Summary of Safety and Effectiveness
IMMULITE and IMMULITE 2000 AFP

I. GENERAL INFORMATION

Device Generic Name:	Enzyme immunometric test system for the quantitative measurement of alpha fetoprotein (AFP) in human serum.
Device Trade Name:	IMMULITE® AFP* IMMULITE® 2000 AFP*
Applicant's Name and Address:	Diagnostic Products Corporation (DPC) 5700 West 96th Street Los Angeles, California 90045
Premarket Approval Application (PMA) Number:	P010007
Date of Notice of Approval to the Applicant:	November 9, 2001

* IMMULITE® and IMMULITE® 2000 are registered trademarks of Diagnostic Products Corporation. All references herein to IMMULITE or IMMULITE 2000 are to the registered trademark.

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II. INDICATIONS FOR USE

The IMMULITE® AFP is intended to be used with the IMMULITE Analyzer and IMMULITE 2000® AFP is intended to be used with the IMMULITE 2000 Analyzer. These devices are indicated for *in vitro* diagnostic use 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.

III. CONTRAINDICATIONS,

No known contraindications.

IV. WARNING AND PRECAUTIONS

See Package Insert for a listing of Warnings and Precautions.

V. DESCRIPTION OF DEVICES

Background

Alpha-fetoprotein (AFP) is a single chain glycoprotein with a molecular weight of approximately 70,000 daltons. Discovery of this embryo-specific protein in human fetal serum was reported in 1956 by Bergstrand and Czar[1]. AFP shares considered sequence homology with albumin, but its precise functional role has not been clearly defined[2]. AFP appears as a major serum protein in the fetus, reaches a peak at 13 weeks of gestation, but its concentration decreases rapidly toward birth[3-5]. The reappearance of the elevated AFP concentrations in adult serum has been observed during pregnancy and in conjunction with several benign liver diseases (hepatitis, cirrhosis) and malignancies such as primary hepatocellular carcinoma and some germ cell tumors.[6-8]

Fetal Open Neural Tube Defects

Since 1972, AFP measurements have become recognized to have clinical value as a screening test to aid in the detection of open NTDs[9-13]. Elevated levels of AFP in maternal serum and amniotic fluid indicate an increased risk for carrying an affected fetus. The prevalence of NTDs, estimated to be approximately 1-2 per 1000 live births in the United States, varies considerably in different population due to a number of factors[13].

NTDs are a consequence of abnormal development of the central nervous system during fetal development. The major types of NTDs are classified as anencephaly

and spina bifida (myelomeningocele) which together constitute about 94% of cases, with the remainder classified as encephaolocele[14].

In anencephaly the forebrain fails to develop normally, the bones of the cranium fail to fuse and the exposed neural tissue degenerates. Anencephaly lesions are considered open lesions and death usually results before or shortly after birth. In spina bifida, the neural tube fails to fuse. Approximately 80% of these lesions are defined as open with neural tissue exposed or covered only by a thin transparent membrane. The remainder is defined as closed and covered completely by skin or by a thick membrane. Closed spina bifida is not amenable to detection by AFP measurement. Spina bifida results in mild to severe disability due to impaired innervation of the lower portion of the body consequent to abnormal development of the spinal column and often the spinal cord. Finally, encephalocele is the least common of the three major NTDs and manifests as a closed lesion in approximately 80% of cases. Most cases of encephalocele are therefore not detectable via AFP measurement[13].

Prenatal Testing for NTDs

During pregnancy, maternal serum alpha-fetoprotein (MSAFP) levels rise until the mid third trimester as the result of diffusion of fetal AFP across the placenta into the maternal circulation. Open fetal NTDs are associated with elevated MSAFP levels during the second trimester of pregnancy.

When assessing the open NTD risk, maternal factors such as race, weight, age, diabetes, multiple births and family history have to be taken into consideration. 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. Normal median MSAFP levels rise from approximately 30 to 65 ng/mL during this time frame. The method of choice in reporting AFP levels is as a Multiple of the Median (MoM). Values greater than 2.0 to 2.5 MoM are cause for further investigation for the presence of fetal NTD. Repeating the AFP measurement in a second sample following an initial elevated MSAFP has proven useful for sorting out false positive results. However, a repeat MSAFP measurement that falls within the normal range does not exclude the possibility of an open fetal malformation, it only reduces the likelihood[13].

To rule out the more common causes of AFP elevation, the second elevated serum test is followed by an ultrasound procedure which allows visualization of multiple pregnancies and enables a more accurate estimate of gestational age. Certain NTDs, if present, can also be visualized during an ultrasound procedure. If ultrasonography does not provide an explanation of an elevated AFP level, the physician may offer the patient an amniocentesis.

Amniotic fluid APF levels peak at about 12 weeks gestation after which they rapidly decline and taper off at about week 22 of gestation. A high level of AFP

in the amniotic fluid indicates that a high probability exists that the fetus has an open NTD, provided other possible explanations have been ruled out. The high probability of an NTD can be confirmed by performing an additional acetylcholinesterase test on the amniotic fluid. Additionally, the location and magnitude of the possible defects can be assessed by means of either high resolution ultrasonography or by amniography.

Nonseminomatous Testicular Cancer Management

The quantitative determination of AFP in human serum when used in conjunction with other evaluation procedures and diagnostic tests has proven to be valuable in the management of patients with nonseminomatous testicular cancer. Serial measurements of serum AFP following therapy or surgery could monitor effectiveness of the therapeutic regimens and disease progression or recurrence.

A. IMMULITE AFP

The IMMULITE AFP is a solid-phase, two-site sequential chemiluminescent immunometric assay. The solid phase, a polystyrene bead enclosed within an IMMULITE Test Unit, is coated with a monoclonal antibody specific for AFP.

The patient sample and a buffer/serum matrix are simultaneously introduced into the Test Unit, and incubated for approximately 30 minutes at 37°C with intermittent agitation. During this time, AFP in the sample binds to the monoclonal, anti-AFP antibody-coated bead. Unbound sample is then removed by a centrifugal wash.

An alkaline phosphatase-labeled polyclonal anti-AFP antibody is introduced, and the Test Unit is incubated for another 30-minute cycle. The unbound enzyme conjugate is removed by a centrifugal wash. Substrate is then added, and the Test Unit is incubated for a further 10 minutes.

The chemiluminescent substrate, a phosphate ester of adamantyl dioxetane, undergoes hydrolysis in the presence of alkaline phosphatase to yield an unstable intermediate. The continuous production of this intermediate results in the sustained emission of light, providing a window for multiple readings. The bound complex - and thus the photon output, as measured by the luminometer - is proportional to the concentration of AFP in the sample. The concentration of AFP in the patient sample is obtained using a stored master calibration curve within the IMMULITE analyzer.

B. IMMULITE 2000 AFP

IMMULITE 2000 AFP is a solid-phase, two-site chemiluminescent immunometric assay. The solid phase is a polystyrene bead coated with a monoclonal antibody specific for AFP.

The patient serum sample and a buffer/serum matrix are introduced into the Reaction Tube containing the bead and incubated for approximately 30 minutes at 37°C with agitation. During this time, AFP in the sample binds to the monoclonal, anti-AFP antibody-coated bead. Unbound sample is then removed by a centrifugal wash.

An alkaline phosphatase-labeled polyclonal anti-AFP antibody is introduced and the Reaction Tube is incubated for another 30 minute cycle. The unbound enzyme conjugate is removed by a centrifugal wash. Substrate is then added, and the Reaction Tube is incubated for a further 5 minutes. The concentration of AFP in the sample is then measured in the same manner as that of IMMULITE AFP.

VI. ALTERNATIVE PRACTICES OR PROCEDURES

The detection of NTDs in fetuses can be achieved by using the following alternative practices or procedures:

1) Ultrasonography;
2) Amniography;
3) Amniotic fluid acetylcholinesterase testing;
4) The use of other legally-marketed AFP devices.

In the management of nonseminomatous testicular cancer, alternative and additional practices include physical evaluation, histological diagnosis after orchiectomy, lymphadenectomy and lymph node biopsy, exploratory laparoscopy, lymphography, chest radiography, ultrasound, computed tomography, magnetic resonance imaging, and other immunological devices for the quantification of other markers in human serum.

VII. MARKETING HISTORY

IMMULITE AFP and IMMULITE 2000 AFP have been marketed in the United States and Internationally as an aid in the management of patients with nonseminomatous testicular cancer since December 1998.

VIII. POTENTIAL ADVERSE EFFECTS OF DEVICE ON HEALTH

The extensive follow-up testing to maternal serum and amniotic fluid AFP measurement reduces to a very low likelihood, but does not completely eliminate the possibility that a healthy fetus may be incorrectly diagnosed as having an NTD. Special consideration needs to be given when interpreting AFP results from pregnancies where one twin appears by ultrasound to have suffered fetal demise or an open NTD while the other appears unaffected in order to avoid a misdiagnosis of the unaffected twin. In addition, an amniocentesis procedure that may be performed as a follow-up to an elevated MSAFP result carries a

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miscarriage risk, estimated to be approximately 1 in 1000. The physician should discuss the risk of miscarriage with the patient.

Patients undergoing treatment for nonseminomatous testicular cancer should not experience any adverse effects if test results from these in vitro devices are used as an aid in managing the cancer in conjunction with other routine medical practices and procedures and all available clinical information. A false positive result would indicate that a person may be incorrectly diagnosed as having testicular cancer whereas a false negative result would indicate no change in the patient's clinical status.

IX. SUMMARY OF STUDIES

A. Nonclinical Studies

The information in the table below provides the performance characteristics of IMMULITE AFP and IMMULITE 2000 AFP.

Characteristics	**IMMULITE AFP**	**IMMULITE 2000 AFP**
Calibration upper limit	300 IU/mL	300 IU/mL
Analytical Sensitivity	0.2 IU/mL	0.2 IU/mL
Precision (within-run)	CV range: 4.5-7.4%	CV range: 2.1-6.3%
Precision (total)	CV range: 5.8-8.4%	CV range: 4.5-12%
Specificity	No clinically significant cross-reactivity	No clinically significant cross-reactivity
Linearity (Serum)	%Observed/Expected range: 97-114%	%Observed/Expected range: 96-111%
Linearity (Amniotic Fluid)	%Observed/Expected range: 81-119%	%Observed/Expected range: 94-106%
Spiking Recovery (Serum)	%Observed/Expected range: 98-108%	%Observed/Expected range: 94-106%
Spiking Recovery (Amniotic)	%Observed/Expected range: 81-127%	%Observed/Expected range: 96-128%
Effect of Bilirubin (unconjugated)	Small but statistically significant effect	Small but statistically significant effect
Effect of Lipemia	No significant effect	No significant effect
Effect of Hemolysis	No significant effect	No significant effect
No "high dose hook" effect up to AFP levels of:	450,000 IU/mL	534,000 IU/mL
Stability	Kit is stable for at least one year when kept in long-term storage conditions.	Kit is stable for at least one year when kept in long-term storage conditions.

B. Clinical studies

1. Expected Values

Clinical studies were performed at three medical institutions involving 2373 maternal serum and 385 amniotic fluid samples prospectively and retrospectively obtained during 15 through 20 weeks of gestation. These samples were assayed by IMMULITE and IMMULITE 2000 AFP.

Of the samples tested, 2287 maternal sera and 341 amniotic fluid specimens were confirmed as unaffected, non-insulin-dependent, singleton pregnancies at 15 through 20 gestational weeks. The following table shows the medians and multiples of medians calculated by the weighted log-linear regression for the 2287 serum samples, tested either by IMMULITE or IMMULITE 2000 AFP.

Gestational Week	No. of Specimens	Regressed 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

The following table shows the medians and multiples of medians calculated by the weighted log-linear regression for these 341 amniotic fluid samples, tested either by IMMULITE or IMMULITE 2000 AFP.

Gestational Week	No. of Specimens	Regressed 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

2. Clinical Specificity and Sensitivity

Using the above cutoffs, the clinical specificity and clinical sensitivity were calculated to evaluate the ability of the IMMULITE and IMMULITE 2000 AFP to confirm unaffected pregnancies (clinical specificity) and to detect open NTD pregnancies (clinical sensitivity).

IMMULITE AFP Specificity (%) for Maternal Serum

Gestational Week	No. of Samples	% <= 2.0 MoM	% <= 2.5 MoM	% <= 3.0 MoM
15	173	96.0%	98.8%	98.8%
16	411	98.1%	99.3%	99.5%
17	372	96.5%	99.7%	100.0%
18	204	95.1%	99.0%	100.0%
19	108	94.4%	99.1%	100.0%
20	50	100.0%	100.0%	100.0%
All (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%

IMMULITE 2000 AFP Specificity (%) for Maternal Serum

Gestational Week	No. 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%	100.0%	100.0%
All (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%

IMMULITE AFP Sensitivity (%) for Maternal Serum

Gestational Week	No. of Samples	% > 2.0 MoM	% > 2.5 MoM	% > 3.0 MoM
15 - 20	13	92.3%	69.2%	69.2%
95% CI for all samples		64.0-99.8%	38.6-90.9%	38.6-90.9%

IMMULITE 2000 AFP Sensitivity (%) for Maternal Serum

Gestational Week	No. of Samples	% > 2.0 MoM	% > 2.5 MoM	% > 3.0 MoM
15 - 20	9	100.0%	77.8%	66.7%
95% CI for all samples		66.4-100.0%	40.0-97.2%	29.9-92.5%

IMMULITE AFP Specificity (%) for Amniotic Fluid

Gestational Week	No. of Samples	% <= 2.0 MoM	% <= 2.5 MoM	% <= 3.0 MoM
15	23	100.0%	100.0%	100.0%
16	39	97.4%	100.0%	100.0%
17	25	100.0%	100.0%	100.0%
18	34	94.1%	97.1%	100.0%
19	33	100.0%	100.0%	100.0%
20	13	100.0%	100.0%	100.0%
All (15 - 20)	167	98.2%	99.4%	100.0%
95% CI for all samples		94.8-99.6%	96.7-100.0%	97.8-100.0%

IMMULITE 2000 AFP Specificity (%) for Amniotic Fluid

Gestational Week	No. of Samples	% <= 2.0 MoM	% <= 2.5 MoM	% <= 3.0 MoM
15	53	100.0%	100.0%	100.0%
16	50	98.0%	100.0%	100.0%
17	28	100.0%	100.0%	100.0%
18	20	100.0%	100.0%	100.0%
19	13	92.3%	100.0%	100.0%
20	10	100.0%	100.0%	100.0%
All (15 - 20)	IMMULITE AFP Sensitivity (%) for Amniotic Fluid			
95% CI for all samples		95.9-99.9%	97.9-100.0%	97.9-100.0%

Gestational Week	No. of Samples	% > 2.0 MoM	% > 2.5 MoM	% > 3.0 MoM
15 - 20	10	90.0%	90.0%	90.0%
95% CI for all samples		55.5-99.8%	55.5-99.8%	55.5-99.8%

IMMULITE 2000 AFP Sensitivity (%) for Amniotic Fluid

Gestational Week	No. of Samples	% > 2.0 MoM	% > 2.5 MoM	% > 3.0 MoM
15 - 20	8	87.5%	87.5%	87.5%
95% CI for all samples		47.3-99.7%	47.3-99.7%	47.3-99.7%

3. Conclusions

In previously conducted studies under the 510(k)s (K983263 & K983263), the IMMULITE and IMMULITE 2000 AFP were cleared for serial measurements in human serum to aid in the management of patients with nonseminomatous testicular cancer and other conditions associated with abnormal AFP production. This indication has been further expanded based upon the additional clinical performance studies to include AFP 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.

C. Method Comparison Studies

1. IMMULITE AFP

a) Nonseminomatous testicular cancer management

The assay was compared to a legally marketed AFP 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 another legally marketed device.

	IMMULITE AFP		Relative Sensitivity (95% CI)	Relative Specificity (95% CI)
Kit C	Pos	Neg		
Pos	74	4	94.9%	97.3%
Neg	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	Pos	Neg		
Pos	62	3	95.4%	97.3%
Neg	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

95% CI	Slope	Intercept
Lower	0.81	-0.65
Upper	0.84	0.31

b) Open Neural Tube Defects

i. Maternal serum

In two separate clinical studies conducted in the United States, IMMULITE AFP results were compared to two legally marketed AFP 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 = 1006

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

ii. Amniotic fluid

In one of the studies above, IMMULITE AFP results were also compared to a 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.

2. IMMULITE 2000 AFP

a) Nonseminomatous testicular cancer management

The IMMULITE 2000 AFP assay was compared to 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% CI	Slope	Intercept
Lower	1.03	-0.51
Upper	1.05	1.20

b) Open NTD

i. Maternal serum

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.

(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 = 1015

95% CI	Slope	Intercept
Lower	0.72	4.66
Upper	0.74	5.79

ii. Amniotic fluid

In one of 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

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c) Comparison of IMMULITE AFP and IMMULITE 2000 AFP

i. Amniotic fluid

IMMULITE 2000 AFP was also compared to IMMULITE AFP on 46 amniotic fluid samples, in a range from approximately 3 to 20 kIU/mL*. By linear regression:

(IML 2000) = 1.03 (IML) + 0. 52 kIU/mL r = 0.982 n = 46

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

Mean of IMMULITE AFP = 10.0 kIU/mL
Mean of IMMULITE 2000 AFP = 10.8 kIU/mL

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

ii. Maternal serum

IMMULITE 2000 AFP was also compared to IMMULITE AFP on 346 maternal serum samples in a range from approximately 10 to 120 IU/mL. By linear regression:

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

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

Mean of IMMULITE AFP = 33.8 IU/mL
Mean of IMMULITE 2000 AFP = 34.3 IU/mL

X. CONCLUSION

A false positive result could result in the possibility that a healthy fetus may be incorrectly diagnosed as having an NTD. However, the follow-up testing to maternal serum and amniotic fluid AFP measurement reduces the risk to a very low likelihood. Therefore, the benefits of AFP testing far outweigh the risk.

The data presented in the submission provide reasonable assurance that the IMMULITE AFP and IMMULITE 2000 devices are safe and effective as an aid in the detection of open neural tube defects and as an aid in the management of nonseminomatous testicular cancer patients.

XI. PANEL RECOMMENDATIONS

Pursuant to Section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not the subject of an FDA Immunology Devices Advisory Panel meeting because the information in the PMA substantially duplicated information previously reviewed by this Panel.

XII. CDRH DECISION

The applicant's manufacturing facilities were inspected in October 4, 2001, and the facilities were found to be in compliance with the Good Manufacturing Practice (GMP) Regulations.

FDA issued an approval order on November 9, 2001.

XIII. APPROVAL SPECIFICATION

Directions for use: See the labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, precautions and Adverse Events in the labeling.

Postapproval Requirements and Restrictions: See approval order

XIV. REFERENCES

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