Leinco Technologies, Inc.™

PRODUCT DATA SHEET

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Alpha-1 Fetoprotein (AFP) MICRO-ELISA Test Kit

Prod. No.: T176
Pkg. Size: 96 Tests

CAUTION: The concentrations of AFP in a given specimen determined with assays from different manufacturers can vary due to differences in assay method and reagent specificity. The results reported by the laboratory to the physician must include identity of the AFP assay used. Values obtained with different assay methods cannot be used interchangeably. If, in the course of monitoring a patient, the assay method used for determining AFP levels serially is changed, additional sequential testing should be carried out to confirm baseline values

Description

The ELISA AFP test is a solid phase sandwich-type enzyme immunoassay (ELISA) Diagnostic Kit for the in vitro quantitative determination of alpha-fetoprotein (AFP) concentration in human serum and amniotic fluid.

Summary and Explanation of the Test

Alpha-fetoprotein (AFP) is a single chain glycoprotein with a molecular weight of approximately 70,000 daltons and alpha electrophoretic mobility.[1] AFP was first identified in 1956 by Bergstrand and Czar in human fetal serum as an embryospecific protein.[2] AFP is a major serum protein in the fetus, but the concentrations decreased rapidly after birth, and by the second year of life and thereafter only trace amounts are normally detected in serum.[3-5] The reappearance of elevated AFP concentrations in adult serum has been observed not only during pregnancy, but also in conjunction with several benign and malignant diseases (Table 1).

AFP in Pregnancy

AMNIOTIC FLUID. Alpha-fetoprotein is normally produced during fetal and neonatal development by the liver, yolk sac, and in small concentrations by the gastrointestinal tract.[1,4] AFP of fetal serum origin is cleared by the fetal kidneys and excreted by urination into amniotic fluid. Amniotic fluid AFP (AF-AFP) levels increase with each week of gestation and peak at about 13-14 weeks at a level of approximately 15-20 µg/ml, after which the levels rapidly decline until about 22 weeks gestation and then gradually decline until term.[6]

If the fetus has an open neural tube defect (NTD), spina bifida or anencephaly, AFP leaks from the lesion, causing elevated AF-AFP concentrations.[7-9] NTD's result from the failure of the developing neural tube to fuse, a process which normally occurs by the fifth week of gestation. NTD's that are not covered by a full thickness of skin or membrane are "open." "Open" NTD's, the causes of which are unknown, account for approximately half of all neural tube defects. Many studies have confirmed the utility of AFP in the early detection of fetal open neural tube defects (NTD).[10-12] Ninety-five percent of births with NTD's are to women with no previous history of an affected fetus.[13] Women with an increased risk for an NTD in the fetus (approximately a 3% risk) are those who have previously delivered a child with a neural tube defect.[14] A family history of neural tube defects increases the risk of a birth with an NTD. Increased concentrations of AF-AFP do not occur to the same degree in fetuses with "closed" NTD's. Therefore, measurements of AFP cannot reliably detect the presence of a closed NTD.

Neural tube defects are among the most commonly occurring birth defects. In spontaneously aborted fetuses, 3-5% will have a neural tube defect. In the United States, the incidence of a neural tube defect is 0.1 to 0.2% of live births.[15] The rate of NTD births is 2-3 times higher in the United Kingdom than in the United States.10 The rate of open spina bifida among Caucasian women is more than twice that of blacks.[16] The rate of NTD births is also affected by geographic location, with incidence in the U.S. generally decreasing form north to south as well as from east to west [17]

Most cases of NTD with anencephaly are stillborn or die soon after birth. The majority of those born with spina bifida die within five years due to the severity of the defect.[18] Moderate to severe handicaps affect more than 95% of those that survive. The remainder, whose handicaps are less severe, can develop and function with relative normality. Impairments associated with spina bifida may include: paralysis of the lower limbs, hydrocephalus, mental retardation, sensory loss, recurrent urinary tract infections, and bladder or bowel incontinence.[18]

AF-AFP assays, to determine the presence of an open NTD, are usually done between the 16th and 20th week of gestation. Because the normal level of AF-AFP is decreasing each week during this period, it is useful to express the results of the assay as a ratio of the concentration observed to the median level of AF-AFP for the specific week of gestation. The U.K. Collaborative Study on over 18,000 pregnancies established multiples of the median (MoM) as the preferred way to expressed AFP results.[10] The median AFP value for each gestational week is first determined; then individual AFP levels are reported as multiples of this value. This method of expression facilitates comparison of AFP test results between gestational weeks and between laboratories.

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The sensitivity of AF-AFP analysis is defined as the percentage of women with an affected fetus who had AF-AFP concentrations greater than or equal to specific MoM cutoff levels. The second U.K. Collaborative study reported a sensitivity of 98% (258/262) using a cutoff of 2.5 MoM for weeks 16 to 21 of gestation. Another study detected 98% (62/63) and 87% (55/63) of open NTD's at cutoffs greater than 2.0 and 2.5 respectively.[11,19-22]

The specificity of AF-AFP analysis is defined as the percentage of women with an unaffected fetus who had AF-AFP concentrations less than or equal to specific MoM cutoff levels. The literature reports a specificity of approximately 99% using a cutoff of 2.5 MoM for weeks 16 to 21 of gestation and a specificity of greater than 99.8% using a cutoff of 3.0 MoM.[11,19-22]

Certain pathological conditions related to pregnancy other than open NTD's can cause elevated AF-AFP concentrations. These include fetal ventral wall defect, congenital nephrosis, Turner's syndrome, and fetal distress, demise or threatened abortion.[11,20,22,23] Multiple fetuses, miscalculated gestational age, and contamination by fetal blood must also be ruled out as the cause of an elevated AF-AFP.[23]

Elevated AF-AFP concentrations indicate additional testing is required, which may include high resolution ultrasonography, amniography, and amniotic fluid AChE analysis.[24-25] High resolution ultrasonography or amniography, but not AFP quantitation, may provide additional information regarding the severity of the defect and the potential degree of impairment. Surgical and other corrective techniques may ameliorate some of these handicaps.[26]

Low levels of AF-AFP are associated with molar pregnancy, missed abortion, pseudocyesis, overestimated gestational age and Down Syndrome.[7]

MATERNAL SERUM. Fetal AFP diffuses across the placenta into the maternal circulation, thus accounting for the increased maternal serum AFP (MS-AFP) concentration observed during pregnancy.[27] Elevated AF-AFP results in elevated MS-AFP, and low AF-AFP results in low MS-AFP.[6] Maternal serum is therefore the primary specimen for neonatal screening for NTDs.

Normal MS-AFP concentrations reach a peak of approximately 250 ng/ml between 32 and 34 weeks of gestation.[6] Maternal serum AFP are usually measured during the 15th to 21st week of gestation. To facilitate the comparison between weeks of gestation and test methods, it is common to report AFP levels as multiples of the median AFP value (MoM) for each week of gestation. Accurate determination of gestational age (by ultrasonography if necessary) is extremely important, since the normal range of MS-AFP concentrations increases each week.

Sensitivity, in the context of AFP testing for neural tube defects, is the percentage of pregnant women with an NTD fetus who has MS-AFP concentrations greater than or equal to a specific MoM cutoff level. For example, using a cutoff of 2.5 MoM, researchers have reported sensitivities of 85% (71/84)[10], 88% (15/17)[19] and 89% (17/19).[10,12,19-21,28] The sensitivity of the test can be increased by selecting a lower MoM cutoff; however, this also increase the number of false positive results obtained. Selecting a higher MoM cutoff increases the specificity of the test and, consequently, the number of false negative results obtained.

Specificity, in the context of AFP testing for open neural tube defects, is expressed as the percentage of pregnant women with singleton fetuses without NTD's at birth that have MS-AFP concentrations below a certain cutoff. Results published in the scientific literature indicate that the specificity of MS-AFP is approximately 96% at a cutoff of 2.0 MoM; 99% at 2.5 MoM and greater than 99% at a cutoff of 3.0 MoM.[10,12,19-21,28]

Additional factors need to be considered when assessing the risk of a NTD being present.[23] One is the effect of maternal weight. Maternal blood volume, as reflected by maternal weigh has been reported to affect maternal serum AFP (MS-AFP) concentrations in maternal circulation; the higher the maternal weight, the lower the MS-AFP concentration.[23-36] Another factor to consider in maternal diabetes. Insulin dependent diabetic women reportedly have MS-AFP levels significantly lower (35-40% lower) than non-diabetic serum, and an increased incidence of NTD.[33-34,37-39] Maternal serum AFP levels in the black population average about 10% higher than MS-AFP values in the non-black population. Adjustment factors or appropriate data bases have been suggested in the literature as a means of normalizing MS-AFP values.[31-32]

Other pregnancy related causes of abnormal MS-AFP concentrations include miscalculated gestational age, multiple fetuses, fetal distress or death, fetal ventral wall defect, congenital nephrosis or maternal liver disease.[10,12,40] In cases in which the fetus does not have an open neural tube defect, the most obvious causes of elevated MS-AFP concentrations are miscalculated gestational age and multiple fetuses. If the second MS-AFP test result is also elevated, ultrasonography can be used to identify the correct gestational age, number of fetuses, fetal distress or demise, and anencephaly. A sample for AF-AFP should be obtained in conjunction with a second MS-AFP determination (to avoid contamination, the serum sample must be obtained before amniocentesis is performed) since MS-AFP can provide independent measures of risk.[11]

Low MS-AFP concentrations and advanced maternal age have been associated with Down's Syndrome.[41]

AFP in Disease

Alpha-fetoprotein (AFP) was first described as a human tumor-associated protein in 1964 by Tatarinov, who observed increased serum AFP concentrations in subjects

and primary hepatocellular carcinoma.[42] Greater than 70% of patients with primary hepatocellular carcinoma have been reported to have elevated levels of serum AFP.[43-45]

Increased serum AFP concentrations have since been observed in patients with nonseminomatous and mixed carcinoma of the testis, i.e., testicular carcinoma with contained both non-semionmatous and seminoma elements. Increase serum AFP concentrations have not been observed in patient with testicular carcinoma which was purely seminoma in origin.[5,47-54] In the case of nonseminomatous testicular cancer, a direct relationship has been observed between the incidence of elevated AFP levels and the stage of disease.[49-52,55-59] Human chorionic gonadotropin (hCG) and AFP are also important prognostic indicators of survival rate among patients with advanced non-seminomatous germ cell testicular tumors.[60] For patient in clinical remission following treatment, AFP levels generally decrease.[31-35,49,52,54,55,61,62] Post-operative AFP values which fail to return to normal strongly suggest the presence of residual tumor.[44,49,52,55,57,58,63] Tumor recurrence is often accompanied by a rise in AFP before progressive disease is clinically evident.[47,49,51,54,55,64,65]

Elevated AFP levels have occasionally been found in association with gastrointestinal tract cancers with and without liver metastases.[61] Elevated serum AFP concentrations have been reported in patients with endodermal sinus tumors and teratocarcinoma with yolk sac components of the ovary and occasionally tumors of other tissues.[43,44,66-68] Elevated serum AFP has been found during pregnancy, in diseases such as ataxia telangiectasia, hereditary tyrosinemia, teratocarcinoma and in benign hepatic conditions such as acute viral hepatitis, chronic active hepatitis and cirrhosis.[43-45,49,50,61,69-75] Elevations of serum AFP in benign hepatic disease in usually transient.[43]

Measurement of serum AFP concentrations is not recommended as a screening procedure for the detection of cancer. Elevated AFP concentrations are also observed in a variety of non-neoplastic disease.[43-45,49,50,53,61,69-75] Furthermore, AFP concentrations within the normal range do no necessarily exclude the presence of malignant disease. However, measurement of AFP concentrations is widely accepted as an aid in the prognosis and management of cancer patients in whom changing concentrations of AFP are observed.

TABLE 1
DISTRIBUTION OF AFP VALUES
REPORTED IN VARIOUS CONDITIONS (%)

	NORMAL 0-7.5 ng/ml	BORDERLINE ELEVATED 7.5-15.0 ng/ml	ELEVATED >15.0 ng/ml
Healthy individuals			
Male	98	2	0
Female non-pregnant	96	4	0
Malignant Disease			
Testicular			
Non-Seminoma	48	8	44
Seminoma	93	7	0
1° Hepatocellular	0	0	100
Gastrointestinal	90	6	4
Pancreatic	72	16	16
Ovarian	86	9	5
Other Cancer	87	11	2
Non-Malignant Diseases			
Cirrhosis	91	2	7
Hepatitis	85	10	5
Pancreatitis	100	0	0

Principle of the Procedure

The test is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes two unique antibodies (goat polyclonal and mouse monoclonal) directed against distinct antigenic determinants on the AFP molecule.

Specifically, plastic wells are coated with anti-AFP (mouse monoclonal). With the addition of a calibrator, test sample or appropriate control containing AFP, immune complexes are formed between AFP in the sample and the solid phase anti-AFP. After a five minute incubation, anti-AFP (goat polyclonal) enzyme-labeled with horseradish peroxidase is added to each well. During an incubation (60 minutes at room temperature), the AFP molecule is sandwiched between the solid phase and enzyme-labeled antibodies. The sample is then decanted and the wells are washed to remove unbound labeled-antibody. An enzyme substrate-chromogen (hydrogen peroxide, H_2O_2, and tetramethylbenzidine, TMB) is added to the well and incubated for 30 minutes at room temperature, resulting in the development of a blue color. The addition of 1.0 N H_2SO_4 stops the reaction and converts the color to yellow. The intensity of the yellow color is directly proportional to the concentration of AFP in the sample. A calibration curve is constructed from the 450 nm absorbance of the 0, 10, 40, 80, 160 and 350 ng/ml calibrators.

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Reagents

Components in Each 96-Test Elisa AFP Kit

1. 96 wells, **AFP ANTIBODY COATED WELLS:** Coated with anti-AFP (mouse monoclonal); contained in a pack with silica gel desiccant.
2. 1 bottle, 22 ml, **AFP ENZYME ANTIBODY CONJUGATE:** anti-AFP (goat polyclonal) labeled with horseradish peroxidase in buffered protein solution; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives; contains FD&C red #40 as coloring agent.
3. 1 bottle, 12 ml, **SUBSTRATE-CHROMOGEN** Buffered hydrogen peroxide and 3,3',5-5'- tetramethylbenzidine (TMB) solution.
4. 1 bottle, 12 ml, **STOP SOLUTION** 1.0 N H_2SO_4.
5. 1 vial, 4 ml, **0 ng/ml AFP CALIBRATOR/SAMPLE DILUENT**: Bovine serum; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives.
6. 5 vials, 1 ml, **AFP CALIBRATORS**: AFP in bovine serum; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives. **10, 40, 80, 160 and 350 ng/ml.**
7. 1 bottle, 50 ml, **WASH BUFFER CONCENTRATE (20X):** Buffered detergent solution, contains 0.1% sodium azide and 0.002% gentamicin sulfate as preservatives. Dilute bottle to 1000 ml with deionized water.

Additional Materials Required

Disposable tip precision pipets - 0.02, 0.1 ml and 0.2 ml.
microtiter plate reader.
Distilled or deionized water.

Storage and Stability

Store all components at 2°-8°C when not in use. The following components may be stored at ambient temperature: WELLS, SUBSTRATE-CHROMOGEN, WASH BUFFER and STOP SOLUTION. Expiration date printed on the kit indicates limits of stability.

The AFP ANTIBODY COATED WELLS are supplied in a resealable bag containing a desiccant and must be stored with the bag sealed to protect from moisture. Wells can be stored at 2° - 30°C.

Chemical or Physical Indications of Instability

Alterations in the physical appearance of reagents, or results consistently outside the acceptable limits for control sera, may be due to reagent contamination or deterioration.

Instruments

Performance of the AFP test requires use of a precision microtiter plate reader at a wavelength of 450 ± 20 nm.

Specimen Collection and Preparation

Serum or amniotic fluid samples are used in the AFP Diagnostic Kit procedure. No special preparation of the patient is necessary; fasting is not required. Maternal blood should be collected prior to amniocentesis. Visibly blood stained amniotic fluid samples should be examined for the presence of fetal blood cells by the Kleinhauer-Betke technique.[31] Repeated freezing and thawing of specimens should be avoided. No additives or preservatives are necessary.

STORAGE: Specimens may be stored in a tightly sealed tube at 2°-8°C for 24 hours. If the sample is not assayed within 1 day, store frozen (-20°C) in a tightly sealed tube for up to 3 weeks. Specimens should be allowed to come to room temperature and should be mixed thoroughly by gentle inversion before assaying.

ELISA AFP Procedure

Reagent Preparation

Dilute bottle of WASH BUFFER CONCENTRATE (50X) solution to 1000 ml with deionized water. Diluted wash solution will be stable until the expiration date stamped on the kit.

Preliminary Comments and Precautions

Patient sample may contain pathogens: treat all samples as potentially infectious.

Reagents contain thimerosal; avoid contact with skin.

Avoid contact with SUBSTRATE-CHROMOGEN (tetramethyl-benzidine) solution. It is harmful if inhaled or absorbed through skin (may cause irritation).

CAUTION: Source material used to prepare Calibrators was derived from human material. The material was tested using FDA-approved methods and found non-reactive for Hepatitis B Surface Antigen (HBsAg) by ELISA and non-reactive for HIV by ELISA. No known test method can offer total assurance that infectious agents are absent. HANDLE THESE REAGENTS AS IF THEY ARE POTENTIALLY INFECTIOUS. Information on handling human serum is provided in the CDC/NIH manual "Bio-safety in Microbiological and Biomedical Laboratories" (1984).

Procedural Notes

1. All test kit components used in the assay must be of the same master lot number. Materials should not be used after the expiration date shown on the package label. Components and test specimens should be at room temperature (18° - 30°C) before testing begins.
2. All calibrators, controls, and samples should be tested in duplicate simultaneously. The test samples and controls must be well mixed before use.
3. A separate disposable tip should be used for each sample to avoid cross-contamination. All pipetting steps should be performed with the utmost care and accuracy. Avoid contaminating the reagent pipette tip

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with the serum sample.

4. The duration of the incubation times must be the same for all wells within a run.
5. Run size should be limited to the number of samples that can be added to antibody coated wells within 5 minutes.
6. Samples should be pipetted to the bottom of the antibody-coated wells.
7. If microtiter reader is not capable of reading absorbances greater than 2.5, the color should be read after a shorter incubation time with the SUBSTRATE /CHROMOGEN, i.e., 15 minutes.
8. **AMNIOTIC FLUID:** Dilute the specimen of amniotic fluid 1:51. Add 10 µl of amniotic fluid to 0.5 ml of the ZERO CALIBRATOR / SAMPLE DILUENT.

Test Procedure

1. Place sufficient COATED WELLS in a holder to run 0.0, 10, 40, 80, 160 and 350 ng/ml CALIBRATORS, Quality Control Sera and patient samples (for amniotic fluid, diluted 1:51) in duplicate. Limit run size to the number of samples that can be pipetted in 5 minutes.
2. Pipet **20 µl** (0.02 ml) of the **CALIBRATORS, Controls** or **Patient Sample** to the corresponding COATED WELL and mix gently.
3. Incubate at room temperature (18°-30°C) for **5 minutes**.
4. Pipet or dispense **200 µl** (0.2 ml) of the ENZYME ANTIBODY **CONJUGATE** solution to all the wells and mix gently.
5. Incubate at room temperature (18°-30°C) for **60 minutes** ± 5 minutes.
6. **Decant** or aspirate and discard liquid contents of all wells. **SLAP** the inverted wells on a clean piece of absorbent paper. Remove **ALL OF THE LIQUID** from the wells.
7. Fill each well with diluted **WASH BUFFER**. Fill the wells to overflowing, you cannot cause any carryover between the wells. Decant or aspirate liquid contents of all wells. SLAP the inverted wells on a fresh clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.

WARNING: WASHING THE WELLS IS OF CRITICAL IMPORTANCE. Fill the wells to overflowing, you CANNOT cause any carryover between wells. You CANNOT over wash the wells. Completely decant or aspirate all of the liquid out of the wells. SLAP the inverted wells on a FRESH clean piece of absorbent paper AFTER EACH WASH. YOU CANNOT SLAP TOO HARD, REMOVE ALL OF THE LIQUID FROM THE WELLS.

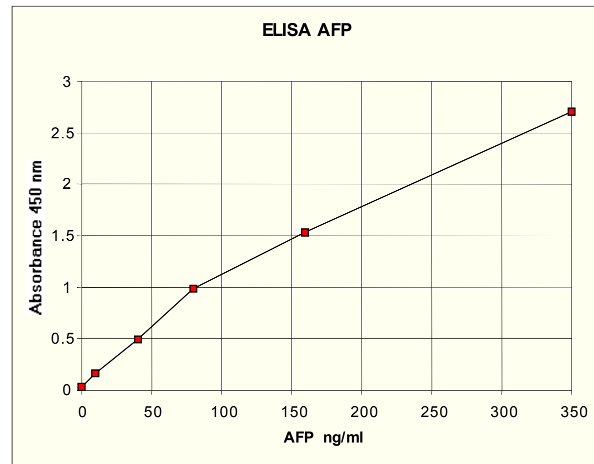
8. Repeat step 7 three more times (for a total of **4 washes**).
9. Fill each well with deionized water. Fill the wells to overflowing. Decant or aspirate liquid contents of all wells. SLAP the inverted wells on a fresh clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.
10. Pipet or dispense **100 µl** (0.1 ml) of SUBSTRATE-CHROMOGEN solution into each well.
11. Mix thoroughly and incubate **30 minutes** at room temperature (18°-30°C).
12. Pipet or dispense **100 µl** (0.1 ml) of **1 N H_2SO_4** into each well and mix thoroughly.
13. Read the absorbance of each well at **450 nm** ± 10 nm against water.

Calculation of Results

1. Calculate the mean value for each duplicate sample absorbance at 450 nm. Values for duplicate absorbances should be within 10% (or 0.02 absorbance units for absorbances less than 0.2).
2. Construct a standard curve by plotting the mean absorbance obtained for each AFP CALIBRATOR on the vertical (Y) axis versus the corresponding AFP concentration on the horizontal (X) axis, using rectilinear graph paper.
3. Connect the points with straight-line segments.
4. Using the mean absorbance for each sample, read the corresponding AFP concentration in ng/ml from the curve. Multiply the value by the dilution factor if required.

EXAMPLE DATA
DO NOT USE IN PLACE OF CUREVE
DETERMINED ATHE THE TIME OF ASSAY.

Specimen I.D.		A_{450}	Mean A_{450}	AFP(ng/ml)
CALIBRATOR				
0 ng/ml		0.027	0.033	0.030
10 ng/ml		0.162	0.164	0.163
40 ng/ml		0.501	0.493	0.497
80 ng/ml		0.956	1.016	0.986
160 ng/ml		1.593	1.476	1.534
350 ng/ml		2.629	2.777	2.703
SAMPLES				
# 1	0.511	0.520	0.516	41.5
# 2	1.861	1.832	1.846	210.7
# 3	2.923	3.126	3.025	402.2



Calculate the MoM (Multiple of Median) for maternal serum and amniotic fluid samples.

$$\text{MoM} = \frac{\textbf{AFP Concentration}}{\textbf{Median Value for Gestation Age}}$$

The range of this assay is 0 - 350 ng/ml. For specimen with AFP concentrations beyond the standard curve (350 ng/ml), repeat the test by diluting the specimen with the 0 ng/ml CAIBRATOR / SAMPLE DILUENT. To obtain the final concentration, multiply the concentration of the diluted sample by the dilution factor**. ALL AMNIOTIC FLUID SAMPLES MUST BE DILUTED 1:51 PRIOR TO BEING ASSAYED.**

Quality Control

Good laboratory practice requires that quality control specimen be run with each calibration curve to check the assay performance. Controls containing azide can not be used. Pooled human serum or commercially available control sera without azide are also suitable. Any material used should be assayed repeatedly to establish mean values and acceptable ranges to assure proper performance.

Read the absorbance of the test solutions against a water blank. If the absorbance of the 0 ng/ml CALIBRATOR exceeds 0.100 at 450 nm it is an indication of careless washing and the assay must be repeated.

Limitations of the Procedure

As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

Serum AFP concentration should not be interpreted as absolute evidence for the presence of absence of malignant disease. Patients with confirmed carcinoma frequently have pretreatment serum AFP concentrations within the range of concentrations observed in healthy individuals. Additionally, elevated AFP concentrations may be observed in the serum obtained from heavy cigarette smokers and patients with nonmalignant diseases. Serum AFP concentrations should be used only in conjunction with information available from the clinical elevation of the patient and other diagnostic procedure.[1-75] Therefore, the ELISA AFP test is not recommended for use as a screening procedure to detect the presence of cancer in the general population.

Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies.[79-81] Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). These samples should not be assayed with the ELISA AFP test as erroneous results may be obtained.[76-78] These conditions should be ruled out prior to clinical evaluation of test results.

The wash procedure (steps 7-9) is critical. Insufficient washing will result in poor precision and falsely elevated absorbances. The use of tap water for washing could result in a higher background absorbance.

FINAL REACTION STABILITY: The spectrophotometric measurement should be made within 30 minutes after the addition of the H_2SO_4 solution.

Samples with elevated levels of AFP (up to 16,000 ng/ml) will always assay as >350 ng/ml when tested, and will not result in a "high dose hook effect". When it is necessary to measure levels of AFP greater than the 350 ng/ml CALIBRATOR, the sample should be diluted with the 0 ng/ml CALIBRATOR and re-assayed.

Expected Values

Serum samples from 101 apparently normal males and non-pregnant females and the following results were obtained:

	AFP (ng/ml)	
Range	0.0 - 10.0	>10.0
% of Observations in Range	99.0%	1.0%

Maternal Serum and Amniotic Fluid:
The expected values for AFP in maternal serum and amniotic fluid are based on the median value for 606 unaffected, singleton pregnancies and are express as the Multiple of Median Value for the number of full gestational weeks.

MATERNAL SERUM

WEEKS GESTATION	**MEDIAN AFP (ng/ml)**	**MULTIPLES OF MEDIAN (ng/ml)**				
		0.5	1.0	2.0	2.5	3.0
15	33.2	16.6	33.2	66.5	83.1	99.7
16	38.6	19.3	38.6	77.3	96.6	115.9
17	45.0	22.5	45.0	90.0	112.5	135.0
18	52.3	26.2	52.3	104.6	130.8	156.9
19	60.8	30.4	60.8	121.6	152.0	182.4
20	70.7	35.4	70.7	141.4	176.8	212.1
21	82.3	41.2	82.3	164.6	205.8	246.9

AMNIOTIC FLUID

WEEKS GESTATION	**MEDIAN AFP (μg/ml)**	**MULTIPLES OF MEDIAN (μg/ml)**				
		0.5	1.0	2.0	2.5	3.0
15	16.0	8.0	16.0	32.0	40.0	48.0
16	13.5	6.8	13.5	27.0	33.8	40.5
17	11.5	5.8	11.5	23.0	28.8	34.5
18	9.7	4.9	9.7	19.4	24.3	29.1
19	8.2	4.1	8.2	16.4	20.5	24.6
20	7.0	3.5	7.0	14.0	17.5	21.0
21	5.9	3.0	5.9	11.8	14.8	17.7

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These values are consistent with those reported in the literature. However, it is recommended that each laboratory determine its own normal range.

Performance Characteristics of the Test

Assay Sensitivity

The sensitivity of this assay is defined as the smallest single value that can be distinguished from the zero calibrator. This value was calculated from the mean + two standard deviations for twenty-two replicates at the zero concentration. The calculated sensitivity is <1.0.

Assay Specificity

Specificity of this test system was determined by assaying 606 serum samples which had been assayed for AFP by either the Abbott (AXSYM) or Hybritech (ELISA) AFP tests. MoM values were calculated for 556 samples with confirmed gestational ages.

ELISA AGREEMENT WITH OTHER (ABBOTT OR HYBRITECH) AFP

	< 7.5 ng/ml	> 7.5 ng/ml
# OTHER AFP	20	586
ELISA AGREEMENT	20	586
% AGREEMENT	**100%**	**100%**

ELISA AGREEMENT WITH OTHER (ABBOTT OR HYBRITECH) MoM

	< 2.5	> 2.5
# OTHER MoM	538	18
ELISA AGREEMENT	537	15
% AGREEMENT	99.8%	83.3%
ELISA DISAGREEMENT	1	3
% DISAGREEMENT	0.2%	16.6%

OVERALL AGREEMENT 552 OUT OF 556 (99.3%)

MoM VALUES IN DISAGREEMENT

OTHER MoM VALUE	ELISA MoM VALUE
2.20	2.57
2.59	2.02
2.66	2.37
2.72	2.37

Assay Reproducibility

Intra-assay reproducibility was determined by measurement of 22 replicates of three serum pools in a single run.

	Mean AFP (ng/ml)	SD	%CV
Serum A	33.13	1.62	4.90
Serum B	158.80	4.42	2.77
Serum C	336.23	9.89	2.94

The interassay reproducibility was determined by duplicate measurement of three serum pools in twenty-four separate runs.

	Mean AFP (ng/ml)	SD	%CV
Serum A	34.01	1.43	4.20
Serum B	167.06	7.91	4.73
Serum C	344.20	12.38	3.60

Comparison to Other AFP Tests

Correlation studies on a group of 330 serum samples with a range of values from 1.0 - 159 ng/ml, were performed using the results from the ELISA AFP Test and the Abbott AXSYM AFP test. The correlation coefficient of the test results was 0.979. Another correlation study on a group of 296 serum samples with a range of values from 2.0 - 169 ng/ml, was performed using the ELISA AFP Test and the Hybritech Tandem-E AFP test. The correlation coefficient of the test results was 0.978. Additionally, the MoM was calculated for each sample. The correlation coefficient for the MoM was 0.938.

	Slope	Y-Intercept	Correlation Coefficient
OVERALL AFP			
n= 606	**0.969**	**1.84**	**0.973**
OVERALL MoM			
n= 556	**0.817**	**0.15**	**0.938**
ABBOTT (AXSYM)			
n= 330	0.968	4.19	0.979
HYBRITECH TANDEM-E			
n= 296	0.991	-0.55	0.978

Assay Linearity

A study was performed diluting a serum sample containing an elevated level of AFP with the 0 ng/ml CALIBRATOR to determine the linearity of the ELISA AFP.

Dilution Factor	Expected Value (ng/ml)	Observed Value (ng/ml)	% of Expected Value
undiluted	-	155.91	-
3:4	116.93	113.57	97 %
1:2	77.96	75.77	97 %
1:4	38.98	41.22	106 %
1:8	19.49	20.29	104 %

Assay Recovery

Three aliquots of human sera with a AFP concentration of 3.66 ng/ml were spiked with 1, 5 and 10 μl of human sample with a AFP value of 26,000 ng/ml. The samples were assayed in duplicate.

AFP ng/ml

Added Serum 26,000 ng/ml	Expected Value	Measured Value	% Recovery
0 μl	-	3.67	
1 μl	29.66	30.97	104 %
5 μl	133.66	129.48	97 %
10 μl	263.66	255.58	97 %

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Leinco Technologies, Inc.

PRODUCT DATA SHEET

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