## 5.3 Proposed Product Literature

### 5.3.1. Product Labeling

# Access®

Immunoassay system

# AFP

**33211**
**300 Test Kit**

## 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.

**Caution: For U.S.A. only, Federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted to by or on the order of a physician.**

**The concentrations 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 AFP assay used. Values obtained with different assay methods cannot be used interchangeably. If, in the course of monitoring a patient, the assay method used for determining AFP levels serially is changed, additional sequential testing should be carried out to confirm baseline values. Prior to changing assays, the laboratory must: 1) for Cancer Management - Confirm baseline values for patients being serially monitored; 2) 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.**

## WARNING

Increased maternal serum AFP levels may also occur with multiple fetuses, low birth weight, fetal demise, and incorrect estimation of gestational age. Diagnostic ultrasonography can aid in defining the course of further clinical evaluations by determining the correct gestational age, the presence of multiple fetuses, ONTD, or other pregnancy problems.

Elevated AFP levels in amniotic fluid can result from ONTD and also from other fetal abnormalities such as congenital nephrosis, omphalocele, Turner's syndrome, gastroschisis, threatened abortion, or fetal demise (3,27). Falsely elevated amniotic fluid AFP levels may be caused by contamination of the fluid with fetal blood (3,27,28). Maternal blood contamination may falsely decrease AFP levels by dilution of the sample. Refer to LIMITATIONS OF PROCEDURE. In the absence of fetal blood contamination, an elevated amniotic fluid AFP level strongly suggests fetal abnormality or complication. Further testing is required to confirm the diagnosis of ONTD.

## SUMMARY AND EXPLANATION

Alpha-fetoprotein (AFP) is a single-chain glycoprotein with a molecular mass of approximately 70,000 daltons (1). AFP is highly similar to albumin, and together, both proteins constitute the two major proteins in fetal circulation. Production of AFP occurs primarily in the fetal liver and yolk sac, and to a lesser degree in other organs (2). AFP is first detected in the fetal circulation approximately 30 days after conception (3). After reaching a peak concentration at 12-15 weeks gestation, levels gradually diminish until birth. By 2 years of age, only trace levels of AFP can be detected in normal individuals (4). Elevated AFP levels reappear in adults in certain malignant diseases and pregnancy.

### Malignant Disease

Tatarinov was the first to identify AFP as a tumor-associated protein (5). Subsequent studies confirmed the finding of elevated AFP levels in primary hepatic carcinoma and extended this observation to other malignancies as well, most importantly non-seminomatous testicular carcinoma (6,7,8,9,10). The finding of elevated levels of AFP in non-seminomatous testicular carcinoma greatly facilitated the differential diagnosis of germ cell tumors, since pure seminoma is not associated with elevated AFP levels (6, 9,11). Changing AFP levels have assisted in the prognosis and management of patients with non-seminomatous testicular carcinoma. For example, AFP, in conjunction with human chorionic gonadotropin (hCG) has served as an important prognosticator of survival in patients with non-seminomatous testicular carcinoma (12,13). Additionally, decreasing levels following therapy generally indicate successful intervention, whereas rising levels following therapy usually indicate residual tumor or recurrence (14,15).

Elevated AFP levels have also been found in association with ataxia telangiectasia, hereditary tyrosinemia, neonatal hyperbilirubinemia, acute and chronic viral hepatitis, cirrhosis, and other malignancies (16,17,18,19,20). Therefore, AFP is not recommended as a screening tool for cancer detection in the general population.

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**Prenatal Testing**

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 (21). In that same year, Brock and Sutcliffe reported the association between increased amounts of amniotic fluid AFP and neural tube defect pregnancies (22). The following year Brock, et al. demonstrated that maternal serum levels were also elevated under these conditions (23).

Neural tube defects result from a failure in the closure of the developing fetal nervous system within the first month of pregnancy. The opening in the fetal neural tube allows AFP in the fetal circulation to leak across the defect causing higher than normal levels of AFP in amniotic fluid and maternal serum. Women carrying fetuses with closed (skin-covered) neural tube defects generally have serum and amniotic fluid AFP levels within normal limits. In these cases, the AFP in the fetal circulation fails to leak across the defect. Closed neural tube defects occur in a small number, approximately 5%, of fetuses affected with neural tube defects (24).

Open neural tube defects (ONTD) are among the most common and serious congenital malformations affecting approximately 1 to 2 newborns per 1000 live births in the United States. Anencephaly and spina bifida each constitute approximately half of all ONTD. Approximately 90% of affected fetuses occur in families with no previous history of ONTD. A family with an ONTD child faces a recurrence risk of approximately 2% (24). Two major studies have demonstrated the overall reliability of AFP testing for the prenatal detection of ONTD; the first in 1977 addressed AFP maternal serum testing (25) and the second in 1979 addressed amniotic fluid AFP testing (26).

## PRINCIPLES OF THE PROCEDURE

The Access AFP assay is a two-site immunoenzymatic ("sandwich") assay. A sample is added to a reaction vessel with mouse monoclonal anti-AFP-alkaline phosphatase conjugate, and paramagnetic particles coated with a second mouse monoclonal anti-AFP antibody. The AFP in the sample binds to the immobilized monoclonal anti-AFP on the solid phase while, at the same time, the monoclonal anti-AFP-alkaline phosphatase conjugate reacts with different antigenic sites on the sample AFP. 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 reaction is measured with a luminometer. The light production is directly proportional to the amount of AFP in the sample. The amount of analyte in the sample is determined by means of a stored, multi-point calibration curve.

## PRODUCT INFORMATION

**Cat. No. 33211:** 300 determinations, 50 tests/pack; 1 set of seven calibrators

- Provided ready to use.
- Store upright at 2 to 10°C.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Packs must be refrigerated at 2 to 10°C for two hours before loading or storing in the instrument.
- After initial use, pack is stable for 28 days at 2 to 10°C.
- Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.
- Mix calibrators well by gently inverting before use. Avoid bubble formation.

**AFP Reagent Packs: 6 packs**

**R1a:** Paramagnetic particles coated with mouse monoclonal anti-AFP antibodies suspended in Tris buffered saline, with surfactant, bovine serum albumin (BSA), < 0.1% sodium azide, and 0.1% ProClin** 300.

**R1b:** Mouse monoclonal anti-AFP alkaline phosphatase (bovine) conjugate diluted in phosphate buffered saline, with surfactant, BSA, proteins (goat, rabbit, mouse), < 0.1% sodium azide, and 0.25% ProClin 300.

**AFP Calibrators: 2.5 ml/vial**

**S0:** Buffered bovine serum albumin (BSA) matrix with surfactant, < 0.1% sodium azide, and 0.1% ProClin 300. Contains 0.0 ng/ml AFP.

**S1, S2, S3, S4, S5, S6:** AFP at levels of approximately 2.5, 5, 25, 100, 500 and 3000 ng/ml, respectively, in buffered BSA matrix with surfactant, < 0.1% sodium azide, and 0.1% ProClin 300.

Refer to vial labels for exact concentrations.

- **Calibration Card: 1**

## WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
2. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up (29).
3. Xi. Irritant: 0.25% ProClin 300.

   R 43: May cause sensitization by skin contact.

   S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.
4. Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines (30).

5. Human source material from human placental cord serum. Human source material used in the preparation of the calibrators has been tested and found non-reactive for hepatitis B surface antigen (HBsAg), antibodies to hepatitis C virus (HCV Ab), and antibodies to human immunodeficiency virus (HIV-1 and HIV-2 Ab). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease (30).
6. The Access AFP reagents and calibrators are packaged as a matched set. **DO NOT** mix materials from different kit lot numbers.

## SPECIMEN COLLECTION AND PREPARATION

1. Serum and amniotic fluid are the recommended sample types.
2. Maternal serum and amniotic fluid samples should be obtained between 15 and 20 weeks of gestation. Valid measurements of AFP in maternal serum CANNOT be made after amniocentesis. Maternal serum samples **MUST** be drawn PRIOR to amniocentesis.
3. Observe the following recommendations for handling, processing, and storing blood samples (31,32):
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow samples to clot adequately before centrifugation.
   - Keep tubes stoppered at all times.
   - Within two hours after centrifugation, transfer at least 500 µl of cell-free serum to a tightly stoppered storage tube.
   - Store sample, tightly stoppered, at room temperature (15 to 30°C) for no longer than eight hours.
   - If the assay will not be completed within eight hours, refrigerate the serum at 2 to 8°C.
   - If the assay will not be completed within 48 hours, or for shipment of samples, freeze at –20°C.
   - Thaw samples only once.
4. Observe the following recommendations for handling, processing and storing amniotic fluid samples:
   - Centrifuge at 1800 rcf or greater in a refrigerated centrifuge for 20 minutes.
   - Remove the supernatant for testing.
   - Centrifugation and removal of the supernatant should be done immediately upon receipt of the sample.
   - Retain the cell pellet from the amniotic fluid sample until the AFP concentration in the amniotic fluid is determined and further testing is not required.
   - Store the sample at 2 to 8° C if performing the test within 48 hours.
   - If a longer time will elapse before performance of the test, freeze the sample at –20° C or colder.
   - Avoid repeated freezing and thawing of the sample.
   - Dilute amniotic fluid samples 1 volume with 10 volumes with Access AFP Sample Diluent (Cat. No. 33216). Multiply the obtained value by 11 to determine the amniotic fluid AFP concentration of the sample. If after diluting, an amniotic sample still measures > 3000 ng/ml, further dilution is required. Re-dilute the already diluted sample as needed to bring the sample value within the calibration curve and calculate appropriately.

## PROCEDURE

**Materials Provided**

R1 AFP Reagent Packs and
S0-S6 AFP Calibrators

**Materials Required But Not Provided**

1. Access AFP Sample Diluent (14 ml)
   Buffered bovine serum albumin (BSA) matrix with surfactant, < 0.1% sodium azide, and 0.1% ProClin 300. Contains 0.0 ng/ml AFP.
   Cat. No. 33216
2. Access Substrate
   Cat. No. 81906
3. Access Wash Buffer
   Cat. No. 81907
4. Access AFP QC (or other commercial control material)
   Cat. No. 33219
5. Access AFP Physician Brochure
   Cat. No. XXXX
6. Access AFP Patient Report Form
   Cat. No. XXXX
7. Access AFP Patient Brochure
   Cat. No. XXXX
   Additional Physician Brochures, Patient Report Forms and Patient Brochures (for prenatal testing only) are available at no charge from Beckman Coulter, Inc. To order, call Beckman Coulter Technical Support at **1-800-666-8121.**

**Procedural Comments**

1. Refer to the Access Immunoassay System Operator's Guide and/or Reference Manual for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Mix contents by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs – mix reagents by swirling gently.
3. Ten (10) µl of sample is used for each determination in addition to the sample cup or sample tube dead volume. Refer to the sample container section of the Operator's Guide for the minimum sample volume required.
4. Sample results are reported in ng/ml. However, the system can report results in International Units (IU/ml). Refer to the System Configuration chapter of the Reference Manual for detailed instructions. To manually convert concentrations units, multiply ng/ml by 0.826.

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Procedure

1. Access the Test Request screen from the Main Menu.
2. For each sample, assign a tray position, enter sample information, and specify which tests to perform.
3. Place patient sample tube in sample tray, or transfer sample into a sample cup and place in designated position in sample tray.
4. Press the Run Key (F11) to initiate processing.
5. The analyzer will prompt the operator to perform any necessary calibrations.
6. The system will calculate test results.
7. Amniotic fluid samples can be reported in µg/ml by changing the test units in the Access Software. Go to the System Configuration screen then press [F3] Tests. Highlight AFP test and press [F5] Configure Units. For sample type [Other], use space bar to select units [µg/ml]. Return to Main menu. For more details refer to the Access Immunoassay System Operators Manual.
8. To test request an amniotic fluid sample, select the sample type [Other] and enter the dilution factor [Dil Fact] of 11 (or greater if further dilutions were required) to obtain the automatically calculated AFP value.

## DETAILS OF CALIBRATION

An active calibration curve is required for all tests. The AFP Calibrators are provided at seven levels – zero, and approximately 2.5, 5, 25, 100, 500, and 3000 ng/ml – prepared gravimetrically from human AFP and buffered BSA matrix, referenced to the WHO 1st International Standard 72/225.

For the AFP assay, calibration is required every 28 days to maintain an active calibration curve. Refer to the Operator's Guide and Reference Manual for complete instructions on calibration procedures. The Operator's Guide describes interim recalibration requirements and system prompts needed to select calibration.

## QUALITY CONTROL

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a "random access" format rather than a "batch" format, control materials should be included in each 24-hour time period (33). Include Access AFP QC tri-level controls (Cat. No. 33219), or other commercially available control materials that cover at least three levels of analyte. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish a mean value and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since the last acceptable quality control test point for this analyte. Refer to the Operator's Guide for information about reviewing control results.

## RESULTS

Patient test results are determined automatically by the system software using a four-parameter logistic curve math model. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration curve. Patient test results can be reviewed using the Sample Results screen. Refer to the Operator's Guide for complete instructions on reviewing results.

## LIMITATIONS OF THE PROCEDURE

1. Samples can be accurately measured within the reportable range of the lower limit of detection and the highest calibrator value approximately 0.50 to 3000 ng/ml (0.41 to 2478 IU/ml)). If a serum sample from a patient with testicular cancer contains more than the stated value of the highest Access AFP calibrator (S6), report the result as > 3000 ng/ml (> 2478 IU/ml). Alternatively, dilute 1 volume of sample with 100 volumes of AFP Sample Diluent (Cat. No. 33216). After assaying the diluted sample, multiply the obtained value by the dilution factor 101. Initially pre-dilute all amniotic fluids, 1 volume sample with 10 volumes diluent, and multiply to obtained value by the dilution factor of 11. If after diluting, an amniotic sample still measures > 3000 ng/ml, further dilution is required. Re-dilute the diluted sample as needed to bring the sample value within the calibration curve and calculate appropriately. Refer to the Operator's Guide for detailed instructions on processing pre-diluted samples. If a sample contains less than the lower limit of detection for the assay, report the results as < 0.50 ng/ml (< 0.41 IU/ml).
2. The Access AFP assay is of value as an aid in the management of patients with non-seminomatous testicular cancer when the results are interpreted in conjunction with the patient's clinical presentation and other diagnostic procedures. Elevated levels of AFP may occur in non-neoplastic conditions including ataxia telangiectasia, hereditary tyrosinemia, nonmalignant hepatic disease (such as acute viral hepatitis, chronic active hepatitis and cirrhosis) and pregnancy. Not all teratocarcinomas of germ cell origin produce AFP. Therefore, the Access AFP assay is not intended for the diagnosis of, or for screening for testicular cancer.
3. Valid measurements of AFP in maternal serum CANNOT be made after amniocentesis. Maternal serum samples MUST be drawn PRIOR to amniocentesis.
4. A reliable AFP evaluation for prenatal testing requires precise determination of the gestational age. Underestimation of the gestational age may lead to a false positive determination, while over estimation of gestational age may result in a false negative interpretation. When gestational age is uncertain, confirmation with ultrasonography is indicated. All samples for prenatal testing should be collected between 15 and 20 weeks gestation.
5. Bloody amniotic fluid samples that have an elevated AFP concentration MUST be tested to determine whether the source of the blood is maternal or fetal. Contamination of amniotic fluid with maternal blood may reflect accurate AFP levels as long as the amount of maternal blood is not sufficient to dilute the amniotic fluid sample. Specimens contaminated with fetal blood may be artificially elevated. False elevations of AFP due to fetal blood contamination can be determined by testing the amniotic fluid sample for fetal hemoglobin (FHb) using the Kleihauer-Betke FHb test, electrophoresis or other appropriate tests.

6. An elevated maternal serum AFP alone is not diagnostic of ONTD, additional clinical factors should be considered. Other conditions that may result in an elevated maternal serum AFP are: miscalculated gestational age, multiple births, fetal death or distress, other fetal malformations and maternal liver disease. Elevated maternal serum AFP values have also been reported in normal viable pregnancies, therefore, confirmatory tests such as amniocentesis, sonography and amniotic fluid acetylcholinesterase are often indicated.
7. Human anti-mouse antibodies (HAMA) may be present in samples from patients who have received immunotherapy utilizing monoclonal antibodies (34). Additionally, heterophile antibodies capable of binding to mouse or other immunoglobulins may be present in patient samples (35). This assay has been specifically formulated to minimize the effects of these antibodies on the assay. However, carefully evaluate results from patients known to have such antibodies.
8. The Access AFP assay has no discernible "hook effect" at 500,000 ng/ml (413,000 IU/ml).

## EXPECTED VALUES

**Cancer**

1. Each laboratory should establish its own reference ranges to assure proper representation of specific populations.
2. The AFP level was measured, using the Access AFP assay, in 1126 serum samples from apparently healthy male and female (non-pregnant) subjects, and patients with known benign and malignant diseases. In this study 98.9% of healthy adults had AFP concentrations less than 9.0 ng/ml (7.4 IU/ml). The distribution of AFP values in each clinical category is listed in the following table:

| Clinical Category | n | 0–9.0 ng/ml | 9.1–100 ng/ml | 101–300 ng/ml | 301–1000 ng/ml | > 1000 ng/ml |
|---|---|---|---|---|---|---|
| Apparently Healthy | 177 | 98.9% | 1.1% | 0.0% | 0.0% | 0.0% |
| Testicular Carcinoma | | | | | | |
| Non-seminomatous | 120 | 57.5% | 25.8% | 3.3% | 5.9% | 7.5% |
| Seminomatous | 24 | 95.8% | 4.2% | 0.0% | 0.0% | 0.0% |
| Hepatocellular Carcinoma | 259 | 22.0% | 38.2% | 13.5% | 9.7% | 16.6% |
| Other GI Malignancies* | 75 | 89.3% | 6.7% | 0.0% | 1.3% | 2.7% |
| Liver Cirrhosis | 88 | 37.5% | 48.9% | 6.8% | 3.4% | 3.4% |
| Hepatitis | 383 | 63.7% | 32.1% | 2.6% | 1.0% | 0.5% |

*category includes non-hepatocellular hepatomas, colorectal, gastric, esophageal, bile duct and pancreatic carcinomas.

**Prenatal Testing**

1. The presence of neural tube defects in the United States among Caucasians is higher than in Blacks. Prevalence also varies geographically. Each laboratory should establish it's own normal range for each gestational week from confirmed unaffected singleton pregnancies. At least 100 maternal sera and 50 amniotic fluids at each week should be assayed to determine the range.
2. Expected ranges for maternal serum and amniotic fluid AFP values were determined using the ACCESS Immunoassay System. Median values were calculated for gestational weeks 15 to 20. Regressed median values were determined using a weighted log linear regression. All samples had confirmed unaffected, singleton pregnancy outcomes. The maternal serum medians were comprised of 2539 specimens obtained from three clinical trial sites. Amniotic fluid medians were comprised of 720 specimens obtained from three clinical sites. Multiples (2.0, 2.5, 3.0) of each median (MoM) are also shown.

**MATERNAL SERUM AFP**

| Gestational Week* | Number of Samples | Median Concentration (ng/ml) | Multiples of Median Concentration (ng/ml) | | |
|---|---|---|---|---|---|
| | | | 2.0 | 2.5 | 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 |

**AMNIOTIC FLUID AFP**

| Gestational Week* | Number of Samples | Median Concentration (µg/ml) | Multiples of Median Concentration (µg/ml) | | |
|---|---|---|---|---|---|
| | | | 2.0 | 2.5 | 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 |

* AFP values have been determined using COMPLETED gestational weeks

3. Clinical Specificity and Sensitivity. The following tables summarize the specificity and sensitivity estimates (and associated 95% confidence intervals) of the ACCESS AFP Immunoassay for maternal serum and amniotic fluid at various multiples of the median (MoM): As defined here, specificity is the probability that the test will be negative in the absence of disease and sensitivity is the probability that the test will be positive in the presence of an ONTD. The specificity table represents data gathered on unaffected singleton pregnancies from 15 – 20 weeks gestation using the ACCESS AFP Immunoassay.

**SPECIFICITY**

| | | Multiples of the Median (MoM) | | |
|---|---|---|---|---|
| Sample Type | Number of Samples | ≥ 2.0 | ≥ 2.5 | ≥ 3.0 |
| Maternal Serum (95% CI) | 2539 | 95.4% (94.4% – 96.1%) | 98.3% (97.7% – 98.7%) | 99.3% (98.9% – 99.6%) |
| Amniotic Fluid (95% CI) | 720 | 97.5% (96.0% – 98.5%) | 98.8% (97.6% – 99.4%) | 99.3% (98.3% – 99.7%) |

**SENSITIVITY**

| | | Multiples of the Median (MoM) | | |
|---|---|---|---|---|
| Sample Type | Number of Samples | ≥ 2.0 | ≥ 2.5 | ≥ 3.0 |
| Maternal Serum (95% CI) | 23 | 91.3% (70.5%– 98.5%) | 73.9% (51.3% – 88.9%) | 69.6% (47.0% – 85.9%) |
| Amniotic Fluid (95% CI) | 15 | 100.0% N/A | 100.0% N/A | 100.0% N/A |

## SPECIFIC PERFORMANCE CHARACTERISTICS

**Accuracy**

**Correlation**

Comparison of serum AFP values using the Access AFP assay and a commercially available immunoassay gives the following statistical data by adjusted least squares regression analysis:

| Sample Type | n | Range of Observations ng/ml | Intercept ng/ml | Slope | Correlation coefficient |
|---|---|---|---|---|---|
| Cancer Serum | 170 | 0.80–2277.84 | 3.86 | 0.91 | 0.988 |
| Maternal Serum | 437 | 3.06-268.56 | 3.59 | 0.86 | 0.989 |
| | | µg/ml | µg/ml | | |
| Amniotic Fluid | 307 | 1.38-32.96 | 0.38 | 0.85 | 0.966 |

**Spiking Recovery**

Recovery was assessed by adding purified AFP into human maternal and non-maternal serum samples and assaying the samples before and after the addition of the exogenous AFP.

| **Non-maternal serum** AFP Added (ng/ml) | Expected Concentration (ng/ml) | Observed Concentration (ng/ml) | Recovery (%) |
|---|---|---|---|
| 0.00 | N/A | 2.05 | N/A |
| 10.00 | 12.05 | 12.52 | 104 |
| 100.00 | 102.05 | 103.15 | 101 |
| 1000.00 | 1002.05 | 1022.10 | 102 |
| 2500.00 | 2502.05 | 2688.87 | 107 |
| **Maternal serum** AFP Added (ng/ml) | Expected Concentration (ng/ml) | Observed Concentration (ng/ml) | Recovery (%) |
| 0.00 | N/A | 4.77 | N/A |
| 60.00 | 64.77 | 68.86 | 106 |
| 120.00 | 124.77 | 128.70 | 103 |
| 240.00 | 244.39 | 255.90 | 105 |

**Dilution Recovery (Linearity)**

Two samples, 1 cancer patient serum and 1 maternal serum containing elevated levels of AFP, were diluted with Access AFP Sample Diluent. The results of these studies are as follows:

| Sample 1 Cancer Serum | Expected Concentration (ng/ml) | Observed Concentration (ng/ml) | Recovery (%) |
|---|---|---|---|
| Neat | N/A | 178.82 | N/A |
| 8/10 | 143.05 | 145.11 | 101 |
| 6/10 | 107.29 | 110.14 | 103 |
| 4/10 | 71.53 | 78.78 | 110 |
| 2/10 | 35.76 | 37.48 | 105 |
| 1/100 | 1.79 | 1.94 | 109 |

| Sample 2 Maternal Serum | Expected Concentration (ng/ml) | Observed Concentration (ng/ml) | Recovery (%) |
|---|---|---|---|
| Neat | N/A | 446.55 | N/A |
| 8/10 | 357.25 | 362.78 | 102 |
| 6/10 | 267.95 | 272.05 | 102 |
| 4/10 | 178.64 | 183.78 | 103 |
| 2/10 | 89.34 | 93.07 | 104 |
| 1/10 | 44.69 | 46.17 | 103 |

**Precision**

Reproducibility was determined for three levels of serum-based controls by performing triplicate measurements in 2 assays per day for 20 days. The data were analyzed via analysis of variance (ANOVA) (36,37) and are as follows:

| Serum-based Control | N | Mean (ng/ml) | Within Run (% CV) | Between Run (% CV) | Total Imprecision (% CV) |
|---|---|---|---|---|---|
| 1 | 120 | 6.53 | 3.22 | 3.22 | 4.44 |
| 2 | 120 | 72.10 | 2.88 | 2.04 | 3.54 |
| 3 | 120 | 1672.88 | 2.71 | 2.07 | 3.41 |

**Analytical Specificity/Interferences**

No significant interference was observed for the Access AFP assay with the following substances in the presence or absence of AFP.

| Substance | Amount added | Substance | Amount added |
|---|---|---|---|
| Acetaminophen | 1500 µg/ml | hFSH | 2 IU/ml |
| Acetylsalicylic acid | 10 mg/ml | hLH | 2 IU/ml |
| Alpha-1 acid glycoprotein | 4.54 mg/ml | hTSH | 6 µg/ml |
| Alpha-1 anti-trypsin | 14.8 mg/ml | Human placental lactogen | 100 µg/ml |
| Ascorbic acid | 1000 µg/ml | Lipemia | 520 mg/dl |
| Bleomycin | 100 µU/ml | Phenacetin | 500 µg/ml |
| Bilirubin | 25 mg/dl | Phenothiazine | 150 µg/ml |
| CEA | 375 µg/ml | Reserpine | 100 µg/ml |
| Chlorothiozide | 1000 µg/ml | Retinoic acid | 500 µg/ml |
| Cisplatin | 1000 µg/ml | Rheumatoid factor | 600 IU/ml |
| Cobalamine | 500 µg/ml | Riboflavin | 50 µg/ml |
| Diazepan | 50 µg/ml | Serum Albumin (BSA) | 6 mg/ml |
| Ethanol | 1.90 % | Spironolactone | 15 µg/ml |
| Fetal hemoglobin | 500 µg/ml | Thiamine | 50 µg/ml |
| Haptoglobin | 20.0 mg/ml | Transferrin | 23.7 mg/ml |
| hCG | 200 µg/ml | Vinblastine | 500 µg/ml |
| Hemoglobin | 1.2 g/dl | | |

**Analytical Sensitivity**

The lowest detectable level of AFP distinguishable from zero (Access AFP Calibrator S0) with 95% confidence is 0.50 ng/ml. This value is determined by processing a complete seven-point calibrator curve along with controls and twenty-five replicates of the zero calibrator. The analytical sensitivity value is interpolated from the curve at the point that is two standard deviations from the average measured zero analytical calibrator signal.

# AFP SAMPLE DILUENT 33216

## INTENDED USE

The Access AFP Diluent (BSA based) is intended for use with the Access AFP assay to dilute patient samples containing AFP concentrations greater than the S6 AFP calibrator.

## SUMMARY AND EXPLANATION

Alpha-fetoprotein (AFP) levels in patient samples may exceed the levels of the Access AFP calibrator S6. If a quantitative value is required, it will be necessary to dilute the sample in order to determine the AFP concentration. All amniotic fluids should be pre-diluted before assaying.

## PRODUCT INFORMATION

**Cat. No. 33216:**
- Provided ready to use.
- Avoid bubble formation.
  Stable to the expiration date stated on the label when stored at 2 to 10°C.

**Access AFP Diluent: 14 ml/vial. Liquid**
Tris buffered bovine serum albumin (BSA) matrix with surfactant, <0.1% sodium azide and 0.1% ProClin 300. Contains 0.0 ng/ml AFP.

## WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
2. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up (29).
3. Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines (30).

## PROCEDURE

Samples can be accurately measured within the reportable range of the lower limit of detection and the highest calibrator value (approximately 0.50 to 3000 ng/ml (0.41 to 2478 IU/ml). If a serum sample from a patient with testicular cancer contains more AFP than the stated value of the S6 calibrator, dilute one volume of sample with 100 volumes of Access AFP Sample Diluent. After assaying the diluted sample, multiply the value by the dilution factor of 101.

**All amniotic fluid samples require a pre-dilution.** Dilute 1 volume of amniotic fluid with 10 volumes of Access AFP Sample Diluent. After assaying the diluted sample, multiply the obtained value by the dilution factor of 11. Alternatively, the Access Analyzer can be configured to automatically calculate the result of the pre-diluted sample. To obtain the automatically calculated result, in the Test Request screen, select the sample type of [Other] and enter the dilution factor [Dil Fact] of 11. If after diluting, an amniotic sample still measures > 3000 ng/ml, further dilution is required. Re-dilute the already diluted sample as needed to bring the sample value within the calibration curve and calculate appropriately. For more details refer to the Access Immunoassay System Operators Guide.



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### 5.3.2. Physician Brochure

# Access®
immunoassay system
# AFP

**Cat. No.: XXX (Physician Brochure)**

## Information on Alpha-fetoprotein Testing

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. Therefore, values obtained with different AFP assay methods cannot be used interchangeably. The results reported by the laboratory to the physician must include the identity of the AFP assay used. Prior to changing assays, the laboratory must establish a range of normal values for the new assay, based on normal sera and amniotic fluids from pregnant women with confirmed gestational age.

### INTRODUCTION

Alpha fetoprotein (AFP) is a fetal specific protein produced mainly in the fetal liver and secreted into the fetal serum. Fetal serum concentrations of AFP peak at the end of the first trimester, then gradually decrease during later gestation. The presence of AFP in the amniotic fluid is due to fetal micturition. AFP reaches the maternal circulation via the placenta or by diffusion across the fetal membranes. Measurable concentrations appear in the maternal serum at the end of the first trimester reaching a maximum level during the second trimester.

Neural tube defects result from a failure in the closure of the developing fetal nervous system within the first month of pregnancy. In open neural tube defects (ONTD) the opening in the fetal neural tube allows AFP in the fetal circulation to leak across the defect causing higher than normal levels of AFP in amniotic fluid and maternal serum. Because of the ease in obtaining a maternal blood sample, the first step in testing for ONTD is to measure levels of AFP in maternal serum.

Women carrying fetuses with closed (skin-covered) neural tube defects generally have serum and amniotic fluid AFP levels within normal limits. In these cases, the AFP in the fetal circulation fails to leak across the defect. Closed neural tube defects occur in a small number (approximately 5%) of fetuses affected by neural tube defects (1).

ONTD are among the most common and serious congenital malformations affecting approximately 1 to 2 newborns per 1000 births in the United States. Greater than 90% of affected fetuses occur in families with no previous history of ONTD. A family that has a previous child with an ONTD faces a recurrence risk of approximately 2% (1).

Two of the more serious ONTD are anencephaly and spina bifida. Newborn infants with anencephaly fail to survive the early neonatal period. With surgery, about 70% of spina bifida newborns survive 5 years or more (2). These patients can demonstrate such problems as 1) paralysis or weakness of the lower limbs, 2) loss of skin sensation below the defect, 3) failure of bowel and bladder, and 4) hydrocephalus (in approximately 70% of the patients) (3). Infants with closed defects are often less handicapped with a relatively good prognosis as compared to those with open defects (4).

### TESTING PROCEDURE

When testing for ONTD, the optimal time for collecting maternal serum to evaluate AFP levels is between 16 and 18 weeks of gestation. Precise gestational age (as calculated from the first day of the last menstrual period) is necessary to accurately evaluate AFP levels. An error in estimating gestational age can lead to misinterpretation of AFP levels.

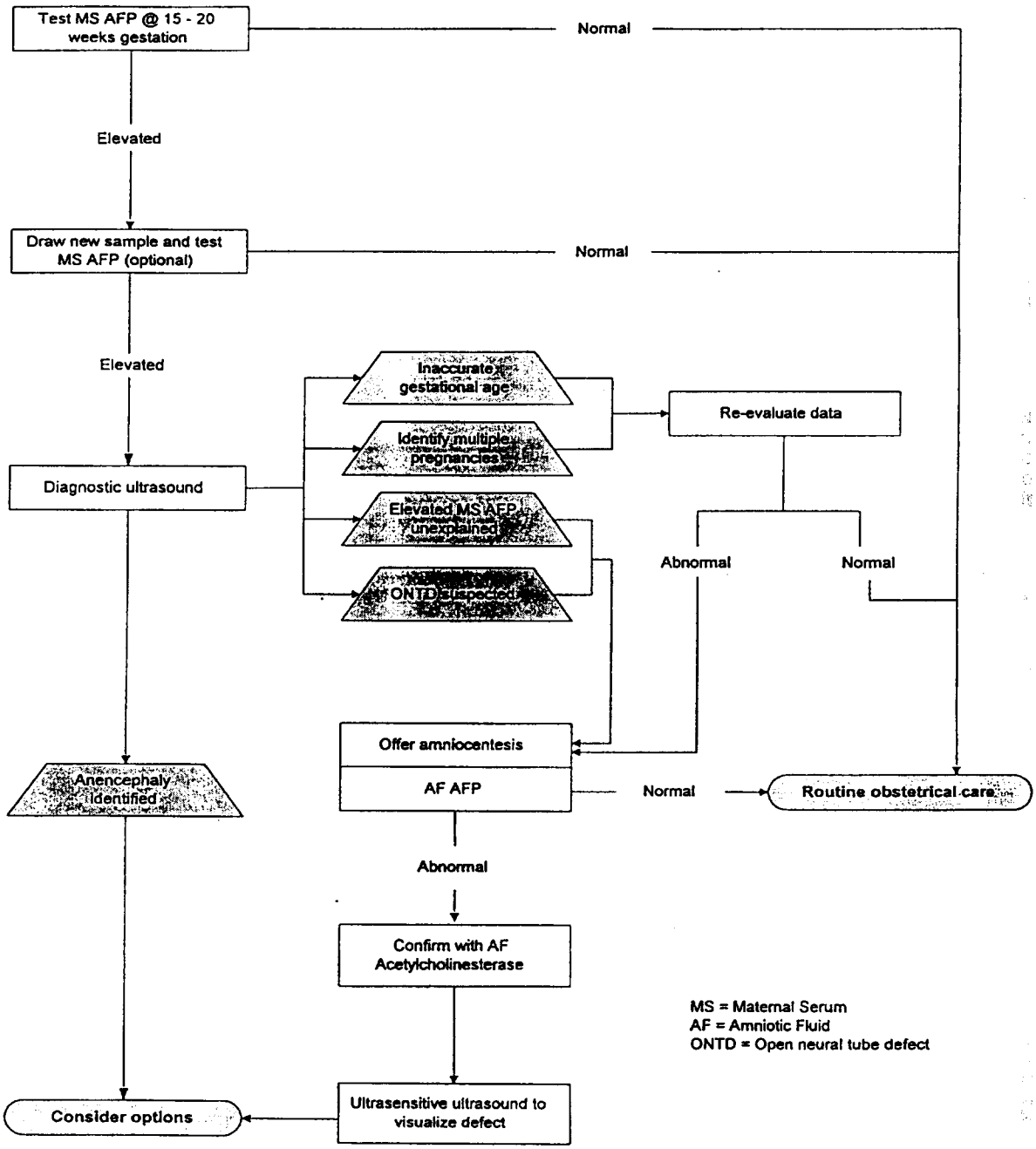
An elevated maternal serum AFP may be confirmed by repeating the test on a second serum sample drawn at least 1 week later. An elevated AFP level in the second sample indicates the need for follow-up procedures. An elevated maternal serum AFP level can be an indirect indication of the possibility of an ONTD in the fetus. Increased maternal serum AFP levels may also occur with multiple fetuses, low birth weight, fetal death or distress, maternal liver disease and incorrect estimation of gestational age.

Diagnostic ultrasound can aid in defining the course of further clinical evaluations by determining the correct gestational age, the presence of multiple fetuses, ONTD, or other pregnancy problems. Women with pregnancies at risk for recurring ONTD should be offered amniocentesis and biochemical evaluation despite normal ultrasound evaluation.

When ultrasound rules out incorrect gestational age or multiple births, the next step is amniocentesis and the determination of amniotic fluid AFP. Fetal blood contamination of amniotic fluid can falsely elevate AFP levels. Maternal blood contamination can falsely decrease AFP levels by dilution of the sample.

Specific tests are available in the laboratory to determine if bloody amniotic fluid samples contain fetal or maternal blood. Elevated levels of amniotic fluid AFP in the absence of fetal blood contamination can indicate an ONTD. Increased amniotic fluid AFP can also result from other fetal abnormalities such as congenital nephrosis, omphalocele, Turner's syndrome, gastroschisis, threatened abortion, or fetal death (5). Amniotic fluid acetylcholinesterase is another laboratory test available to confirm ONTD and rule out the potential for false positive results (6).

**Recommended ONTD Testing Protocol**



### SAMPLE COLLECTION

Serum and amniotic fluid are the recommended sample types. There are no special collection procedures for determination of AFP in serum samples. Collect blood samples observing routine precautions for venipuncture, avoiding hemolysis. Allow samples to clot and separate serum by centrifugation. **Maternal serum samples must be collected prior to amniocentesis to prevent contamination by fetal blood and falsely elevated results. Maternal serum and amniotic fluid samples should be obtained between 15 and 20 weeks of gestation.**

Amniotic fluid should be obtained by trained personnel using acceptable medical technique. If the sample shows any evidence of contamination with blood, the source of the blood must be determined using the Kleihauer-Betke fetal hemoglobin test or other appropriate method. The presence of fetal blood can cause a falsely elevated amniotic fluid AFP concentration and any such sample should not be used for evaluating the presence of fetal ONTD.

Both serum and amniotic samples may be stored refrigerated at 2-8° C for up to 48 hours. Specimens that are to be stored longer than 48 hours, or shipped, should be frozen at –20° C or colder.

### SPECIFICITY

The following table shows data gathered on unaffected singleton pregnancies using the ACCESS AFP Immunoassay. These data represent the specificity of maternal serum and amniotic fluid specimens using the upper limits of normal set at various multiples of the median (MoM). As defined here, specificity is the probability that the test will be negative in the absence of disease.

| | | Multiples of the Median (MoM) | | |
|---|---|---|---|---|
| | Number of Samples | ≥ 2.0 | ≥ 2.5 | ≥ 3.0 |
| **Maternal Serum** | 2539 | 95.4% | 98.3% | 99.3% |
| **Amniotic Fluid** | 720 | 97.5% | 98.8% | 99.3% |

### SENSITIVITY

The following table summarizes the sensitivity of the ACCESS AFP Immunoassay for maternal serum and amniotic fluid at various multiples of the median (MoM). As defined here, sensitivity is the probability that the test will be positive in the presence of an ONTD.

| | | Multiples of the Median (MoM) | | |
|---|---|---|---|---|
| | Number of Samples | ≥ 2.0 | ≥ 2.5 | ≥ 3.0 |
| **Maternal Serum** | 23 | 91.3% | 73.9% | 69.6% |
| **Amniotic Fluid** | 15 | 100.00% | 100.00% | 100.00% |

### GUIDE FOR INTERPRETING AFP RESULTS

The distribution of AFP levels in the normal population is non-symmetrical. When AFP levels have been studied, the number of samples higher than the 97th percentile is greater than one would statistically expect. The use of multiples of the median (MoM) to express AFP results minimizes the effect of this non-symmetry and allows better laboratory to laboratory comparison (7).

The suggested** median values for maternal serum using the ACCESS AFP Immunoassay are:

| Gestational Week* | Number of Samples | Median Concentration (ng/ml) | Multiples of the Median (MoM) Concentration (ng/ml) | | |
|---|---|---|---|---|---|
| | | | 2.0 | 2.5 | 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 suggested** median values for amniotic fluid using the ACCESS AFP Immunoassay are:

| Gestational Week* | Number of Samples | Median Concentration (µg/ml) | Multiples of the Median (MoM) Concentration (µg/ml) | | |
|---|---|---|---|---|---|
| | | | 2.0 | 2.5 | 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 |

* Gestational weeks were assigned using completed weeks of gestation.

****These suggested values are based on clinical studies using the ACCESS AFP Immunoassay and are intended only as a guideline. Prevalence of ONTD can vary by race and geographical regions. Each testing laboratory should collect data and develop its own normal ranges for each gestational week from confirmed normal pregnancies. Data from at least 100 maternal sera and 50 amniotic fluids at each of these weeks should be gathered to establish median values and cut-off values that will yield appropriate sensitivity and specificity.**

### RACIAL AND GEOGRAPHICAL FACTORS

The prevalence of ONTD in the United States among Caucasian births is higher than in Black births. For Caucasian births, the highest incidence of spina bifida occurs in the Southeast at a rate of 8 per 10,000 births. The rates of spina bifida for Black births are highest along the Atlantic Coast and South Central region with 3 per 10,000 births. A study of all geographical areas shows the overall rate of ONTD for both races decreases from the east to the west (8).

### PATIENT REPORT FORM

Beckman Coulter, Inc. provides a Patient Report Form (Cat. No XXX) for the testing laboratory to assist the physician in the interpretation of serum and amniotic fluid AFP results. The Patient Report Form includes AFP results expressed as the Median (ng/ml) and Multiples of the Median (MoM).

### PATIENT BROCHURE

As an aid to inform patients about AFP testing and ONTD, Patient Brochures are available from Beckman Coulter, Inc., (Cat. No. XXX). The physician should distribute copies of the Patient Brochure to all patients who are considering AFP testing.

**Note: Additional Physician Brochures, Patient Brochures and Patient Report Forms are available upon request from Beckman Coulter, Inc Technical Support by calling 1-800-666-8121.**

### REFERENCES

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### 5.3.3. Patient Report Form

# BECKMAN COULTER, INC

# PATIENT REPORT FORM FOR AFP TESTING

## Guidelines for Interpretation of Results

These charts show the normal values for each week of gestation for normal singleton pregnancies. Normal values are expressed as Multiples of the Median (MoM) for maternal serum and amniotic fluid. Compare the patient results to the Median values appropriate for the patient's completed weeks of gestation.

The suggested* median values for maternal serum using the ACCESS AFP Immunoassay are:

| Gestational Week* | Number of Samples | Median Concentration (ng/ml) | Multiples of the Median (MoM) Concentration (ng/ml) | | |
|---|---|---|---|---|---|
| | | | 2.0 | 2.5 | 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 suggested* median values for amniotic fluid using the ACCESS AFP Immunoassay are:

| Gestational Week* | Number of Samples | Median Concentration (µg/ml) | Multiples of the Median (MoM) Concentration (µg/ml) | | |
|---|---|---|---|---|---|
| | | | 2.0 | 2.5 | 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 |

*These suggested values are based on clinical studies using the ACCESS AFP Immunoassay and are intended as a guideline. Prevalence of ONTD varies by race and geographic regions. Each testing laboratory should collect data and develop its own normal ranges for each gestational week from confirmed normal pregnancies. Data from at least 100 maternal sera and 50 amniotic fluids at each of these weeks should be gathered to establish median values and cut-off values that will yield appropriate sensitivity and specificity.

For further information, refer to the Beckman Coulter, Inc. Physician's Brochure entitled Information on Alpha-Fetoprotein Testing. This brochure is available from Beckman Coulter, Inc., Cat. No. XXXX. Additional Patient Brochures and Patient Report forms are also available upon request from Beckman Coulter, Inc..

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Printed in USA

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Chaska, MN 55318

# PATIENT REPORT FORM FOR AFP TESTING

## To be completed by Physician

**Physician Name** _____ Date _____

Address _____

Telephone _____

**Patient Name** _____ Patient ID _____

Date of Birth _____

Weight _____

Height _____

Race _____

First Day of LMP _____

Gestational Age (Last completed week of pregnancy) _____

Determined by: o LMP o PE o Ultrasonography

Patient Type 1 Diabetic: o Yes o No

Type of Samples: o 1st Maternal Serum
o Repeat (2nd) Maternal serum
o Amniotic Fluid

Date Collected _____

Other Relevant Information _____

_____

## To be completed by Laboratory

Date Sample Received _____ Sample ID _____

Date Sample Tested _____

AFP Results:

1st Maternal Serum _____ng/ml Repeat (2nd) Maternal Serum _____ng/ml

_____MoM* _____MoM*

Amniotic Fluid _____µg/ml

_____MoM*

Other Tests:

Test _____

Interpretation _____

Comments: _____

*To calculate MoM (multiple of the median), divide the AFP result by the median AFP value for the appropriate gestational age.

### 5.3.4. Patient Brochure

# Access®

**immunoassay system**

# AFP

**Cat. No.: XXX**

**Patient Brochure for Alpha-Fetoprotein Testing**

## WHAT IS AFP TESTING?

Alpha-fetoprotein (al-fah-fee-toe-pro-teen) (AFP) test is a blood test that helps identify a small number of women whose unborn babies may have certain birth defects of the brain and spinal cord, called neural tube defects. It also offers to the remaining majority of women tested added assurance that their babies are not likely to have this type of birth defect.

## WHAT SHOULD I CONSIDER WHEN DECIDING WHETHER OR NOT TO HAVE AN AFP TEST?

*If you are thinking of having the AFP blood test, you should be aware that the test itself is not a guarantee. It is possible that neural tube defects may be missed (false negative result), and there is a very slight chance, even with extensive follow-up testing, that a healthy unborn baby may be incorrectly identified as having a neural tube defect (false positive result).* After reading this brochure, discuss the possibility of false positive or negative results with your doctor before you ask for an AFP test.

## WHAT ARE NEURAL TUBE DEFECTS?

Neural tube defects are defects of the central nervous system. This system includes the spinal cord and the brain and is derived from a structure in the developing unborn baby called the neural tube. As a baby develops in its mother, the neural tube normally closes completely within the first month of pregnancy. If all or part of the neural tube fails to close, leaving an opening, the baby has a neural tube defect. In some cases, the opening in the neural tube may be left exposed (open); in others, this defect may be covered with bone and skin (closed). Most closed neural tube defects are not detected by the AFP test.

## WHAT ARE SOME TYPES OF NEURAL TUBE DEFECTS?

Two common and serious types of neural tube defects are anencephaly (an-en-sef-a-lee) and spina bifida (spi-nah biff-i-da).

**Anencephaly.** In this disorder, much of the brain, head, and possibly the spinal cord do not develop normally. Newborn children with this severe disorder usually die shortly after birth.

**Spina Bifida.** This disorder is a defect of the spinal column which is also called "open spine." Normal development of a child with spina bifida is possible. Often, however, paralysis of the lower limbs, repeated urinary tract infections, hydrocephalus ("water on the brain"), mental retardation, and incontinence (inability to control bladder and bowel movements) occur.

The spinal malformation and nerve damage caused by this disorder hamper movement in varying degrees. Some individuals with spina bifida can walk by themselves. Others use braces and crutches or wheelchairs. Modern surgical and corrective techniques can help many children born with spina bifida lead healthy and productive lives.

The cause of neural tube defects is not known. The defects may be inherited; they may also be caused by environmental factors. More research must be conducted to find out the cause of these disorders. Recent studies have demonstrated that sufficient intake of folic acid (a B Vitamin) before conception and during early pregnancy can reduce the risk of having a child with an open neural tube defect. Therefore, the U.S. Department of Health and Human Services has recommended a minimum daily intake of 0.4 mg of folic acid for every women of childbearing age.

In the United States, about 1 to 2 in every 1000 live births involves a neural tube defect. About 90% of these births will be open neural tube defects, evenly split between anencephaly and spina bifida. Of the births that result in an open neural tube defect, approximately 90 to 95% of the babies with such a defect will be born to women who have no "special risk" characteristics. The remaining 5 to 10% of babies with open neural tube defects will be born to parents who have "special risk" characteristics such as:

- women who have already given birth to a child with a neural tube defect;
- one or both parents have neural tube defects; and
- women whose mothers or fathers have a history of neural tube defects.

## WHAT IS ALPHA-FETOPROTEIN(AFP)?

Alpha-fetoprotein(AFP) is a substance that is produced by the unborn baby as it grows. When the neural tube is not properly formed, large amounts of alpha-fetoprotein pass into the amniotic fluid and reach the mother's blood. By measuring AFP in the mother's blood and amniotic fluid (the fluid filling the sac around the unborn baby), it is possible to tell whether or not there is a chance that the unborn baby has an open neural tube defect.

## WHAT IS THE AFP BLOOD TEST?

The AFP blood test is a simple laboratory procedure. A sample of blood is taken from your arm and sent to a laboratory. The laboratory tests the blood to see how much AFP is present and then sends the results to your doctor.

If the result of the first blood test is elevated, then other tests are required before your doctor can determine whether your unborn baby has an open neural tube defect.

## WHEN SHOULD A PREGNANT WOMAN HAVE THE AFP BLOOD TEST?

The AFP blood test should be done between 15–20 weeks after the last menstrual period. The best time is 16–18 weeks. Unreliable results may be obtained if the blood sample is taken too early during the pregnancy.

## WHAT DO THE TEST RESULTS MEAN?

If your blood has normal levels of AFP, the test is considered negative and there is no need for further tests. Parents should understand that an AFP blood test result in the normal range cannot guarantee a normal baby at birth. The first AFP blood test may miss as many as 20% of those babies affected with neural tube defects. It can offer much greater assurance, however, that the baby is not likely to have an open neural tube defect.

An elevated result indicates that there are high levels of AFP in your blood. This does not always mean a neural tube defect is present. It means that you should have additional tests to see whether or not your unborn baby has an open neural tube defect.

If the first blood sample has an elevated level of AFP, your doctor may ask that a second sample of blood will be taken from your arm. If the result of the second test is also elevated, there is still only about a 4% to 10% chance that your unborn baby has an open neural tube defect. More likely, you may be carrying twins or your pregnancy may be further advanced than you think. For these reasons, the doctor will want to conduct additional tests.

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. Therefore, values obtained with different AFP assay methods cannot be used interchangeably.

## WHAT ARE OTHER TESTS THAT MAY BE NEEDED IF THE AFP BLOOD TEST IS ELEVATED?

**Sonography (so-nog-rah-fee) or Ultrasound.** This is a procedure in which sound waves are used to obtain a television-like picture of the unborn baby. The picture will enable your doctor to tell whether the blood test was elevated because of twins, or because the length of pregnancy was estimated incorrectly. Sonography can also show if an unborn baby has died, and often whether a neural tube defect is present. If sonography does not give a reason for the elevated AFP values, amniocentesis may be recommended by your doctor.

**Amniocentesis (am-nee-o-sen-tee-sis).** In this test, the doctor will take a sample of the amniotic fluid and send it to the laboratory to measure the AFP level in the fluid. If the AFP level is high and other possible causes (twins or an incorrect estimate of the length of pregnancy) have been ruled out, there is a high chance that the unborn baby has an open neural tube defect. This test cannot tell how severe the defect will be or the possible degree of the handicap.

Although relatively safe, amniocentesis presents a small risk. You may want to discuss the nature of the risk with your doctor.

**Some Other Tests.** If the level of AFP in the amniotic fluid is elevated, the doctor may wish to attempt to locate the possible defect by means of high resolution sonography (a more advanced and accurate type of ultrasound) or by amniography, in which the unborn baby is outlined by radio-opaque dye injected into the uterus. To further reduce the chance of a wrong diagnosis, a chemical test may be used in which amniotic fluid is analyzed for the presence of the enzyme acetylcholinesterase.

## COUNSELING

This test is being offered for those who want it. You are free to choose an AFP test or you may say that you do not want the test.

You should ask your doctor about these tests, the risks they present, how much they cost, where these tests are given, genetic counseling that is available, and other questions that may concern you. Your doctor has received a more detailed brochure about AFP testing and should be able to provide you with additional information.

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