody and biotinylated monoclonal mouse IgG specific for PSA in buffer, orange dye, and preservative. Store at 2-8°C.

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

F. Signal Reagent A – 2 x 7ml/vial - Icon C[A]
Two (2) vials containing luminol in buffer. Store at 2-8°C.

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

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

I. 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.**

4.1 Required But Not Provided:
1. Pipette(s) capable of delivering 0.025 ml (25µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5% (optional).
3. Microplate washer 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 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 plasma 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 redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or 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 2-8oC 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 temperatures of -20oC for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required for each tumor marker assayed.

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 indicated 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 diluted buffer at 2-30°C for up to 60 days.
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, 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 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 or specimen into the assigned well.
3. Add 0.100ml (100µl) of the appropriate tracer 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 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 reagent 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 for five (5) minutes at room temperature in the dark.
10. Read the Relative Light Units (RLUs) in each well in a microplate luminometer for at least 0.2 seconds per well. **The results can be read within 30 minutes of adding the signal solution.**

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 each corresponding marker 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 versus the corresponding marker concentration in appropriate units on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of corresponding cancer marker 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 ng/ml) from the horizontal axis of the graph.

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

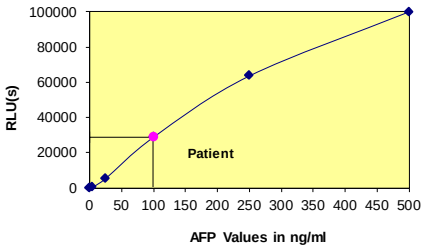
EXAMPLE 1 (AFP)

Sample I.D.	Well Number	RLUs	Mean RLUs	Value (ng/ml)
Cal A	A1	11	12	0
	B1	12		
Cal B	C1	464	452	5
	D1	441		
Cal C	E1	5473	5334	25
	F1	5196		
Cal D	G1	28548	29370	100
	H1	29037		
Cal E	A2	65649	63595	250
	B2	61542		
Cal F	C2	100477	100000	500
	D2	99523		
Ctrl 1	E2	4251	4187	**21.2**
	F2	4124		
Ctrl 2	G2	49533	48995	**182.2**
	H2	48456		
Patient	A3	28371	28731	**99.8**
	B3	29091		

*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.

Note: 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.

Figure 1



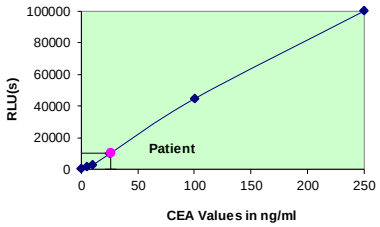
EXAMPLE 2 (CEA)

Sample I.D.	Well Number	RLUs	Mean RLUs	Value (ng/ml)
Cal A	A1	89	80	0
	B1	70		
Cal B	C1	1267	1252	5
	D1	1238		
Cal C	E1	2886	2879	10
	F1	2872		
Cal D	G1	9727	9983	25
	H1	10239		
Cal E	A2	45185	44692	100
	B2	44200		
Cal F	C2	100351	100000	250
	D2	99649		
Ctrl 1	E2	258	259	**1.04**
	F2	259		
Ctrl 2	G2	3366	3403	**11.3**
	H2	3440		
Patient	A3	10152	10464	**26.0**
	B3	10775		

*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.

Note: CEA has a low clinical sensitivity and specificity as a tumor marker. Clinically an elevated **CEA 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. There are patients with colorectal cancer that do not exhibit elevated CEA values and elevated CEA values do not always change with progression or regression of disease. Smokers demonstrate a higher range of baseline values than non-smokers.

Figure 2



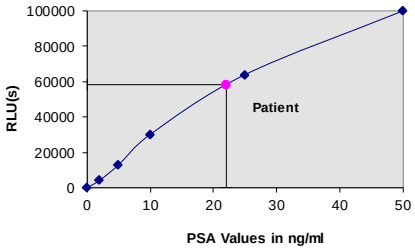
EXAMPLE 3 (tPSA)

Sample I.D.	Well Number	RLUs	Mean Abs (B)	Value (ng/ml)
Cal A	A1	29	30	0
	B1	30		
Cal B	C1	4297	4323	2
	D1	4349		
Cal C	E1	12912	13108	5
	F1	13304		
Cal D	G1	29581	30244	10
	H1	30906		
Cal E	A2	65541	63996	25
	B2	62451		
Cal F	C2	101082	100000	50
	D2	98918		
Ctrl 1	E2	1101	1101	**0.66**
	F2	1101		
Ctrl 2	G2	8479	8698	**3.59**
	H2	8917		
Patient	A3	58984	58232	**22.0**
	B3	57481		

*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.

Note: PSA is elevated in benign prostrate hypertrophy (BPH). Clinically an elevated **PSA value alone is not of diagnostic value as a specific test for cance**r and should only be used in conjunction with other clinical manifestations (observations) and diagnostic procedures (prostate biopsy). Free PSA determinations may be helpful in regard to the discrimination of BPH and prostrate cancer conditions.[5]

Figure 3



11.0 QC PARAMETERS

In order for the assay results to be considered valide 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 interactions 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 immunopassays.' 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.
7. Please refer to the notes in the results section for each cancer marker concerning the limitations for their specific use as a stand alone diagnostic tool.

13.0 EXPECTED VALUES (AFP, CEA & tPSA)

A study of an apparent normal adult population was undertaken to determine expected values for the Cancer Panel VAST® AccuLite® CLIA Test System. A total number of 486 apparently normal samples were taken for the study to establish values for these analytes. The expected values are presented in Table 1.

TABLE I
Expected Values for the Cancer Panel VAST®

Adult *Population*	AFP *(ng/ml)*	CEA *(ng/ml)*	tPSA *(ng/ml)*
Smokers	< 8.5	< 10.0	< 4.0
Non-Smokers	< 8.5	< 5.0	< 4.0

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 Cancer Panel VAST® AccuLite® CLIA Test System were determined by analyses on three different levels of pooled sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 - 7.

TABLE 2
(AFP) Intra- Assay Precision (Values in ng/ml)

Sample	N	X	σ	CV%
Level 1	24	16.99	1.34	7.9
Level 2	24	83.09	4.77	5.7
Level 3	24	182.16	11.94	6.6

TABLE 3
(AFP) Inter- Assay Precision* (Values in ng/ml)

Sample	N	X	σ	CV%
Level 1	10	17.00	1.56	9.2
Level 2	10	84.82	4.88	5.8
Level 3	10	182.00	11.39	6.3

*As measured in ten experiments in duplicate.

TABLE 4
(CEA) Intra- Assay Precision (Values in ng/ml)

Sample	N	X	σ	CV%
Level 1	22	1.20	0.13	11.2
Level 2	22	10.35	0.60	5.8
Level 3	22	16.53	1.11	6.7

TABLE 5
(CEA) Inter- Assay Precision* (Values in ng/ml)

Sample	N	X	σ	CV%
Level 1	10	1.79	0.28	15.9
Level 2	10	12.22	0.61	5.0
Level 3	10	17.21	1.06	6.2

*As measured in ten experiments in duplicate.

TABLE 6
(tPSA) Intra- Assay Precision (Values in ng/ml)

Sample	N	X	σ	CV%
Level 1	20	0.7	0.05	7.1
Level 2	20	4.5	0.20	4.4
Level 3	20	28.3	1.07	3.7

TABLE 7
(tPSA) Inter- Assay Precision* (Values in ng/ml)

Sample	N	X	σ	CV%
Level 1	10	0.8	0.09	11.3
Level 2	10	4.3	0.25	5.8
Level 3	10	27.5	1.42	5.2

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The Cancer Panel VAST® AccuLite® CLIA Test System has sensitivity for different analytes as listed in the following Table 11. 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

Analyte	Sensitivity (ng/ml)
AFP	0.168
CEA	0.018
tPSA	0.004

14.3 Accuracy

This Cancer Panel VAST® AccuLite® CLIA Test System was compared with reference methods. Clinical and non-clinical specimens were assayed. The total number of such specimens was 486. The least square regression equation and the correlation coefficient were computed for AFP, CEA and PSA assays in comparison with the reference method. The data obtained is displayed in Tables 8 - 10.

TABLE 8 (AFP)

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method	112.2	Y = 1.002x – 0.7881	0.997
Reference	112.7		

TABLE 9 (CEA)

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method	5.04	Y = 1.084x – 0.379	0.997
Reference	4.92		

TABLE 10 (tPSA)

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method	5.04	Y = 1.084x – 0.379	0.997
Reference	4.92		

Only slight amounts of bias between the Cancer 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

The cross-reactivity of the Cancer Panel VAST® AccuLite® CLIA Test System 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 analyte needed to produce the same absorbance. The cross reactivity for different analytes is listed in the table below.

TABLE 11
% Cross Reaction

Analyte	AFP	CEA	tPSA
AFP	100	0.0001	0.0002
CEA	ND*	100	ND
PSA	ND	ND	100
CA-125	ND	ND	ND
hCG	0.0001	0.0004	ND
hLH	ND	ND	ND
hTSH	ND	ND	ND
hPRL	0.0002	ND	ND
Acetylsalicylic Acid	ND	ND	ND
Amethopterin	ND	ND	ND
Ascorbic Acid	ND	ND	ND
Atropine	ND	ND	ND
Caffeine	ND	ND	ND

*ND = not detectable

14.5 Linearity & Hook Effect:

Three different lots of reagent preparations of the Cancer Panel VAST® AccuLite® CLIA Test System were used to assess the linearity and hook effect.

The test showed a good dose recovery of 97.0 to 109.4% when linear dilutions of very high concentrations, in pooled sera were assayed with the Cancer Panel VAST® AccuLite® CLIA Test System.

Massive concentrations were used for spiking in pooled human patient sera. The Cancer Panel VAST® AccuLite® CLIA Test System did not show any high dose hook effect with following concentrations of respective analytes.

Analyte	Dose (ng/ml)
AFP	100,000
CEA	60,000
PSA	10,000

15.0 REFERENCES

1. Wild D, *The Immunoassay Handbook*, Stockton Press p44, (1994).
2. Henry J.B., *Clinical Diagnosis and Management by Laboratory Methods,* W.B. Saunders Company, p1075, (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. Gold P, Freedman SO, *J Exp Med,* 121, 439 (1965).
6. Mizejewski GJ, "Alfa-fetoprotein structure and function; relevant to isoforms, epitopes and conformational variants", *Exp Biol Med*, 226, 337-408 (2001).
7. Johnson OJ, Williams R, 'Cirrhosis and etiology of hepatocellular carcinoma.' *J. Hepatology,* 4, 140-147 (1987).
8. Javadpour N, 'The role of biologic tumor markers in testicular cancer*', Cancer,* 45, 1755-61 (1980).
9. Sikorska H, Schuster J, Gold P. "Clinical applications of carcinoembryonic antigen", *Cancer Detection Preview,* 12, 321-355 (1988).
10. Minton JP, Martin EW Jr." The use of serial CEA determinations to predict recurrence of colon cancer and when to do a second-look surgery", *Cancer* 42, 1422-27 (1978).
11. Staab HJ, Anderer FA, Stumpf E, Fischer R," Slope analysis of the postoperative CEA time course and its possible application as an aid in diagnosis of disease progression in gastrointestinal carcinoma", *Am J Surgery,* 136, 322-327 (1978).
12. Thomas P, Toth CA, Saini KS, Jesup JM, Steele G. Jr," The structure, metabolism and function of carcinoembryonic antigen gene family*", Biochem Biophys Acta,* 1032,177-189 (1990).
13. Yamashita K, Totami K, Kuroki M, Ueda I, Kobata A, "Structural studies of the carbohydrate moieties of carcinoembryonic antigens", *Cancer Research,* 47, 3451-3459 (1987).
14. Hammerstrom S, Shively JE, Paxton RJ, Beatty BG, Larson A, Ghosh R, et al, "Antigenic sites in carcinoembryonic antigen" , *Cancer Research*, 49, 4852-58 (1989).
15. National Institute of Health. Carcinoembryonic Antigen: Its role as a marker in the management of cancer. A national Institute of Health Consensus Development Conference., *Ann Inter Med*, 94, 407-409 (1981).
16. Prestigiacomo AF, Stamey TA, 'Physiological variations of serum prostate antigen in the (4-10 ng/ml) range in male volunteers', J Urol, 155, 1977-80 (1996).
17. Stamey TA, McNeal JE, Yemoto CM, Sigal BM, Johnstone IM, 'Biological determinants of cancer progression in men with prostate cancer' , *JAMA,* 281, 1395-1400 (1999).
18. Chen Z, Prestigiacomo A, Stamey T, 'Purification and characterization of Prostate Specific Antigen (PSA) Complexed to α_1-Anticymotrypsin:Potential *reference* Material for International Standardization of PSA Immunoassays' ,*Clin Chem*, 41/9, 1273-1282 (1995).
19. Horton GL, Bahnson RR, Datt M, Cfhan KM, Catalona WJ and Landenson JH, "Differences in values obtained with two assays of Prostate Specific Antigen*", J Urol*, 139, 762-72 (1988).
20. Stenman UH, Leinonen J, Alfthan H, Rannikko S, Tuhkanen K and Alfthan O,"*A* complex between prostate specific antigen and α1-anticymotrypsin is the major form of prostate specific antigen in serum of patients with prostate cancer; assay of complex improves clinical sensitivity for cancer*", Cancer Res*, 51, 222-26 (1991).

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Glossary of Symbols
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IVD — In Vitro - Diagnostic Medical Device
2° 8° — Temperature Limitation Storage Condition (2-8° C)
Consult Instructions for Use
REF — Catalogue Number
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