SECTION III: COMPREHENSIVE SUMMARY OF SAFETY AND EFFECTIVENESS

I. General Information

Device Generic Name: Chemiluminescent immunoassay for in vitro diagnostic quantitation of alpha fetoprotein (AFP) in maternal serum and amniotic fluid.

Device Trade Name: Access® AFP Immunoassay System

Applicant's Name and Address:

Beckman Coulter, Inc.
1000 Lake Hazeltine Drive
Chaska, MN 55318

Premarket Approval Application (PMA) Number: P980041

Date of Panel Recommendation: 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 Advisory Panel meeting because the information in the PMA substantially duplicates information previously reviewed by this panel.

Date of Notice of Approval to the Application: FEB - 8 1999

II. Intended Use

The Access® AFP assay is a paramagnetic particle, chemiluminescent immunoassay for use with the Access® Immunoassay System for the quantitative determination of alpha-fetoprotein (AFP) in:

1) Human serum, as an aid in the management of patients with non-seminomatous testicular cancer.
2) Maternal serum and amniotic fluid at 15 to 20 weeks gestation, to aid in the detection of fetal open neural tube defects (ONTD). The assay is intended for use in conjunction with other diagnostic tools such as ultrasound and amniography.

Background

Open neural tube defects (ONTD) are among the most common and serious congenital malformation affecting approximately 1 to 2 newborns per 1000 births in the United States (1).

Alpha-fetoprotein (AFP) is a feto-specific protein consisting of a single polypeptide chain with a molecular weight of approximately 70,000 Daltons, slightly larger than albumin. Unlike albumin, AFP is a glycoprotein containing approximately 4% carbohydrate and is, after albumin, the major protein in fetal circulation (2). Production occurs primarily in the fetal liver and to a lesser degree in the yolk sac and other fetal organs (3). AFP is secreted into the fetal serum where it is detectable approximately 30 days after conception (4). Fetal serum concentrations of AFP peak at the end of the first trimester then gradually decrease during later gestation (5).

During gestation, AFP is present in the amniotic fluid as a result of fetal micturition. AFP reaches the maternal circulation via the placenta or by diffusion across the fetal membranes. Measurable concentrations appear in the maternal serum beginning at the end of the first trimester reaching a maximum level during the second trimester.

The presence of AFP in maternal sera was recognized by Seppala and Ruoslahti in 1972 (6). In that same year, Brock and Sutcliffe reported the association between increased amounts of AFP in the amniotic fluid and pregnancies affected with neural tube defects (7). The following year Brock, et al. demonstrated that AFP concentrations in maternal serum were also elevated under these conditions (8).

Increased AFP concentrations in maternal serum may occur in multiple fetuses, low birth weight and fetal demise. An incorrect estimation of gestational age may also lead to misinterpretation of the Multiple of the Median (MoM) result. Ultrasonography can be used to aid in the determination of gestational age, the presence of multiple fetuses, open neural tubes, or other pregnancy problems.

Prenatal measurement of AFP concentrations are an effective aid to identify women potentially at risk for carrying a fetus with an ONTD. Two United Kingdom Collaborative studies have demonstrated the overall reliability of AFP testing for the prenatal detection of ONTD; the first in 1977 addressed AFP maternal serum testing (12) and the second addressed amniotic fluid AFP testing (13).

III. Device Description

The Access® AFP reagents and the Access® Immunoassay Analyzer comprise the Access® Immunoassay System for the quantitative determination of AFP. The Access® AFP assay is a paramagnetic particle, chemiluminescent immunoassay for use with the Access® Immunoassay System for the quantitative determination of alpha-fetoprotein (AFP) in maternal human serum and amniotic fluid at 15 and 20 weeks of gestation, to aid in the detection of fetal open neural tube defects (ONTD). Maternal serum and amniotic fluid test results, when used in conjunction with ultrasonography, or amniography, and amniotic fluid acetylcholinesterase testing, aid in the detection of fetal ONTD.

The Access® AFP assay is a two-site immunoenzymatic (sandwich) assay that measures AFP concentrations between approximately 0.50 and 3000 ng/ml (0.40 and 2478 IU/ml). Prior to assaying, amniotic fluid samples require a dilution of 1 volume sample with 10 volumes of Access® AFP Sample Diluent. The assay features a simultaneous format in which the sample is incubated for 4.8 minutes at a controlled temperature with paramagnetic particles coated with monoclonal anti-human AFP antibody and alkaline phosphatase anti-human AFP antibody conjugate.

Separation in a magnetic field, and washing, removes materials not bound to the solid phase. A chemiluminescent substrate, Lumi-Phos® 530, is added to the reaction vessel and light generated by the dephosphorylation of the substrate is measured with a luminometer. The light production is proportional to the quantity of AFP in the sample. The quantity of AFP in the sample is determined by means of a stored, multi-point calibration curve.

Access® Analyzer

The Access® Immunoassay Analyzer is a bench top, microcomputer controlled, random and continuous access instrument. The analyzer performs enzyme immunoassays utilizing paramagnetic particle solid phase and chemiluminescent detection. The Access® Immunoassay Analyzer consists of the following functional modules:

Sample / Reagent Carousel Module
Main Pipettor Module

Analytical Module
Fluidics Module
Electronics / System Computer
Peripheral Module

The Access® Immunoassay Analyzer system software controls all instrument functions:

Facilitates operator input through user interface
Schedules assays
Runs assays
Monitors and controls environment within the instrument
Drives instrument motors, valves, and hardware modules
Stores data in data base files
Facilitates Laboratory Information System (LIS) communication, optional

WARNINGS AND PRECAUTIONS:

Warnings and precautions for use of the device are stated in the attached product labeling. (See labeling)

IV. Alternative Practices and Procedures

Alternative and additional practices or procedures for aiding in the detection of fetal ONTD *in utero* include ultrasonography and amniography. In addition, there are other AFP immunological in vitro diagnostic devices for which there are approved PMAs for use as an aid in the detection of fetal ONTD.

V. Marketing History

The Access® AFP Immunoassay System, has been marketed in the US as an aid in the management of patients with non-seminomatous testicular cancer since September 1998.

VI. Potential Adverse Effects of the Device on Health

As with any *in vitro* diagnostic device, a possible adverse effect may be either a false positive or false negative result.

In the event of an initial positive result, confirmatory testing is recommended in the package insert. In order for a false positive result to be reported (incorrect

diagnosis of an ONTD), the confirmatory test would have to report a positive result which would also be incorrect.

In the event of a false negative report, a fetal abnormality would not be detected because no further testing would be done and the pregnancy outcome could potentially be a birth with an ONTD condition.

VII. Summary of Studies

A. Pre-Clinical Studies

Pre-Clinical studies were conducted at Beckman Coulter–Chaska to determine the purity and specificity of the reagents, as well as the performance characteristics of the assay.

Characterization of the Antigen

The AFP antigen used as the immunogen in the development of the monoclonal antibodies found in the Access® AFP assay was purified from human placenta. The AFP used to prepare the calibrators and controls for the Access® AFP assay was manufactured from human cord serum.

Characterization of the Antibodies

The antibodies chosen were characterized using the following tests:

- SDS-PAGE - the molecular weight of both purified monoclonal antibodies was determined to be 150 kD.
- Isoelectric focusing - the isoelectric points of each monoclonal antibody were determined. The isoelectric points for the monoclonal antibodies were pI = 6.2 – 6.6 for the capture antibody and pI = 6.5 – 6.9 for the conjugate antibody.
- Immunoglobulin subtyping by ELISA - the subtypes of the selected monoclonal antibodies were heavy chain IgG_1 and light chain K for both antibodies.
- Western Blot analysis - one band was observed against amniotic fluid and placental AFP, with no reactivity observed towards CEA or HSA with both antibodies.

Performance Characteristics

1. **Reproducibility**

   Within-run, between-run and total reproducibility (precision) of the Access® AFP assay were determined at Beckman Coulter-Chaska. Three serum based controls containing AFP concentrations spanning the assay range were assayed in triplicate twice a day for 20 days. One kit lot was used in the testing. The summary of results are presented in Table 1. The percent coefficients of variation ranged from 3.41 to 4.44.

Table 1. Intra-assay (Within-run), Inter-assay (Between-run) and Total Precision

Sample	Grand Mean (n = 120) (ng/ml)	Within Run 1 SD	Within Run (%CV)	Between run 1 SD	Between Run %CV	Total 1 SD	Total (%CV)
1	6.53	0.21	3.22	0.21	3.22	0.29	4.44
2	72.10	2.08	2.88	1.47	2.04	2.55	3.54
3	1672.88	45.34	2.71	34.55	2.07	57.00	3.41

2. **Analytical Sensitivity**

   The analytical sensitivity, or minimum detectable concentration (MDC), of the Access® AFP assay is defined as that concentration of AFP that corresponds to the relative light unit (RLU) that is 2 standard deviations greater than the mean RLU of 25 determinations of the Access® AFP Calibrator S0.

   The analytical sensitivity was determined by assaying the Access® AFP Calibrator S0 in replicates of 25 in 9 assays using 3 different reagent lots. The mean value of all of the calculated sensitivities (from the 9 separate assays) was 0.06 ng/ml which is below the product labeling claim of 0.50 ng/ml of AFP.

3. **Dilution Recovery**

   Three maternal serum samples and 5 amniotic fluid samples containing elevated concentrations of AFP were diluted using the Access® AFP Sample Diluent to yield several concentrations. Each dilution study was analyzed by means of linear regression, yielding correlation coefficients of 1.000 for all 3 maternal serum samples and 0.977 to 1.000 for amniotic fluid samples.

4. **Cross Reactivity and Interfering Substances(Specificity)**

The analytical specificity of the Access® AFP assay was evaluated with the substances listed in Table 2. Each of these substances was added to the calibrator matrix containing no AFP (control) and to calibrator matrix containing AFP (test). Each control and test sample was assayed in quadruplicate. The results of the interfering substances analysis demonstrate that there is no significant interference from any of the substances tested.

Table 2. Cross Reactivity and Interfering Substances Tested

Substance	Concentration
Bilirubin	25 mg/dl
Hemoglobin	1.2 g/dl
Triglycerides	520 mg/dl
Albumin (BSA)	6 mg/dl
Rheumatoid Factor	600 IU/ml
Acetaminophen	1500 µg/ml
Acetylsalicylic Acid	10 mg/ml
Alpha-1-Acid Glycoprotein	4.54 mg/ml
Alpha-1-antitrypsin	14.8 mg/ml
Ascorbic Acid	1000 µg/ml
Bleomycin	100 µU/ml
CEA	375 µg/ml
Chlorothiozide	1000 µg/ml
Cisplatin	1000 µg/ml
Cobalamin	500 µg/ml
Diazepan	50 µg/ml
Ethyl Alcohol	1.90%
Fetal Hemoglobin	500 µg/ml
Haptoglobin	500 mg/ml
HCG	200 µg/ml
HFSH	2 IU/ml
HLH	2 IU/ml
HTSH	6 µg/ml
Phenacetin	500 µg/ml
Phenothizine	150 µg/ml
Placental Lactogen	100 µg/ml
Reserpine	100 µg/ml
Retinoic Acid	500 µg/ml
Riboflavin	50 µg/ml
Spironolactone	15 µg/ml
Thiamine HCl	50 µg/ml
Transferrin	23.7 mg/ml
Vinblastine	500 µg/ml

5. Stability

Three lots of Access® AFP reagents were stored between 2 and 10°C and were tested at specified time intervals. The results of this testing support expiration dating of at least 18 months.

6. Method Correlation

To determine the correlation between the Access® AFP and another AFP assay for which there is an approved PMA, a total of 437 maternal serum and 307 amniotic fluid samples were analyzed. Samples were run at 3 external sites and each site utilized 3 Access® AFP reagent kit lots. The data, presented in Table 3, demonstrate correlation between the Access® AFP and the other AFP assay.

Table 3. Access® AFP assay correlation.

Sample type	N	Range of Observations	Intercept	Slope	Correlation Coefficient
Maternal Serum	437	3.06 – 268.56 ng/ml	3.59 ng/ml	0.86	0.989
Amniotic Fluid	307	1.38 – 32.96 µg/ml	0.38 µg/ml	0.85	0.966

B. Clinical Study

A multicenter, retrospective clinical trial was conducted to evaluate the safety and effectiveness the Access® AFP assay when used used in conjunction with ultrasonography, or amniography to aid in the detection of fetal open neural tube defects in maternal serum and amniotic fluid at 15 to 20 weeks gestation.

1. Study Sites

The study was performed at 3 well-established, geographically diverse AFP testing centers in North America. The principal investigators and their institutions are shown in Table 4.

Table 4. Study Sites and Principal Investigators

Site No.	Institution	Abbr.	Principal Investigators	Lab Certifications
1	Pavillon- St. Francois d' Assise (CHUQ) Quebec City, Quebec, Canada	QUEBEC	Jean-Claude Forest, MD, Ph.D., FRCPC Biochimistre	Canadian Council
2	Foundation for Blood Research Scarborough, ME	FBR	George Knight, Ph.D. Director, Prenatal Screening Laboratory	CLIA
3	University of Texas Southwestern Medical Center Dallas, TX	UTSMC	Leland Baskin, MD Associate Professor, Dept. of Pathology Frank H. Wians, Jr. Ph.D. Associate Professor, Dept. of Pathology	CLIA and CAP

2. Study Objective

The clinical trial study objective was to:

- Establish median AFP values for amniotic fluid samples obtained from pregnant women at 15 through 20 weeks gestation with subsequent viable, unaffected singleton pregnancy outcomes
- Determine the diagnostic sensitivity and specificity, and their 95% confidence intervals for the Access® AFP assay
- Compare the Access® AFP assay to another AFP assay for which there is an approved PMA, utilizing both maternal serum and amniotic fluid samples
- Determine the performance characteristics (precision, linearity, and analytical sensitivity) of the Access® AFP assay in a clinical prenatal testing environment.

3. Subject Selection, Exclusion Criteria, and Study Population

Each site selected maternal serum and amniotic fluid samples from their laboratory AFP specimen storage bank utilizing a selection process that insured an unbiased population of maternal serum and amniotic fluid samples that met the inclusion and exclusion criteria. All subjects meeting the inclusion and exclusion criteria over the specified period were enrolled in the study.

The inclusion criteria were: 1) maternal serum samples obtained from pregnant women at 14 through 21 weeks gestation and who elected to

have AFP testing done as part of their obstetrical care; 2) amniotic fluid samples obtained from pregnant women at 15 through 21 weeks of gestation and who elected to have AFP testing done as part of their obstetrical care; and 3) the samples were stored in a continuous state (-20° C or colder), frozen less than 4 years in tightly sealed containers, and subjected to a maximum of one freeze-thaw cycle.

The exclusion criteria included: 1) the amniocentesis was performed prior to drawing the serum sample; 2) the serum samples were hemolyzed, icteric or lipemic; or 3) the amniotic fluid samples were visibly contaminated with blood.

Table 5 lists the number of samples included in this study.

Table 5. Study Population

Outcome	Weeks	Number of Maternal Serum Samples	Number of Amniotic Fluid Samples	Total Number of Case Report Forms
Normal	15 – 20 14 & 21	2,539 328	720 55	3,259 383
Multiple Birth	14 –21	17	2	19
Abnormal	14 - 21	40	36	76
All	14 – 21	2,924	813	3,737

4. Study Results

The 3 clinical sites assayed a total of 2539 maternal serum and 720 amniotic fluid specimens with confirmed normal singleton pregnancies at 15 to 20 gestational weeks. Table 6 contains the combined maternal serum AFP regressed medians (ng/mL) for all three clinical sites.

Table 6
Maternal Serum AFP Medians
Quebec, FBR, UTSMC Combined

Gestational Week	Number of Observations	Median (ng/mL)	Multiple Of Median (2.0)	Multiple Of Median (2.5)	Multiple Of Median (3.0)
15	435	31.1	62.2	77.8	93.4
16	506	36.0	72.0	90.0	108.0
17	452	41.6	83.2	104.1	124.9
18	425	48.1	96.3	120.3	144.4
19	413	55.7	111.3	139.2	167.0
20	308	64.4	128.8	161.0	193.2

The Table 7 below contains the combined amniotic fluid AFP regressed medians (ng/mL) for all three clinical sites.

Table 7
Amniotic Fluid AFP Medians
Quebec, FBR, UTSMC Combined

Gestational Week	Number of Observations	Median (ng/mL)	Multiple Of Median (2.0)	Multiple Of Median (2.5)	Multiple Of Median (3.0)
15	157	16.5	33.0	41.3	49.5
16	107	13.4	26.9	33.6	40.3
17	105	10.9	21.8	27.3	32.8
18	117	8.9	17.8	22.2	26.6
19	111	7.2	14.4	18.1	21.7
20	123	5.9	11.7	14.7	17.6

Clinical specificity, defined as the probability that the test will be negative in the absence of disease is shown in tables 8 and 9.

Table 8
Clinical Specificity of the Access® AFP Assay for Maternal Serum
All sites combined

Gestational Week	Number of Samples	2.0 MoM (%)	2.5 MoM (%)	3.0 MoM (%)
15	435	92.6	97.2	99.3
16	506	95.3	98.2	98.8
17	452	95.1	98.0	98.9
18	425	96.7	98.8	99.5
19	413	96.4	98.8	99.8
20	308	96.4	98.7	99.7

Using a cutoff of 2.0 MoM for the maternal serum specimens from the 3 clinical sites, the clinical specificity was 95.4% (2421/2539) with a 95% confidence interval of 94.4% - 96.1%. Using a cutoff of 2.5 MoM for the maternal serum specimens, the clinical specificity was 98.3% (2495/2539) with a 95% confidence interval of 97.7% - 98.7%.

Table 9
Clinical Specificity of the Access® AFP Assay for Amniotic Fluid
All sites combined

Gestational Week	Number of Samples	2.0 MoM (%)	2.5 MoM (%)	3.0 MoM (%)
15	157	99.4	99.4	99.4
16	107	97.2	99.1	99.1
17	105	98.1	100.0	100.0
18	117	97.4	98.3	98.3
19	111	96.4	97.3	99.1
20	123	95.9	98.4	100.0

Using a cutoff of 3.0 MoM for the amniotic fluid samples from the 3 clinical sites, the clinical specificity was 99.3% (715/720) with a 95% confidence interval of 98.3% - 99.7%.

Clinical sensitivity is defined as the probability that the test will be positive in the presence of an ONTD. The following tables summarize the clinical sensitivity of maternal serum AFP and amniotic fluid AFP.

Table 10
Clinical Sensitivity of the Access® AFP Assay for Maternal Serum
All sites combined

Category	Number of Defects	≥ 2.0 Mom	≥ 2.5 Mom	≥ 3.0 Mom
ONTD Affected Outcome	23	91.3%	73.9%	69.6%
95% Confidence Interval		(70.5%-98.5%)	(51.3%-88.9%)	(47.0%-85.9%)

A total of 23 maternal serum specimens tested at the 3 clinical sites were associated with confirmed ONTD birth outcomes. Using a cutoff of 2.0 MoM, the clinical sensitivity of the Access® AFP assay for maternal serum was 91.3% (21/23) with a 95% confidence interval of 70.5% - 98.5%. Using a cutoff of 2.5 MoM for the maternal serum specimens, the clinical sensitivity was 73.9% (17/23) with a 95% confidence interval of 51.3% - 88.9%.

A total of 15 amniotic fluid specimens were associated with confirmed ONTD birth outcomes. Using a cutoff of 3.0 MoM, the clinical sensitivity of the Access® AFP assay for amniotic fluid was 100% (15/15).

Table 11
Clinical Sensitivity of the Access® AFP Assay for Amniotic Fluid
All sites combined

Category	Number of Defects	≥ 2.0 Mom	≥ 2.5 Mom	≥ 3.0 Mom
ONTD Affected Outcome	15	100.0%	100.0%	100.0%

VIII. Conclusions Drawn from Studies

Study results demonstrate that the Access® AFP assay is safe and effective when used in conjunction with ultrasonography, or amniography, to aid in the

detection of fetal open neural tube defects in maternal serum and amniotic fluid at 15 to 20 weeks gestation.

IX. Panel Recommendation

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 Advisory Panel meeting because the information in the PMA substantially duplicates information previously reviewed by this panel.

X. CDRH Action on the Application

CDRH issued an approval order for the applicant's PMA Access® AFP Immunoassay System on February 8, 1999.

The applicant's manufacturing and control facilities were inspected on January 25, 1999 and the facilities were found to be in compliance with the Good Manufacturing Practice Regulations (GMPs). The shelf-life of Access® AFP Immunoassay System has been established at 18 months for product stored between 2° and 10° C.

XI. Approval Specifications

Directions for Use: See labeling

Conditions of Approval: FDA approval of this PMA is subject to full compliance with the conditions described in the approval order.

XIII. References

1. Macri JN, Baker DA, and Baim RS. Diagnosis of neural tube defects by evaluation of amniotic fluid. Clin Obstet Gynecol 1981; 24: 1089.

2. Ruoslahti E, Seppala M. Studies of carcino-fetal proteins: Physical and chemical properties of human alpha-fetoprotein. Int J Cancer 1971; 7: 218.

3. Gitlin D, Perricelli A, Gitlin GM. Synthesis of alpha-fetoprotein by liver, yolk sac, and gastrointestinal tract of the human conceptus. Cancer Res 1972; 32: 979.

4. Crandall BF. Alpha-fetoprotein: The diagnosis of neural tube defects. Ped Annals 1981; 10: 78.

5. Kjessler B, Johansson SGO. Monitoring of the development of early pregnancy by determination of alpha-fetoprotein in maternal serum and amniotic fluid samples. Acta Obstet Gynecol Scand 1977; 69: 5.

6. Seppala M, Ruoslahti E. Radioimmunoassay of maternal serum alpha-feto protein during pregnancy and delivery. Am J Obstet Gynecol 1972; 112: 208.

7. Brock DJH and Sutcliffe RG. Alpha-fetoprotein in the antenatal diagnosis of anencephaly and spina bifida. Lancet 1972; 2(7770): 197.

8. Brock DJH, Bolton AE, Monaghan JM. Prenatal diagnosis of anencephaly through maternal serum alpha-fetoprotein measurements. Lancet 1973; 2(7835): 923.

9. Smith AD, Wald NJ, Cuckle HS, Stirrat GM, Bobrow M, Lagercrantz H. Amniotic fluid acetylcholinesterase as a possible diagnostic test for neural tube defects in early pregnancy. Lancet 1979; 1(8118): 685.

10. Macri JN and Weiss RR. The utilization of alpha-fetoprotein in prenatal diagnosis. Birth Defects 1977; 13: 191.

11. Macri JN, Haddow JE, Weiss RR. Screening for neural tube defects in the United States: A summary of the Scarborough Conference. Am J Obstet Gynecol 1979; 133: 119.

12. Wald NJ, Cuckle H, Brock JH, Peto R, Polani PE, Woodford FP. Maternal serum alpha-fetoprotein measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Report of UK Collaborative Study on Alpha-fetoprotein in Relation to Neural Tube Defects. Lancet 1977; 1(8026): 1323.

13. Amniotic fluid alpha-fetoprotein measurement in antenatal diagnosis of anencephaly and open spina bifida in early pregnancy. Second Report of the UK Collaborative Study on Alpha-fetoprotein in Relation to Neural Tube Defects. Lancet 1979; 2(8144): 651.