IMMULITE®

AFP

DPC®

IMMULITE® AFP

English

Intended Use: For *in vitro* diagnostic use with the IMMULITE Analyzer – for the quantitative measurement of alpha-fetoprotein (AFP) in either of two contexts: (a) serial measurements in human serum to aid in the management of patients with nonseminomatous testicular cancer; or (b) measurements in maternal serum and amniotic fluid during gestational weeks 15 through 20 – used in conjunction with ultrasonography or amniography – to aid in detection of fetal open neural tube defects.

Catalog Numbers: **LKAP1** (100 tests), **LKAP5** (500 tests)
Test Code: **AF** Color: **Light Gray**

Caution: In the United States, federal law restricts this device to sale by or on the order of a physician.

> The concentration of AFP in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity. **The results reported by the laboratory to the physician must include the identity of the assay used. Values obtained with different AFP assays cannot be used interchangeably.** Before changing assays, the laboratory must: (a) for cancer management – confirm baseline values for patients being serially monitored; (b) for prenatal testing – establish a range of normal values for the new assay based on normal sera and amniotic fluids from pregnant women with confirmed gestational age.

Summary and Explanation

Alpha-fetoprotein (AFP) is a single-chain glycoprotein with a molecular mass of approximately 70,000 daltons. AFP shares considerable sequence homology with albumin, and is produced by the fetus primarily in cells of the yolk sac, gastrointestinal tract and liver. AFP appears as a major serum protein in the fetus, but its concentration decreases rapidly toward birth.[1,2,3] 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.

Testicular Cancer

Elevated levels of AFP have been observed not only in patients with nonseminomatous testicular cancer, but also in patients with other malignancies such as hepatocellular carcinoma, ovarian cancer, gastrointestinal cancer and pulmonary cancer.[8-15] Serum AFP is frequently elevated in benign hepatic conditions such as acute viral hepatitis, chronic active hepatitis and cirrhosis. Conditions of pregnancy, ataxia telangiectasia and hereditary tyrosinemia have also presented with elevated concentrations of AFP.[8-15]

Seminomas, in pure form, do not present with elevated concentrations of AFP. However, elevated concentrations of serum AFP have been observed in patients diagnosed with seminomatous testicular cancer accompanied by nonseminomatous metastases.[8,16,18,19] During chemotherapy, patients with advanced seminoma and hepatic dysfunction have also presented with elevated serum AFP concentrations.[20] The interpretation of elevated AFP concentrations in patients with seminoma requires special consideration and should assist the clinician in the selection of appropriate therapy.[8,15,21]

The clinical utility of AFP measurement as an aid in the management of patients with nonseminomatous testicular cancer is well documented.[8,16,17,18,22] AFP measurement has found clinical application as an aid in assessing the extent of disease.[16,22-26]

Serial measurements of serum AFP have been shown to reflect the effectiveness of therapeutic regimens in patients with nonseminomatous testicular tumors.[8,15,17,26,27] Post-

surgical determinations of AFP are particularly valuable. The presence of residual tumor is strongly suggested if post-operative AFP concentrations fail to return to normal.[9,15,28,29] The accurate interpretation of post-surgical changes in AFP concentration requires consideration of its metabolic decay rate.[21,22,24,25] When utilizing AFP for monitoring therapy or disease recurrence during chemotherapy, it should be noted that levels often fall rapidly during chemotherapy, reaching normal levels while tumor masses are still evident.[17,21] In such instances, completion of the planned therapy has been recommended.[21]

Following therapy or surgery, serial measurements of AFP have also proved clinically useful when monitoring for progression or recurrence of disease in patients with nonseminomatous testicular cancer. It has been reported that AFP levels frequently rise during disease progression and fall during disease remission.[9,17,18] Elevated AFP levels have frequently been observed to accompany tumor recurrence before progressive disease is clinically evident.[9,18]

Fetal Open Neural Tube Defects

AFP is detectable not only in fetal serum, but also in amniotic fluid and maternal serum. A concentration gradient exists such that when the fetal serum AFP level is 2,000 kIU/mL, the amniotic fluid AFP (AFAFP) level is 20 kIU/mL, and the maternal serum AFP (MSAFP) is 0.02 kIU/mL. In normal pregnancy, the fetal serum AFP concentration peaks at 14 weeks gestation.[34] The AFAFP concentration peaks at about 12 weeks and the MSAFP peaks at approximately 28 – 32 weeks gestation.[36] The fall in AFAFP concentration reflects the fall in fetal serum AFP concentration which results from increased fetal size and fluid volume.[34] Elevated levels of MSAFP and AFAFP may occur most often due to multiple pregnancy and due to incorrect gestational age.

Measurement of AFP concentrations is clinically valuable in screening for open NTDs and other fetal abnormalities;[35] pregnancies associated with open NTDs present with elevated AFP levels. Excess AFP gains access to amniotic fluid, and to a lesser extent to the maternal serum, by transudation across the exposed surface of the fetus or across damaged glomeruli.[35, 37] These conditions are found in open NTDs including open spina bifida and anencephaly, omphalocele, and congenital nephrosis.[32,36] Additional causes of elevated AFP concentrations including both maternal and fetal sources are impending spontaneous abortion, fetal distress or death, oligohydramnios, toxemia, gastroschisis, Meckel's syndrome, sacrococcygeal teratoma, Turner's syndrome and maternal hepatic and oncologic disorders.[35]

Recommended protocols for open NTD screening have been published.[33,35] The cutoff levels for maternal serum and amniotic fluid can be chosen to optimize the needs of the populations being tested based upon varying prevalence of open NTDs. Cutoffs commonly utilize multiples of the median of 2.0 or 2.5 for MSAFP and AFAFP testing. The optimal time for screening MSAFP is between the 16th and 18th weeks of pregnancy, although screening is still effective before or after this period. Elevated AFP concentrations may be subjected to a repeat sampling and analysis to exclude transient rises.

More commonly, ultrasonography is employed to rule out multiple pregnancies and to confirm gestational age. Ultrasonography may also identify signs of open NTDs, particularly anencephaly which is a large, easily visualized lesion. If correction for gestational age or multiple pregnancy does not result in an AFP concentration within the normal range, then diagnostic ultrasonography and/or amniotic fluid sampling is indicated. The greatest diagnostic power can be achieved by combining biochemical analysis of amniotic fluid and diagnostic ultrasonography in cases of a positive MSAFP screen.[35]

Elevated MSAFP results are not diagnostic for NTDs and should not be considered a cause for termination of pregnancy. An overlap exists in the distributions of AFP concentrations from pregnancies with and without open NTDs. Closed NTDs, for example, are not usually associated with increased MSAFP or AFAFP concentrations. Thus, further testing is required to define fetal status. In light of these considerations and the multiple causes for elevated AFP concentrations, all clinical information should be evaluated and confirmatory tests performed wherever possible before reaching a diagnosis.

AFP can be measured by several immunologic techniques, depending on the degree of sensitivity desired. Radial immunodiffusion, countercurrent immunoelectrophoresis, and rocket immunoelectrophoresis are three techniques well suited for research applications. Enzyme-linked immunosorbent assays and radioimmunoassays of both competitive and non-competitive designs have been successfully employed clinically both for maternal serum and amniotic fluid measurements.

Note: IMMULITE AFP Physician Brochure (Cat. #ZS1105) and Patient Brochure (Cat. #ZS1106), explaining the use of AFP prenatal testing to aid in the detection of fetal open NTD are available by calling DPC Customer Services 1-800-372-1782 or your National Distributor.

Principle of the Procedure

IMMULITE AFP is a solid-phase, two-site sequential chemiluminescent immunometric assay.

Incubation Cycles: 2 x 30 minutes.

Specimen Collection

Serum: Collect blood by venipuncture[31] into plain tubes and separate the serum from the cells as soon as possible. Specimens must be obtained before amniocentesis to obtain a valid specimen. Lipemic samples should be clarified with the help of an ultracentrifuge before assay of serum.

Centrifuging serum samples before a complete clot forms may result in the presence of fibrin. To prevent erroneous results due to the presence of fibrin, ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increased clotting time.

Amniotic Fluid: Collect amniotic fluid by amniocentesis into plain tubes. Samples should be obtained by aseptic transabdominal amniocentesis performed by an experienced obstetrician during the second trimester of pregnancy in women with confirmed gestational age. Centrifuge the specimen, retaining a portion of the clear supernatant. Inspect both supernatant and sediment for signs of blood or hemoglobin, as contamination by even trace amounts of fetal material will raise the apparent AFP concentration of the sample, rendering it unsuitable for analysis. The origin of the fetal material should be determined by a test for fetal hemoglobin. If fetal contamination has occurred and the AFP concentration is elevated, an additional specimen should be obtained after 7 to 10 days for evaluation. Amniotic fluid contamination by maternal serum may reflect accurate AFP levels provided the degree of contamination is not sufficient to dilute the sample. Henceforth in this package insert, *amniotic fluid* refers to the clear supernatant obtained from amniotic fluid by centrifugation.

Timing: It is essential to know the gestational age to evaluate AFP results. The recommended time for collection is 16 to 18 weeks for serum, 16 to 20 weeks for amniotic fluid. Serum samples must be collected before amniocentesis since this procedure may lead to spuriously elevated maternal serum levels persisting for 2 to 3 weeks.

Volume Required

Serum: 10 µL serum. (Sample cup must contain at least 100 µL more than the total volume required.)

Amniotic Fluid: 10 µL of prediluted amniotic fluid specimen. (Sample cup must contain at least 100 µL more than the total volume required.)

All amniotic fluid samples must first be diluted 1-in-101 using AFP Sample Diluent, e.g. by adding 10 µL of amniotic fluid to 1.0 mL of AFP Sample Diluent. Results of the diluted sample must be multiplied by the dilution factor to obtain the final AFP concentration.

Storage

Serum: 3 days at 2–8°C. Freeze at –20°C if not assayed within 3 days.

Amniotic Fluid: Amniotic fluid samples should be stored at –20°C. Aliquot if necessary to avoid repeated freezing and thawing. Allow the sample to come to room temperature (15–28°C) before assay, and mix by *gentle* swirling or inversion. Do not attempt to thaw specimens by heating them in a waterbath. If specimens are to be mailed, samples should be packed in dry ice if the time in transit exceeds 72 hours, or if elevated temperatures are a concern, as in warm climates or during the summer. If a repeat analysis is required, the original type of specimen should be taken to maintain consistency of results.

Warnings and Precautions

For *in vitro* diagnostic use.

Reagents: Store at 2–8°C. Dispose of in accordance with applicable laws.

Follow universal precautions, and handle all components as if capable of transmitting infectious agents. Source materials derived from human blood were tested and found nonreactive for syphilis; for antibodies to HIV 1 and 2; for hepatitis B surface antigen; and for antibodies to hepatitis C.

Sodium azide, at concentrations less than 0.1 g/dL, has been added as a preservative. On disposal, flush with large volumes of water to prevent the buildup of potentially explosive metal azides in lead and copper plumbing.

Chemiluminescent Substrate: Avoid contamination and exposure to direct sunlight. (See insert.)

Water: Use distilled or deionized water.

Materials Supplied

Components are a matched set. The barcode labels are needed for the assay.

AFP Test Units (LAP1)
Each barcode-labeled unit contains one bead coated with murine monoclonal anti-AFP. Stable at 2–8°C until expiration date.
LKAP1: 100 units. **LKAP5:** 500 units.

Allow the Test Unit bags to come to room temperature before opening. Open by cutting along the top edge, leaving the ziplock ridge intact. Reseal the bags to protect from moisture.

AFP Reagent Wedges (LAPA, LAPB)
Two barcode-labeled wedges. **LAPA:** one wedge (6.5 mL) of a protein buffer/nonhuman serum matrix. **LAPB:** one wedge (6.5 mL) of alkaline phosphatase (bovine calf intestine) conjugated to polyclonal rabbit anti-AFP in buffer. Store capped and refrigerated: stable at 2–8°C until expiration date. Recommended usage is within 30 days after opening when stored as indicated.
LKAP1: 1 set. **LKAP5:** 5 sets.

AFP Adjustors (LAPL, LAPH)
Two vials (Low and High), 2.0 mL each, of AFP in a bovine serum matrix. Stable at 2–8°C for 30 days after opening, or for 6 months (aliquotted) at –20°C.
LKAP1: 1 set. **LKAP5:** 2 sets.

Kit Components Supplied Separately

AFP Sample Diluent (LAPZ)
For the manual dilution of high serum samples and amniotic fluid samples. 25 mL of an AFP-free bovine serum matrix, with preservative. Stable at 2–8°C for 30 days after opening, or for 6 months (aliquotted) at –20°C.

Analysis of amniotic fluid requires a 1-in-101 dilution of the sample (manual dilution with AFP Sample Diluent). Results of the diluted sample must be multiplied by the dilution factor to obtain the final AFP concentration.

LSUBX: Chemiluminescent Substrate
LPWS2: Probe Wash
LKPM: Probe Cleaning Kit
LCHx-y: Sample Cup Holders (barcoded)
LSCP: Sample Cups (disposable)
LSCC: Sample Cup Caps (optional)

TMCO: Tri-level, multi-constituent control.

Also Required
Sample transfer pipets; distilled or deionized water.

Assay Procedure

Note that for optimal performance, it is important to perform all routine maintenance procedures as defined in the IMMULITE Operator's Manual.

See IMMULITE Operator's Manual for: preparation, setup, adjustment, assay and quality control procedures.

Adjustment Interval: 2 weeks.

Quality Control Samples: Use controls or serum pools with at least two levels (low and high) of AFP.

Expected Values

AFP Values in Testicular Cancer Patients

In a study involving two clinical sites, 119 serum samples from men in apparent good health (median age: 61, central 95%: 27 to 79 years) were processed by the IMMULITE AFP assay. The results ranged from 0.5 to 5.5 IU/mL, with a median of 1.6 IU/mL and a 99th percentile of 5 IU/mL.

The study also included men with testicular cancer; patients with other malignancies (of liver, bladder, kidney, pancreas, lung, prostate and colon); patients with nonmalignant conditions (such as cirrhosis, hepatitis B and C, ulcerative colitis, emphysema, colon and rectal polyps); and a few women in apparent good health. The distribution of IMMULITE AFP results is tabulated below (with the total number for each group in parentheses).

IU/mL:	<5	5–15	15–100	>100
Males				
Healthy Males *(119)*	118	1	—	—
Seminomatous Testicular Cancer *(6)*	6	—	—	—
Nonseminomatous Testicular Cancer *(60)*	14	8	15	23
Liver Cancer *(10)*	3	—	2	5
Other Malignant Diseases *(40)*	36	1	—	3
Cirrhosis *(4)*	3	1	—	—
Hepatitis *(24)*	19	4	1	—
Other Nonmalignant Diseases *(6)*	5	—	—	1

IU/mL:	<5	5–15	15–100	>100
Females				
Healthy Females *(29)*	29	—	—	—
Malignant Diseases *(20)*	18	—	1	1
Nonmalignant Diseases *(16)*	15	—	1	—

Consider these limits as *guidelines* only. Each laboratory should establish its own reference ranges.

Patients with nonseminomatous testicular cancer can be expected to have a distribution of AFP values both within and above the reference range for apparently healthy adult male subjects. In pure form, seminomas do not present with elevated serum AFP levels. However, elevated AFP levels have been observed in patients diagnosed with seminomas accompanied by metastases of nonseminomatous testicular cancer.[8]

A significant increase of AFP levels in patients considered free of metastatic tumor may indicate the development of metastasis. Elevated levels after surgery may indicate incomplete removal of the tumor or the presence of metastases.

Elevated levels of serum AFP are associated with benign liver conditions such as hepatitis and cirrhosis. Most (95%) of the patients with these benign diseases have AFP levels lower than 200 ng/mL (165 IU/mL).[8–15]

AFP Values in Maternal Serum and Amniotic Fluid

Due to potential variation in testing at different laboratories, it is recommended that a particular testing center determine its own set of median AFP values for weeks 15 to 20 of gestation, measured in the population to be screened. Cutoff values commonly utilize multiples of the medians (MoM) of 2.0 or 2.5 for maternal serum and amniotic fluid testing. Each AFP test result can then be expressed as a multiple of the unaffected population median value. This is obtained by dividing the AFP value by the median value for its corresponding gestational week. Gestational weeks are defined as completed gestational weeks; e.g., 16 weeks, 6 days would be considered the 16th week. It has been recommended that median and MoM values determined for each gestational week be based upon at least 100 maternal sera and 50 amniotic fluids from unaffected singleton pregnancies with confirmed gestational age.

Provided below are medians for *maternal serum* samples, calculated by a weighted log-linear regression from data collected from unaffected, singleton pregnancies at three clinical sites in the United States:

Gestational Week	No. of Specimens	Medians IU/mL*	Multiples of Regressed Medians (IU/mL) 2.0	2.5	3.0
15	370	24.9	49.8	62.3	74.7
16	605	28.5	57.0	71.3	85.5
17	569	32.6	65.2	81.5	97.8
18	431	37.2	74.4	93.0	111.6
19	221	42.5	85.0	106.3	127.5
20	91	48.6	97.2	121.5	145.8

*Regressed

Provided below are medians for *amniotic fluid* samples, calculated by a weighted log-linear regression from data collected from unaffected, singleton pregnancies at two clinical sites in the United States:

Gestational Week	No. of Specimens	Medians kIU/mL*	Multiples of Regressed Medians (kIU/mL) 2.0	2.5	3.0
15	76	13.0	26.0	32.5	39.0
16	89	10.7	21.4	26.8	32.1
17	53	8.73	17.5	21.8	26.2
18	54	7.14	14.3	17.9	21.4
19	46	5.84	11.7	14.6	17.5
20	23	4.78	9.56	12.0	14.3

*Regressed

Limitations

Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with *in vitro* immunoassays. [See Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988:34:27-33.] Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference potentially causing an anomalous result. These reagents have been formulated to minimize the risk of interference; however, potential interactions between rare sera and test components can occur. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

Diagnosis: The occurrence of elevated serum AFP levels in conditions other than nonseminomatous testicular cancer precludes the use of AFP measurements in the diagnosis of nonseminomatous testicular cancer.

Screening: AFP measurements cannot be recommended as a screening procedure to detect cancer in the general population. Elevated concentrations of serum AFP have been observed not only in patients with nonseminomatous testicular cancer but also in malignant conditions such as hepatocellular carcinoma, ovarian cancer, and gastrointestinal and pulmonary cancer. Benign hepatic conditions such as acute viral hepatitis, chronic active hepatitis and cirrhosis may present with elevated concentrations of serum AFP. Elevated AFP concentrations have also been observed in pregnancy, ataxia telangiectasia and hereditary tyrosinemia.

Prenatal Testing: A reliable AFP evaluation for prenatal testing requires precise determination of the gestational age. Underestimation of the gestational age may lead to false positive determination, while overestimation of gestational age may result in a false negative interpretation. When gestational age is uncertain, confirmation with ultrasonography is indicated.

Performance Data

See tables and graphs for data *representative* of the assay's performance. Results are expressed in IU/mL. Unless otherwise noted, all were generated on serum samples collected from testicular cancer patients.

Conversion Factor:
IU/mL × 1.21 → ng/mL

Calibration Range: up to 300 IU/mL
(363 ng/mL) (WHO 1st IS 72/225).

Analytical Sensitivity: 0.2 IU/mL
(0.24 ng/mL).

High-dose Hook:
None up to 450,000 IU/mL.

Precision: Seven samples were processed in duplicate over the course of 20 days, two runs per day, for a total of 40 runs and 80 replicates. (See "Precision" table.)

Linearity: Serum and amniotic fluid samples were assayed under various dilutions. (See "Linearity" table for representative data.)

Recovery: Serum samples spiked 1-in-20 with three AFP solutions (9.8, 31 and 67 IU/mL) were assayed. Amniotic samples spiked 1-in-20 with three high amniotic fluid samples (10,000, 20,000 and 36,000 IU/ML) were also assayed. (See "Recovery" tables for representative data.)

Specificity: The assay is highly specific for AFP. (See "Specificity" table. ND: not detectable.)

Bilirubin (unconjugated): A small but (by *t*-test) statistically significant effect. (See "Bilirubin" table.)

Hemolysis: No significant effect.

Lipemia: No significant effect.

Method Comparison – Testicular Cancer Studies: The assay was compared to a legally marketed assay (Kit C), at two clinical sites. At the first (in the northwestern United States), a total of 264 specimens were evaluated, including samples from male patients with nonseminomatous testicular cancer and other malignant and nonmalignant conditions, as well as samples from a few female patients. The results were compared qualitatively, relative to cutoffs based on the stated 99th percentiles for healthy males – namely, 5 IU/mL for IMMULITE, and 8.9 ng/mL (7.36 IU/mL) for Kit C.

	IMMULITE AFP		Relative Sensitivity (95% CI)	Relative Specificity (95% CI)
Kit C	Positive	Negative		
Positive	74	4	94.9%	97.3%
Negative	5	181	(87.4 – 98.6%)	(93.8 – 99.1%)

Agreement: 96.6%

At the second site (in the southern United States), a total of 213 specimens were studied, including samples from male patients with seminomatous and nonseminomatous testicular cancer, and other malignant conditions, as well as samples from a few female patients.

	IMMULITE AFP		Relative Sensitivity (95% CI)	Relative Specificity (95%CI)
Kit C	Positive	Negative		
Positive	62	3	95.4%	97.3%
Negative	4	144	(87.1– 99.0%)	(93.2 – 99.3%)

Agreement: 96.7%

The 424 results from the two sites which were within range by both assays were also compared by linear regression analysis:

(IML) = 0.83 (Kit C) – 0.17 IU/mL r = 0.99
n = 424

95% Confidence Interval (CI)	Slope	Intercept
Lower	0.81	-0.65
Upper	0.84	0.31

Method Comparison – Neural Tube Defect Studies: In two separate clinical studies conducted in the United States, IMMULITE AFP results were compared to two legally marketed assays (Kit A and Kit B) in a linear regression for maternal serum samples, in the range from nondetectable to 300 IU/mL.

(IML) = 0.83 (Kit B) + 6.51 IU/mL r = 0.97
n = 1,006

95% CI	Slope	Intercept
Lower	0.82	5.93
Upper	0.85	7.09

(IML) = 0.87 (Kit A) + 2.72 IU/mL r = 0.96
n = 346

95% CI	Slope	Intercept
Lower	0.84	1.64
Upper	0.89	3.79

In one of the studies above, IMMULITE AFP results were also compared to Kit B in a linear regression for amniotic fluid samples, in the range from nondetectable to 173 kIU/mL*.

(IML) = 0.78 (Kit B) + 0.17 kIU/mL r = 0.99
n = 185

95% CI	Slope	Intercept
Lower	0.76	-0.19
Upper	0.79	0.53

* Amniotic fluid samples were diluted 1-in-101 off-line before they were tested by the IMMULITE instrument.

The assay was also compared to DPC's IMMULITE 2000 AFP on amniotic fluid samples, in the range from approximately 3 to 20 kIU/mL*. (See Graph). By linear regression:

(IML 2000) = 1.03 (IML) + 0.52 kIU/mL r = 0.96
n = 46

Means:
10.0 kIU/mL (IML)
10.8 kIU/mL (IML 2000)

95% CI	Slope	Intercept
Lower	0.93	-0.50
Upper	1.12	1.54

* Amniotic fluid samples were diluted 1-in-101 off-line before they were tested by the IMMULITE instrument.

The assay was also compared to DPC's IMMULITE 2000 AFP on maternal serum samples, in the range from approximately 10 to 120 IU/mL. By linear regression:

(IML 2000) = 1.01 (IML) + 0.154 IU/mL r = 0.982
n = 346

Means
33.8 IU/mL (IML)
34.3 IU/mL (IML 2000)

95% CI	Slope	Intercept
Lower	0.99	-0.60
Upper	1.03	0.91

Clinical Sensitivity for Maternal Serum, *n* = 13:

Gestational Week	% > 2.0 MoM	% > 2.5 MoM	% > 3.0 MoM
15 – 20	92.3%	69.2%	69.2%
95% CI for All Samples	64.0% – 99.8%	38.6% – 90.9%	38.6% – 90.9%

Clinical Specificity for Maternal Serum:

Gestation. Week	*n* of Samples	% ≤ 2.0MoM	% ≤ 2.5MoM	% ≤ 3.0MoM
15	173	96.0%	98.8%	98.8%
16	411	98.1%	99.3%	99.5%
17	372	96.5%	99.7%	100%
18	204	95.1%	99.0%	100%
19	108	94.4%	99.1%	100%
20	50	100%	100%	100%
15 – 20	1318	96.7%	99.3%	99.7%
95% CI for All Samples		95.5% – 97.6%	98.7% – 99.7%	99.2% – 99.9%

Clinical Sensitivity for Amniotic Fluid, *n* = 10:

Gestational Week	% > 2.0 MoM	% > 2.5 MoM	% > 3.0 MoM
15 – 20	90.0%	90.0%	90.0%
95% CI for All Samples	55.5% – 99.8%	55.5% – 99.8%	55.5% – 99.8%

Clinical Specificity for Amniotic Fluid:

Gestation. Week	*n* of Samples	% ≤ 2.0MoM	% ≤ 2.5MoM	% ≤ 3.0MoM
15	23	100%	100%	100%
16	39	97.4%	100%	100%
17	25	100%	100%	100%
18	34	94.1%	97.1%	100%
19	33	100%	100%	100%
20	13	100%	100%	100%
15 – 20	167	98.2%	99.4%	100%
95% CI for All Samples		94.8% – 99.6%	98.7% – 100%	97.8% – 100%

References

Testicular Cancer

1) Herberman, edd. Immunodiagnosis of cancer. 1979:101. 2) Int J Cancer 1971;7:218. 3) Cancer Res 1972;32:979. 4) Scand J Clin Lab Investig 1956;8:174. 5) Acta Unio Internationalis Contra Cancrum 1963;19:80. 6) Vopr Med Khim 1964;10:90. 7) Int J Cancer 1966;3:364. 8) Hum Pathol 1979;10:557. 9) Lancet 1976;2:433. 10) Adv Cancer Res 1971;14:295. 11) Proc Natl Acad Sci USA 1973;70:526. 12) Kirkpatrick, edd. Alpha-fetoprotein. 1981:115. 13) Cancer 1979;44:984. 14) Cancer Res 1975;35:991. 15) Cancer 1974;34:1510. 16) Med Intelligence 1976;295:1237. 17) Cancer 1976;37:215. 18) J Urol 1977;118:994. 19) Cancer 1978;42:2768. 20) J Clin Oncol 1986;4:41. 21) Rose, edd. Manual of clinical laboratory immunology. 1986:810. 22) Cancer 1981;47:328. 23) Urol Clin North Am 1977;4:393. 24) Ravitch, edd. Current problems in surgery. 1978:1. 25) Cancer 1980;45:1755. 26) Pavone-Macaluso, edd. Testicular cancer and other tumors. 1983:63. 27) J Urol 1978;119:759. 28) Kirkpatrick, edd. Alpha-fetoprotein. 1981:135. 29) N Eng J Med 1977;296:693. 30) Eur J Clin Chem Biochem 1993;31:517. 31) NCCLS H3-A3, 1991.

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32) Brock DJH. Prenatal diagnosis – chemical methods. Br Med Bull 1976;32:16. 33) Crandall BF. Alpha-fetoprotein: a review. CRC Crit Rev Clin Lab Sci 1981:Sept:127-85. 34) Gillin D. Normal biology of AFP. Ann NY Acad Sci 1975:259:17-28. 35) Haddow JE, et al. Fetal disorders associated with elevated MSAFP values. Foundation Blood Res 1990:1. 36) Leek AE, Chard T. Proceedings of colloquium on alpha-fetoprotein. In: Masseyeff R, editor. Paris: L'Institut National de la Sante et de la Recherche Medicale 563, (Nice) 1974. 37) Second Report of the Collaborative Study on Alpha-Fetoprotein in Relation to Neural Tube Defects. Amniotic fluid alpha-fetoprotein measurement in antenatal diagnosis of anencephaly and open spina bifida in early pregnancy. Lancet 1979;ii:651. 38) Seppälä M. Fetal pathophysiology of human α-fetoprotein. Ann N Y Acad Sci 1975;259:59.

Tables and Graphs

Precision (IU/mL)

		Within-Run		Total	
	Mean	SD	CV	SD	CV
1	0.70	0.047	6.7%	0.059	8.4%
2	2.63	0.195	7.4%	0.217	8.3%
3	12.9	0.63	4.9%	1.04	8.1%
4	32.3	1.46	4.5%	2.65	8.2%
5	45.1	2.48	5.5%	3.69	8.2%
6	60.9	2.81	4.6%	4.22	6.9%
7	187	8.64	4.6%	10.9	5.8%

Recovery (IU/mL) — Serum

	Solution	Observed	Expected	%O/E
1	—	10.6	—	—
	A	11.4	10.6	108%
	B	12.3	11.6	106%
	C	13.7	13.4	102%
2	—	59	—	—
	A	55	57	96%
	B	58	58	100%
	C	58	59	98%
3	—	117	—	—
	A	112	112	100%
	B	109	113	96%
	C	113	115	98%

Recovery (IU/mL) — Amniotic Fluid

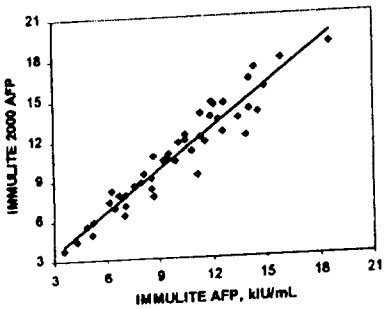
Low Amniotic Sample	Spiking High Amniotic Sample	Observed	Expected	%O/E
1	—	244	—	—
	A	813	732	111%
	B	1,371	1,232	111%
	C	1,943	2,032	96%
2	—	4,483	—	—
	A	4,519	4,759	95%
	B	6,678	5,259	127%
	C	4,896	6,059	81%
3	—	9,050	—	—
	A	9,054	9,098	100%
	B	10,042	9,598	105%
	C	10,640	10,398	102%

Specificity

Compound	Amount Added	% Cross-reactivity
Human IgG	10 mg/mL	ND
Human Hemoglobin	192 mg/dL	ND
Human Transferrin	400 mg/dL	ND
Human Serum Albumin	60 mg/mL	ND
Cisplatin	100 µg/mL	ND
Cyclophosphamide	1,000 µg/mL	ND
Doxorubicin	100 µg/mL	ND
5-Fluorouracil	1,000 µg/mL	ND
Mitomycin C	100 µg/mL	ND
Vincristine	1,000 ng/mL	ND

ND: not detectable.

Method Comparison – Amniotic Fluid:



(IML 2000) = 1.03 (IML) + 0.52 kIU/mL r = 0.96

Linearity (IU/mL) — Serum

	Dilution	Observed	Expected	%O/E
1	32 in 32	160	—	—
	16 in 32	80	80	100%
	8 in 32	40	40	100%
	4 in 32	21	20	105%
	2 in 32	11	10	110%
	1 in 32	5.3	5.0	106%
2	64 in 64	184	—	—
	32 in 64	91	92	99%
	16 in 64	48	46	104%
	8 in 64	24	23	104%
	4 in 64	12	11	109%
	2 in 64	6.2	5.7	109%
	1 in 64	3.3	2.9	114%
3	64 in 64	231	—	—
	32 in 64	118	115	103%
	16 in 64	57	58	98%
	8 in 64	29	29	100%
	4 in 64	14	14	100%
	2 in 64	7.3	7.2	101%
	1 in 64	3.5	3.6	97%
4	64 in 64	235	—	—
	32 in 64	114	117	97%
	16 in 64	61	59	103%
	8 in 64	30	29	103%
	4 in 64	16	15	107%
	2 in 64	7.7	7.3	105%
	1 in 64	3.9	3.7	105%

Linearity (IU/mL) — Amniotic Fluid

	Total Dilution	Observed	Expected	%O/E
1	1 in 100	138	—	—
	1 in 200	66	69	96%
	1 in 400	35	35	100%
	1 in 800	14	17	82%
	1 in 1600	9.0	8.6	105%
	1 in 3200	4.3	4.3	99%
2	1 in 100	175	—	—
	1 in 200	81	88	92%
	1 in 400	46	44	105%
	1 in 800	23	22	105%
	1 in 1600	12	11	109%
	1 in 3200	6.5	5.5	119%
3	1 in 100	281	—	—
	1 in 200	119	141	85%
	1 in 400	60	70	86%
	1 in 800	31	35	89%
	1 in 1600	16	18	89%
	1 in 3200	8.4	8.8	95%

Bilirubin

	Bilirubin (unconjugated)				
		100 mg/L		200 mg/L	
	Expected	Observed	%O/E	Observed	%O/E
1	5.3	4.9	92%	5.1	96%
2	5.7	5.3	93%	5.6	98%
3	26	24	94%	25	97%
4	48	46	95%	46	95%
5	49	46	93%	46	93%

Technical Assistance

In the United States, contact DPC's Technical Services department.
Tel: 800.372.1782 or 973.927.2828.
Fax: 973.927.4101. Outside the United States, contact your National Distributor.

Manufactured by EURO/DPC Ltd. under a Quality System registered to BS EN ISO 9002:1994 and EN 46002:1996.

EURO/DPC LTD
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Diagnostic Products Corporation
5700 West 96th Street
Los Angeles, CA 90045-5597

2001-10-05 (ISO 8601)

October 5, 2001

PILKAP – 6

IMMULITE 2000

AFP

DPC

IMMULITE® 2000 AFP

English

Intended Use: For *in vitro* diagnostic use with the IMMULITE 2000 Analyzer – for the quantitative measurement of alpha-fetoprotein (AFP) in either of two contexts: (a) serial measurements in human serum to aid in the management of patients with nonseminomatous testicular cancer; or (b) measurements in maternal serum and amniotic fluid during gestational weeks 15 through 20 – used in conjunction with ultrasonography or amniography – to aid in detection of fetal open neural tube defects.

Catalog Numbers: **L2KAP2** (200 tests)
Test Code: **AF** Color: **Light Gray**

Caution: In the United States, federal law restricts this device to sale by or on the order of a physician.

The concentration of AFP in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity. **The results reported by the laboratory to the physician must include the identity of the assay used. Values obtained with different AFP assays cannot be used interchangeably.** Before changing assays, the laboratory must: (a) for cancer management – confirm baseline values for patients being serially monitored; (b) for prenatal testing – establish a range of normal values for the new assay based on normal sera and amniotic fluids from pregnant women with confirmed gestational age.

Summary and Explanation

Alpha-fetoprotein (AFP) is a single-chain glycoprotein with a molecular mass of approximately 70,000 daltons. AFP shares considerable sequence homology with albumin, and is produced by the fetus primarily in cells of the yolk sac, gastrointestinal tract and liver. AFP appears as a major serum protein in the fetus, but its concentration decreases rapidly toward birth.[1,2,3] 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.

Testicular Cancer

Elevated levels of AFP have been observed not only in patients with nonseminomatous testicular cancer, but also in patients with other malignancies such as hepatocellular carcinoma, ovarian cancer, gastrointestinal cancer and pulmonary cancer.[8-15] Serum AFP is frequently elevated in benign hepatic conditions such as acute viral hepatitis, chronic active hepatitis and cirrhosis. Conditions of pregnancy, ataxia telangiectasia and hereditary tyrosinemia have also presented with elevated concentrations of AFP.[8-15]

Seminomas, in pure form, do not present with elevated concentrations of AFP. However, elevated concentrations of serum AFP have been observed in patients diagnosed with seminomatous testicular cancer accompanied by nonseminomatous metastases.[8,16,18,19] During chemotherapy, patients with advanced seminoma and hepatic dysfunction have also presented with elevated serum AFP concentrations.[20] The interpretation of elevated AFP concentrations in patients with seminoma requires special consideration and should assist the clinician in the selection of appropriate therapy.[8,15,21]

The clinical utility of AFP measurement as an aid in the management of patients with nonseminomatous testicular cancer is well documented.[8,16,17,18,22] AFP measurement has found clinical application as an aid in assessing the extent of disease.[18,22-26]

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Serial measurements of serum AFP have been shown to reflect the effectiveness of therapeutic regimens in patients with nonseminomatous testicular tumors.[9,15,17,26,27] Post-surgical determinations of AFP are particularly valuable. The presence of residual tumor is strongly suggested if post-operative AFP concentrations fail to return to normal.[9,15,28,29] The accurate interpretation of post-surgical changes in AFP concentration requires consideration of its metabolic decay rate.[21,22,24,25] When utilizing AFP for monitoring therapy or disease recurrence during chemotherapy, it should be noted that levels often fall rapidly during chemotherapy, reaching normal levels while tumor masses are still evident.[17,21] In such instances, completion of the planned therapy has been recommended.[21]

Following therapy or surgery, serial measurements of AFP have also proved clinically useful when monitoring for progression or recurrence of disease in patients with nonseminomatous testicular cancer. It has been reported that AFP levels frequently rise during disease progression and fall during disease remission.[9,17,18] Elevated AFP levels have frequently been observed to accompany tumor recurrence before progressive disease is clinically evident.[9,18]

Fetal Open Neural Tube Defects

AFP is detectable not only in fetal serum, but also in amniotic fluid and maternal serum. A concentration gradient exists such that when the fetal serum AFP level is 2,000 kIU/mL, the amniotic fluid AFP (AFAFP) level is 20 kIU/mL, and the maternal serum AFP (MSAFP) is 0.02 kIU/mL. In normal pregnancy, the fetal serum AFP concentration peaks at 14 weeks gestation.[34] The AFAFP concentration peaks at about 12 weeks and the MSAFP peaks at approximately 28 – 32 weeks gestation.[36] The fall in AFAFP concentration reflects the fall in fetal serum AFP concentration which results from increased fetal size and fluid volume.[34] Elevated levels of MSAFP and AFAFP may occur most often due to multiple pregnancy and due to incorrect gestational age.

Measurement of AFP concentrations is clinically valuable in screening for open NTDs and other fetal abnormalities;[35] pregnancies associated with open NTDs present with elevated AFP levels. Excess AFP gains access to amniotic fluid, and to a lesser extent to the maternal serum, by transudation across the exposed surface of the fetus or across damaged glomeruli.[36, 37] These conditions are found in open NTDs including open spina bifida and anencephaly, omphalocele, and congenital nephrosis.[32,38] Additional causes of elevated AFP concentrations including both maternal and fetal sources are impending spontaneous abortion, fetal distress or death, oligohydramnios, toxemia, gastroschisis, Meckel's syndrome, sacrococcygeal teratoma, Turner's syndrome and maternal hepatic and oncologic disorders.[35]

Recommended protocols for open NTD screening have been published.[33,35] The cutoff levels for maternal serum and amniotic fluid can be chosen to optimize the needs of the populations being tested based upon varying prevalence of open NTDs. Cutoffs commonly utilize multiples of the median of 2.0 or 2.5 for MSAFP and AFAFP testing. The optimal time for screening MSAFP is between the 16th and 18th weeks of pregnancy, although screening is still effective before or after this period. Elevated AFP concentrations may be subjected to a repeat sampling and analysis to exclude transient rises.

More commonly, ultrasonography is employed to rule out multiple pregnancies and to confirm gestational age. Ultrasonography may also identify signs of open NTDs, particularly anencephaly which is a large, easily visualized lesion. If correction for gestational age or multiple pregnancy does not result in an AFP concentration within the normal range, then diagnostic ultrasonography and/or amniotic fluid sampling is indicated. The greatest diagnostic power can be achieved by combining biochemical analysis of amniotic fluid and diagnostic ultrasonography in cases of a positive MSAFP screen.[35]

Elevated MSAFP results are not diagnostic for NTDs and should not be considered a cause for termination of pregnancy. An overlap exists in the distributions of AFP concentrations from pregnancies with and without open NTDs. Closed NTDs, for example, are not usually associated with increased MSAFP or AFAFP concentrations. Thus, further testing is required to define fetal status. In light of these considerations and the multiple causes for

elevated AFP concentrations, all clinical information should be evaluated and confirmatory tests performed wherever possible before reaching a diagnosis.

AFP can be measured by several immunologic techniques, depending on the degree of sensitivity desired. Radial immunodiffusion, countercurrent immunoelectrophoresis, and rocket immunoelectrophoresis are three techniques well suited for research applications. Enzyme-linked immunosorbent assays and radioimmunoassays of both competitive and non-competitive designs have been successfully employed clinically both for maternal serum and amniotic fluid measurements.

Note: IMMULITE 2000 AFP Physician Brochure (Cat. #ZS1105) and Patient Brochure (Cat. #ZS1106), explaining the use of AFP prenatal testing to aid in the detection of fetal open NTD are available by calling DPC Customer Services 1-800-372-1782 or your National Distributor.

Principle of the Procedure

IMMULITE 2000 AFP is a solid-phase, two-site sequential chemiluminescent immunometric assay.

Incubation Cycles: 2 x 30 minutes.

Specimen Collection

Serum: Collect blood by venipuncture[31] into plain tubes and separate the serum from the cells as soon as possible. Specimens must be obtained before amniocentesis to obtain a valid specimen. Lipemic samples should be clarified with the help of an ultracentrifuge before assay of serum.

Centrifuging serum samples before a complete clot forms may result in the presence of fibrin. To prevent erroneous results due to the presence of fibrin, ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increased clotting time.

Amniotic Fluid: Collect amniotic fluid by amniocentesis into plain tubes. Samples should be obtained by aseptic transabdominal amniocentesis performed by an experienced obstetrician during the second trimester of pregnancy in women with confirmed gestational age. Centrifuge the specimen, retaining a portion of the clear supernatant. Inspect both supernatant and sediment for signs of blood or hemoglobin, as contamination by even trace amounts of fetal material will raise the apparent AFP concentration of the sample, rendering it unsuitable for analysis. The origin of the fetal material should be determined by a test for fetal hemoglobin. If fetal contamination has occurred and the AFP concentration is elevated, an additional specimen should be obtained after 7 to 10 days for evaluation. Amniotic fluid contamination by maternal serum may reflect accurate AFP levels provided the degree of contamination is not sufficient to dilute the sample. Henceforth in this package insert, *amniotic fluid* refers to the clear supernatant obtained from amniotic fluid by centrifugation.

Timing: It is essential to know the gestational age to evaluate AFP results. The recommended time for collection is 16 to 18 weeks for serum, 16 to 20 weeks for amniotic fluid. Serum samples must be collected before amniocentesis since this procedure may lead to spuriously elevated maternal serum levels persisting for 2 to 3 weeks.

Volume Required

Serum: 10 µL serum.

Amniotic Fluid Dilution Factor: 100.
All amniotic fluid samples must first be diluted 1-in-100 using on-board Multi-Diluent 2 before being assayed. Select 100 in the Dilution Factor window.

A single determination uses 10 µL prediluted amniotic fluid specimen.

Storage

Serum: 3 days at 2–8°C. Freeze at –20°C if not assayed within 3 days.

Amniotic Fluid: Amniotic fluid samples should be stored at –20°C. Aliquot if necessary to avoid repeated freezing and thawing. Allow the sample to come to room temperature (15–28°C) before assay, and mix by *gentle* swirling or inversion. Do not attempt to thaw specimens by heating them in a waterbath. If specimens are to be mailed, samples should be packed in dry ice if the time in transit exceeds 72 hours, or if elevated temperatures are a concern, as in warm climates or during the summer. If a repeat analysis is required, the original type of specimen should be taken to maintain consistency of results.

Warnings and Precautions

For *in vitro* diagnostic use.

Reagents: Store at 2–8°C. Dispose of in accordance with applicable laws.

Follow universal precautions, and handle all components as if capable of transmitting infectious agents. Source materials derived from human blood were tested and found nonreactive for syphilis; for antibodies to HIV 1 and 2; for hepatitis B surface antigen; and for antibodies to hepatitis C.

Sodium azide, at concentrations less than 0.1 g/dL, has been added as a preservative. On disposal, flush with large volumes of water to prevent the buildup of potentially explosive metal azides in lead and copper plumbing.

Chemiluminescent Substrate: Avoid contamination and exposure to direct sunlight. (See insert.)

Water: Use distilled or deionized water.

Materials Supplied

Components are a matched set. Labels on the inside box are needed for the assay.

AFP Bead Pack (L2AP12)
With barcode. 200 beads, coated with murine monoclonal anti-AFP. Stable at 2–8°C until expiration date.
L2KAP2: 1 pack.

AFP Reagent Wedge (L2APA2)
With barcode. 11.5 mL of a protein buffer/nonhuman serum matrix; and 11.5 mL of alkaline phosphatase (bovine calf intestine) conjugated to polyclonal rabbit anti-AFP, in buffer. Stable at 2–8°C until expiration date.
L2KAP2: 1 wedge.

Before use, tear off the top of the label at the perforations, without damaging the barcode. Remove the foil seal from the top of wedge; snap the sliding cover down into the ramps on the reagent lid.

AFP Adjustors (L2APJ3, L2APJ4)
Two vials (Low and High), 2.0 mL each, of AFP in a bovine serum matrix. Stable at 2–8°C for 30 days after opening, or for 6 months (aliquotted) at –20°C.
L2KAP2: 1 set.

Before making an adjustment, place the appropriate Aliquot Labels (supplied with the kit) on test tubes, so that the barcodes can be read by the on-board reader.

Kit Components Supplied Separately

Multi-Diluent 2 (L2M2Z, L2M2Z4)
For the on-board dilution of high serum samples and for amniotic fluid samples. One vial of concentrated (ready-to-use), nonhuman protein/buffer matrix, with preservative. Stable at 2–

8°C for 30 days after opening, or for 6 months (aliquotted) at –20°C.
L2M2Z: 25 mL. **L2M2Z4:** 55 mL.

Barcode labels are provided for use with the diluent. Before use, place an appropriate label on a 16 × 100 mm test tube, so that the barcode can be read by the on-board reader.
L2M2Z: 3 labels **L2M2Z4:** 5 labels

Analysis of amniotic fluid requires a 1-in-100 dilution of the sample (on-board dilution with Multi-Diluent 2).

L2SUBM: Chemiluminescent Substrate
L2PWSM: Probe Wash
L2KPM: Probe Cleaning Kit
L2RXT: Reaction Tubes (disposable)
L2ZT: 250 Sample Diluent Test Tubes (16 × 100 mm)
L2ZC: 250 Sample Diluent Tube Caps

TMCO: Tri-level, multi-constituent control.

Also Required
Distilled or deionized water; test tubes; controls.

Assay Procedure

Note that for optimal performance, it is important to perform all routine maintenance procedures as defined in the IMMULITE 2000 Operator's Manual.

See the IMMULITE 2000 Operator's Manual for: preparation, setup, dilutions, adjustment, assay and quality control procedures.

Adjustment Interval: 4 weeks.

Quality Control Samples: Use controls or serum pools with at least two levels (low and high) of AFP.

Expected Values

AFP Values in Testicular Cancer Patients

Based on its relationship to DPC's IMMULITE AFP (see Method Comparison), the assay can be expected to have essentially the same reference ranges.

In a study involving two clinical sites, 119 serum samples from men in apparent good health (median age: 61; central 95%: 27 to 79 years) were processed by the IMMULITE AFP assay. The results ranged from 0.5 to 5.5 IU/mL, with a median of 1.6 IU/mL and a 99th percentile of 5 IU/mL.

The study also included men with testicular cancer; patients with other malignancies (of liver, bladder, kidney, pancreas, lung, prostate and colon); patients with nonmalignant conditions (such as cirrhosis, hepatitis B and C, ulcerative colitis, emphysema, colon and rectal polyps); and a few women in apparent good health. The distribution of IMMULITE AFP results is tabulated below (with the total number for each group in parentheses).

IU/mL:	<5	5–15	15–100	>100
Males				
Healthy Males *(119)*				
	118	1	—	—
Seminomatous Testicular Cancer *(6)*				
	6	—	—	—
Nonseminomatous Testicular Cancer *(60)*				
	14	8	15	23
Liver Cancer *(10)*				
	3	—	2	5
Other Malignant Diseases *(40)*				
	36	1	—	3
Cirrhosis *(4)*				
	3	1	—	—
Hepatitis *(24)*				
	19	4	1	—
Other Nonmalignant Diseases *(6)*				
	5	—	—	1
Females				
Healthy Females *(29)*				
	29	—	—	—
Malignant Diseases *(20)*				
	18	—	1	1
Nonmalignant Diseases *(16)*				
	15	—	1	—

Consider these limits as *guidelines* only. Each laboratory should establish its own reference ranges.

Patients with nonseminomatous testicular cancer can be expected to have a distribution of AFP values both within and above the reference range for apparently healthy adult male subjects. In pure form, seminomas do not present with elevated serum AFP levels. However, elevated AFP levels have been observed in patients diagnosed with seminomas accompanied by metastases of nonseminomatous testicular cancer.[8]

A significant increase of AFP levels in patients considered free of metastatic tumor may indicate the development of metastasis. Elevated levels after surgery may indicate incomplete removal of the tumor or the presence of metastases.

Elevated levels of serum AFP are associated with benign liver conditions such as hepatitis and cirrhosis. Most (95%) of the patients with these benign diseases have AFP levels lower than 200 ng/mL (165 IU/mL).[8–15]

AFP Values in Maternal Serum and Amniotic Fluid

Due to potential variation in testing at different laboratories, it is recommended that a particular testing center determine its own set of median AFP values for weeks 15 to 20 of gestation, measured in the population to be screened. Cutoff values commonly utilize multiples of the medians (MoM) of 2.0 or 2.5 for maternal serum and amniotic fluid testing. Each AFP test result can then be expressed as a multiple of the unaffected population

median value. This is obtained by dividing the AFP value by the median value for its corresponding gestational week. Gestational weeks are defined as completed gestational weeks; e.g., 16 weeks, 6 days would be considered the 16th week. It has been recommended that median and MoM values determined for each gestational week be based upon at least 100 maternal sera and 50 amniotic fluids from unaffected singleton pregnancies with confirmed gestational age.

Provided below are medians for *maternal serum* samples, calculated by a weighted log-linear regression from data collected from unaffected, singleton pregnancies at three clinical sites in the United States:

Gestational Week	No. of Specimens	Medians IU/mL*	Multiples of Regressed Medians (IU/mL) 2.0	2.5	3.0
15	370	24.9	49.8	62.3	74.7
16	605	28.5	57.0	71.3	85.5
17	569	32.6	65.2	81.5	97.8
18	431	37.2	74.4	93.0	111.6
19	221	42.5	85.0	106.3	127.5
20	91	48.6	97.2	121.5	145.8

*Regressed

Provided below are medians for *amniotic fluid* samples, calculated by a weighted log-linear regression from data collected from unaffected, singleton pregnancies at two clinical sites in the United States:

Gestational Week	No. of Specimens	Medians kIU/mL*	Multiples of Regressed Medians (kIU/mL) 2.0	2.5	3.0
15	76	13.0	26.0	32.5	39.0
16	89	10.7	21.4	26.8	32.1
17	53	8.73	17.5	21.8	26.2
18	54	7.14	14.3	17.9	21.4
19	46	5.84	11.7	14.6	17.5
20	23	4.78	9.56	12.0	14.3

*Regressed

Limitations

Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with *in vitro* immunoassays. [See Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988:34:27-33.] Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference potentially causing an anomalous result. These reagents have been formulated to minimize the risk of interference; however, potential interactions between rare sera and test components can occur. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

Diagnosis: The occurrence of elevated serum AFP levels in conditions other than nonseminomatous testicular cancer precludes the use of AFP measurements in the diagnosis of nonseminomatous testicular cancer.

Screening: AFP measurements can not be recommended as a screening procedure to detect cancer in the general population. Elevated concentrations of serum AFP have been observed not only in patients with nonseminomatous testicular cancer but also in malignant conditions such as hepatocellular carcinoma, ovarian cancer, and gastrointestinal and pulmonary cancer. Benign hepatic conditions such as acute viral hepatitis, chronic active hepatitis and cirrhosis may present with elevated concentrations of serum AFP. Elevated

AFP concentrations have also been observed in pregnancy, ataxia telangiectasia and hereditary tyrosinemia.

Prenatal Testing: A reliable AFP evaluation for prenatal testing requires precise determination of the gestational age. Underestimation of the gestational age may lead to false positive determination, while overestimation of gestational age may result in a false negative interpretation. When gestational age is uncertain, confirmation with ultrasonography is indicated.

Performance Data

See tables and graphs for data *representative* of the assay's performance. Results are expressed in IU/mL. Unless otherwise noted, all were generated on serum samples collected from testicular cancer patients.

Conversion Factor:
IU/mL × 1.21 → ng/mL

Calibration Range: up to 300 IU/mL
(363 ng/mL) (WHO 1st IS 72/225).

Analytical Sensitivity: 0.2 IU/mL
(0.24 ng/mL).

High-dose Hook: No effect up to 534,000 IU/mL.

Precision: Seven samples were processed in duplicate over the course of 20 days, two runs per day, for a total of 40 runs and 80 replicates. (See "Precision" table.)

Linearity: Serum and amniotic fluid samples were assayed under various dilutions. (See "Linearity" table for representative data.)

Recovery: Serum samples spiked 1-in-20 with three AFP solutions (286, 700 and 1,324 IU/mL) were assayed. Amniotic samples spiked 1-in-20 with three high amniotic fluid samples (10,000, 20,000 and 36,000 IU/ML) were also assayed. (See "Recovery" tables for representative data.)

Specificity: The assay is highly specific for AFP. (See "Specificity" table.)

Bilirubin (unconjugated): Based on the assay's relationship to IMMULITE AFP, bilirubin has a small but (by *t*-test) statistically significant effect. (See "Bilirubin" table for the IMMULITE AFP study.)

Hemolysis: No significant effect.

Lipemia: No significant effect.

Method Comparison – Testicular Cancer Studies: The assay was compared to DPC's IMMULITE AFP on a total of 205 samples from male patients in different clinical stages, pre- and post-surgery, of nonseminomatous testicular cancer. (Concentration range: approximately 0.3 to 280 IU/mL.) By linear regression:

(IML 2000) = 1.04 (IML) + 0.34 IU/mL
r = 0.998
n = 205

95% Confidence Interval (CI)	Slope	Intercept
Lower	1.03	-0.51
Upper	1.05	1.20

Method Comparison – Neural Tube Defect Studies: In two separate clinical studies conducted in the United States, IMMULITE 2000 AFP results were compared to two legally marketed assays (Kit A and Kit B) in a linear regression for maternal serum samples, in the range from nondetectable to 300 IU/mL. By linear regression:

(IML 2000) = 0.91 (Kit A) + 1.81 IU/mL
r = 0.98
n = 346

95% CI	Slope	Intercept
Lower	0.89	0.94
Upper	0.93	2.68

(IML 2000) = 0.73 (Kit B) + 5.22 IU/mL
r = 0.97
n = 1,015

95% CI	Slope	Intercept
Lower	0.72	4.66
Upper	0.74	5.79

In one of the the studies above, IMMULITE 2000 AFP results were compared to Kit B in a linear regression for **amniotic fluid** samples, in the range from nondetectable to 286 kIU/mL*.

(IML 2000) = 0.79 (Kit B) + 2.27 kIU/mL
r = 0.99
n = 200

95% CI	Slope	Intercept
Lower	0.77	1.78
Upper	0.81	2.76

* Amniotic fluid samples were diluted 1-in-101 automatically by the IMMULITE 2000 instrument.

The assay was also compared to DPC's IMMULITE AFP on **amniotic fluid** samples, in the range from approximately 3 to 20 kIU/mL*. By linear regression:

(IML 2000) = 1.03 (IML) + 0.52 kIU/mL
r = 0.96
n = 46

Means
10.0 kIU/mL (IML)
10.8 kIU/mL (IML 2000)

95% CI	Slope	Intercept
Lower	0.93	-0.50
Upper	1.12	1.54

* Amniotic fluid samples were diluted 1-in-101 automatically by the IMMULITE 2000 instrument.

The assay was also compared to DPC's IMMULITE AFP on **maternal serum** samples, in the range from approximately 10 to 120 IU/mL. By linear regression:

(IML 2000) = 1.01 (IML) + 0.154 (IU/mL)
r = 0.982
n = 346

Means
33.8 IU/mL (IML)
34.3 IU/mL (IML 2000)

95% CI	Slope	Intercept
Lower	0.99	-0.60
Upper	1.03	0.91

Clinical Sensitivity for Maternal Serum, *n* = 9:

Gestational Week	% > 2.0 MoM	% > 2.5 MoM	% > 3.0 MoM
15 – 20	100%	77.8%	66.7%
95% CI for All Samples	66.4% – 100%	40.0% – 97.2%	29.9% – 92.5%

Clinical Specificity for Maternal Serum:

Gestational Week	*n* of Samples	% ≤ 2.0 MoM	% ≤ 2.5 MoM	% ≤ 3.0 MoM
15	276	94.2%	97.5%	98.6%
16	304	96.1%	99.0%	99.7%
17	272	97.1%	99.3%	99.6%
18	287	95.8%	98.6%	99.3%
19	152	93.4%	98.0%	99.3%
20	41	95.1%	100%	100%
15 – 20	1332	95.5%	98.6%	99.3%
95% CI for All Samples		94.2% – 96.5%	97.8% – 99.1%	98.7% – 99.7%

Clinical Sensitivity for Amniotic Fluid, *n* = 8:

Gestational Week	% > 2.0 MoM	% > 2.5 MoM	% > 3.0 MoM
15 – 20	87.5%	87.5%	87.5%
95% CI for All Samples	47.3% – 99.7%	47.3% – 99.7%	47.3% – 99.7%

Clinical Specificity for Amniotic Fluid:

Gestation. Week	*n* of Samples	% ≤ 2.0 MoM	% ≤ 2.5 MoM	% ≤ 3.0 MoM
15	53	100%	100%	100%
16	50	98.0%	100%	100%
17	28	100%	100%	100%
18	20	100%	100%	100%
19	13	92.3%	100%	100%
20	10	100%	100%	100%
15 – 20	174	98.9%	100%	100%
95% CI for All Samples		95.9% – 99.9%	97.9% – 100%	97.9% – 100%

References

Testicular Cancer

1) Herberman, edd. Immunodiagnosis of cancer. 1979:101. 2) Int J Cancer 1971;7:218. 3) Cancer Res 1972;32:979. 4) Scand J Clin Lab Investig 1956;8:174. 5) Acta Unio Internationalis Contra Cancrum 1963;19:80. 6) Vopr Med Khim 1964;10:90. 7) Int J Cancer 1968;3:364. 8) Hum Pathol 1979;10:557. 9) Lancet 1976;2:433. 10) Adv Cancer Res 1971;14:295. 11) Proc Natl Acad Sci USA 1973;70:526. 12) Kirkpatrick, edd. Alpha-fetoprotein. 1981:115. 13) Cancer 1979;44:984. 14) Cancer Res 1975;35:991. 15) Cancer 1974;34:1510. 16) Med Intelligence 1976;295:1237. 17) Cancer 1976;37:215. 18) J Urol 1977;118:994. 19) Cancer 1978;42:2768. 20) J Clin Oncol 1986;4:41. 21) Rose, edd. Manual of clinical laboratory immunology. 1986:810. 22) Cancer 1981;47:328. 23) Urol Clin North Am 1977;4:393. 24) Ravitch, edd. Current problems in surgery. 1978:1. 25) Cancer 1980;45:1755. 26) Pavone-Macaluso, edd. Testicular cancer and other tumors. 1983:63. 27) J Urol 1978;119:759. 28) Kirkpatrick, edd. Alpha-fetoprotein. 1981:135. 29) N Eng J Med 1977;296:693. 30) Eur J Clin Chem Biochem 1993;31:517. 31) NCCLS H3-A4, 1998.

Fetal Open Neural Tube Defects

32) Brock DJH. Prenatal diagnosis – chemical methods. Br Med Bull 1976;32:16. 33) Crandall BF. Alpha-fetoprotein: a review. CRC Crit Rev Clin Lab Sci 1981:Sept:127-85. 34) Gitlin D. Normal biology of AFP. Ann NY Acad Sci 1975:259:17-26. 35) Haddow JE, et al. Fetal disorders associated with elevated MSAFP values. Foundation Blood Res 1990:1. 36) Leek AE, Chard T. Proceedings of colloquium on alpha-fetoprotein. In: Masseyeff R, editor. Paris: L'Institut National de la Sante et de la Recherche Medicale 563, (Nice) 1974. 37) Second Report of the Collaborative Study on Alpha-Fetoprotein in Relation to Neural Tube Defects. Amniotic fluid alpha-fetoprotein measurement in antenatal diagnosis of anencephaly and open spina bifida in early pregnancy. Lancet 1979;ii:651. 38) Seppälä M. Fetal pathophysiology of human α-fetoprotein. Ann N Y Acad Sci 1975;259:59.

Tables and Graphs

Precision (IU/mL)

		Within-Run		Total	
	Mean	SD	CV	SD	CV
1	0.80	0.05	6.3%	0.10	12%
2	2.8	0.10	3.6%	0.20	7.1%
3	13	0.27	2.1%	0.72	5.5%
4	31	0.82	2.7%	1.71	5.5%
5	44	0.96	2.2%	2.1	4.8%
6	60	1.5	2.5%	2.7	4.5%
7	182	4.4	2.4%	8.4	4.6%

Specificity

Compound	Amount Added	% Cross reactivity
Human Serum Albumin	60 mg/mL	ND
Human Transferrin	400 mg/dL	ND
Human Hemoglobin	192 mg/dL	ND
Cyclophosphamide	1,000 µg/mL	ND
Doxorubicin HCl	100 µg/mL	ND
Cisplatin	100 µg/mL	ND
Vincristine	1,000 ng/mL	ND
5-Fluorouracil	1,000 µg/mL	ND
Mitomycin C	100 µg/mL	ND

ND: not detectable.

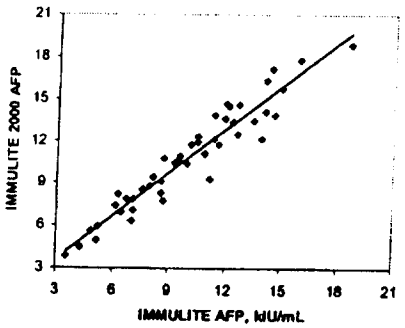
Linearity (IU/mL) - Serum

	Dilution	Observed	Expected	%O/E
1	8 in 8	7.8	—	—
	4 in 8	4.0	3.9	103%
	2 in 8	2.2	2.0	110%
	1 in 8	1.1	1.0	110%
2	8 in 8	23	—	—
	4 in 8	12	12	100%
	2 in 8	6.3	5.8	109%
	1 in 8	3.1	2.9	107%
3	8 in 8	90	—	—
	4 in 8	50	45	111%
	2 in 8	23	23	100%
	1 in 8	12	11	109%
4	8 in 8	143	—	—
	4 in 8	73	72	101%
	2 in 8	37	36	103%
	1 in 8	20	18	111%
5	8 in 8	288	—	—
	4 in 8	138	144	96%
	2 in 8	79	72	110%
	1 in 8	39	36	108%

Linearity (IU/mL) - Amniotic Fluid

	Total Dilution	Observed	Expected	%O/E
1	1 in 100	153	—	—
	1 in 200	76	77	99%
	1 in 400	37	38	97%
	1 in 800	19	19	100%
	1 in 1600	9.0	9.6	94%
	1 in 3200	4.6	4.8	96%
2	1 in 100	190	—	—
	1 in 200	91	95	96%
	1 in 400	47	48	98%
	1 in 800	24	24	100%
	1 in 1600	13	12	108%
	1 in 3200	5.9	5.9	99%
3	1 in 100	268	—	—
	1 in 200	139	134	104%
	1 in 400	68	67	101%
	1 in 800	33	34	97%
	1 in 1600	17	17	100%
	1 in 3200	8.6	8.4	103%

Method Comparison – Amniotic Fluid:



(IML 2000) = 1.03 (IML) + 0.52 kIU/mL $r = 0.96$

Recovery (IU/mL) - Serum

	Solution	Observed	Expected	%O/E
1	—	7.8	—	—
	A	21	22	96%
	B	40	42	95%
	C	75	74	101%
2	—	32	—	—
	A	44	48	92%
	B	61	65	94%
	C	96	97	99%
3	—	65	—	—
	A	76	76	100%
	B	98	97	101%
	C	135	128	106%
4	—	124	—	—
	A	125	132	95%
	B	147	153	96%
	C	183	184	100%
5	—	151	—	—
	A	159	158	101%
	B	182	179	102%
	C	216	210	103%
6	—	250	—	—
	A	247	252	98%
	B	261	273	96%
	C	300	304	99%

Recovery (IU/mL) - Amniotic Fluid

Low Amniotic Sample	Spiking High Amniotic Sample	Observed	Expected	%O/E
1	—	303	—	—
	A	805	788	102%
	B	1,347	1,288	105%
	C	2,142	2,088	103%
2	—	4,203	—	—
	A	4,306	4,493	96%
	B	6,404	4,993	128%
	C	5,714	5,793	99%
3	—	8,398	—	—
	A	8,849	8,478	104%
	B	9,437	8,978	105%
	C	10,567	9,778	108%

Bilirubin

Bilirubin (unconjugated)

		100 mg/L		200 mg/L	
	Expected	Observed	%O/E	Observed	%O/E
1	5.3	4.9	92%	5.1	96%
2	5.7	5.3	93%	5.6	98%
3	26	24	94%	25	97%
4	48	46	95%	46	95%
5	49	46	93%	46	93%

Technical Assistance

In the United States, contact DPC's Technical Services department.
Tel: 800.372.1782 or 973.927.2828
Fax: 973.927.4101. Outside the United States, contact your National Distributor.

The Quality System of Diagnostic Products Corporation is registered to ISO 9001:1994.

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