Access
Immunoassay Systems

AFP
REF 33211 (300 TEST KIT)

Caution **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.[1,2] Falsely elevated amniotic fluid AFP levels may be caused by contamination of the fluid with fetal blood.[1,2,3] Maternal blood contamination may falsely decrease AFP levels by dilution of the sample. Refer to LIMITATIONS OF THE 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.

Intended Use The Access AFP assay is a paramagnetic particle, chemiluminescent immunoassay for use with the Access Immunoassay Systems 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). Test results, when used in conjunction with ultrasonography, are safe and effective aids in the detection of fetal ONTD. The assay is intended for use in conjunction with other diagnostic tools such as ultrasound and amniography.

Summary and Explanation Alpha-fetoprotein (AFP) is a single-chain glycoprotein with a molecular mass of approximately 70,000 daltons.[4] 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.[5] AFP is first detected in the fetal circulation approximately 30 days after conception.[1] 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.[6] 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.[7] 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.[8,9,10,11,12] 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.[8,11,13] 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.[14,15] Additionally, decreasing levels following therapy generally indicate successful intervention, whereas rising levels following therapy usually indicate residual tumor or recurrence.[16,17]

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.[18,19,20,21,22] Therefore, AFP is not recommended as a screening tool for cancer detection in the general population.

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.[23] In that same year, Brock and Sutcliffe reported the association between increased amounts of amniotic fluid AFP and neural tube defect pregnancies.[24] The following year Brock, et al. demonstrated that maternal serum levels were also elevated under these conditions.[25]

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.[26]

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%.[26] 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 [27]and the second in 1979 addressed amniotic fluid AFP testing.[28]

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. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the

concentration of AFP in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

Traceability

The measurand (analyte) in the Access AFP Calibrators is traceable to the WHO 1st International Standard 72/225. Traceability process is based on EN ISO 17511.

The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the Access reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Product Information

Access AFP Reagent and Calibrator kit

Cat. No. 33211: 300 determinations, 6 reagent packs, 50 tests/packs; 1 set of seven calibrators, S0–S6, 2.5 mL/vial

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Reagent pack is stable at 2 to 10°C for 28 days after initial use.
- 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.
- If the reagent pack is damaged (i.e. broken elastomer), discard the pack.
- All antisera are polyclonal unless otherwise indicated.
- Refer to calibration card for exact calibrator concentrations.

R1a:	Paramagnetic particles coated with mouse monoclonal anti-AFP antibodies suspended in TRIS buffered saline, with surfactant, bovine serum albumin (BSA) matrix, < 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 matrix, proteins (goat, rabbit, mouse), < 0.1% sodium azide, and 0.25% ProClin 300.
S0:	Buffered 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, 5.0, 25, 100, 500 and 3000 ng/mL, respectively (2.1, 4.1, 20, 82, 413, and 2478 IU/mL), in buffered BSA matrix with surfactant, < 0.1% sodium azide, and 0.1% ProClin 300.
Calibration Card:	1

Warnings and Precautions

- For *in vitro* diagnostic use.
- 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.
- Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV-1 and HIV-2). 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.[29]

- 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.[30]
- 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.

- The Material Safety Data Sheet (MSDS) is available upon request.
- 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 samples.
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]
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - Within two hours after centrifugation, transfer at least 500 µL of cell-free sample to a storage tube. Tightly stopper the tube immediately.
   - Store samples 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 samples at 2 to 8°C.
   - If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder.
   - Thaw samples only once.
4. Use the following guidelines when preparing specimens:
   - Ensure residual fibrin and cellular matter has been removed prior to analysis.
   - Follow blood collection tube manufacturer's recommendations for centrifugation.
5. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.
6. 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.

Materials Provided

R1 Access AFP Reagent Packs
S0–S6 Access AFP Calibrators

Materials Required But Not Provided

1. Quality Control (QC) materials: commercial control material.
2. Access AFP Sample Diluent
   Cat. No. 33216
3. Access Substrate
   Cat. No. 81906
4. **Access, Access 2, SYNCHRON LXi, UniCel DxC 600i:**
   Access Wash Buffer II, Cat. No. A16792
   UniCel DxI:
   UniCel DxI Wash Buffer II, Cat. No. A16793
   UniCel DxI Access Immunoassay Systems Wash Buffer II, Cat. No A79784 (Diluent pack for use with the UniCel DxI system onboard dilution feature.)

Procedural Comments

1. Refer to the appropriate system manuals and/or Help system 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 of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
3. Use ten (10) µL of sample for each determination in addition to the sample container and system dead volumes. Use thirty-five (35) µL of sample in addition to the sample container and system dead volumes for each determination run with the DxI system onboard dilution feature. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.
4. The system default unit of measure for sample results is ng/mL. To change sample reporting units to the International System of Units (SI units), IU/mL, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply ng/mL by multiplication factor 0.826.

Procedure

Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

Amniotic fluid samples can be reported in mg/mL by changing the test units in the Access Software. Refer to the appropriate system manuals and/or Help system for instructions on changing test units.

To request an amniotic fluid sample, select the sample type (e.g., Other or Amniotic).

For assaying samples containing < 3000 ng/mL **AFP**, select **AFP** as the test name. Select **Dil-AFP** as the test name for assaying samples containing > 3000 ng/mL. Alternatively, DxI users may use the DxI onboard dilution feature by selecting **d-AFP** as the test name for assaying samples containing > 3000 ng/mL. The same reagent pack and calibration curve is used for all assays. Both AFP and Dil-AFP assays will report values between 2700 ng/mL and approximately 3000 ng/mL. The d-AFP assay will report values between 2550 ng/mL and approximately 3000 ng/mL.

Calibration Details

An active calibration curve is required for all tests. For the Access AFP assay, calibration is required every 28 days. Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

The Access AFP Calibrators are provided at seven levels - zero and approximately 2.5, 5.0, 25, 100, 500 and 3000 ng/mL- prepared gravimetrically from human AFP and buffered BSA matrix. Assay calibration data are valid up to 28 days.

Calibrators run in duplicate.

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, quality control materials should be included in each 24-hour time period.[32] Include commercially available quality control materials that cover at least three levels of analyte. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values 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 obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

Results

Patient test results are determined automatically by the system software using a weighted four parameter logistic curve (4PLC) math model. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

Limitations of the Procedure

1. Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value (approximately 0.50–3000 ng/mL [0.41–2478 IU/mL]). The analytic range for Dil-AFP is 2700 ng/mL to approximately 51,000 ng/mL.
   - If a sample contains less than the lower limit of detection for the assay, report the results as less than that value (i.e., < 0.50 ng/mL [< 0.41 IU/mL]). When the DxI system onboard dilution feature is used, the system will report results as less than 2550 ng/mL (2107 IU/mL).
2. To accurately measure samples containing approximately 3000 to 51,000 ng/mL, select the **Dil-AFP** test. This test uses the **AFP** pack. When **Dil-AFP** is requested, the system autodilutes the sample and reads the resulting dose off the **AFP** calibration curve. The system multiplies by the dilution factor defined in the software to calculate final test results. Any neat sample reading < 2700 ng/mL in the Dil-AFP assay should be retested in the AFP assay.
3. For UniCel DxI systems:
   Samples containing > 3000 ng/mL can be processed using the DxI onboard dilution feature. The DxI system onboard dilution feature automates the dilution process, using one volume of sample with 100 volumes of UniCel DxI Access Immunoassay Systems Wash Buffer II, allowing samples to be quantitated up to 303,000 ng/mL. The system reports the results adjusted for the dilution.
4. Alternatively, samples containing > 3000 ng/mL for AFP or > 51,000 ng/mL for Dil-AFP can also be processed via **off-line pre-dilution** following these examples:
   - For AFP values > 3000 ng/mL, dilute as follows:
     - Dilute one volume of sample with 100 volumes of Access Wash Buffer II or Access AFP Sample Diluent for serum samples. The pre-dilution factor is 101.
     - Dilute one volume of sample with 10 volumes of Access Wash Buffer II or Access AFP Sample Diluent for amniotic samples. The pre-dilution factor is 11.
   - For Dil-AFP values > 51,000 ng/mL, dilute as follows:
     - For serum or amniotic samples, dilute one volume of sample with 10 volumes of

Access Wash Buffer II or Access AFP Sample Diluent. The pre-dilution factor is 11.

- Type in the pre-dilution factor when entering the test request. Order the AFP or Dil-AFP test. The system will automatically multiply the result by the pre-dilution factor and report that value.
- If the pre-dilution factor is not used when entering the request, multiply the calculated value by the pre-dilution factor after assaying the diluted sample using the Access AFP assay or the Dil-AFP assay.
- If the system reports a pre-diluted AFP result as < 0.50 ng/mL or a pre-diluted Dil-AFP result < 2700 ng/mL, then re-dilute with a lesser dilution.
  - Refer to the appropriate system manuals and/or Help system for additional instructions on processing pre-diluted samples.

5. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples.[33,34]

   Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
6. The Access AFP results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate information.
7. The Access AFP assay does not demonstrate any "hook" effect up to 500,000 ng/mL (413,000 IU/mL).
8. 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.
9. Valid measurements of AFP in maternal serum CANNOT be made after amniocentesis. Maternal serum samples MUST be drawn PRIOR to amniocentesis.
10. 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.
11. 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.
12. 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.
13. The Access Dil-AFP assay is not available on the Access Immunoassay Analyzer.

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 its 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.

Maternal serum medians were comprised of 2539 specimens obtained from three clinical trial sites. Multiples (2.0, 2.5, 3.0) of each median (MoM) are also shown in the table below.

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

[††] AFP values have been determined using COMPLETED gestational weeks.

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 in the table below.

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

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

Sensitivity

Sample Type	Number of Samples	Multiples of the Median (MoM) ≥ 2.0	≥ 2.5	≥ 3.0
Maternal Serum (95% Cl)	23	91.3% (70.5%–98.5%)	73.9% (51.3%–88.9%)	69.6% (47.0%–85.9%)
Amniotic Fluid (95% Cl)	15	100% N/A	100% N/A	100% N/A

Specific Performance Characteristics

Methods Comparison

A comparison of serum AFP values using the Access AFP assay on the Access Immunoassay System and a commercially available immunoassay kit gave the following statistical data by adjusted least squares regression analysis:

Sample Type	n	Range of Observations	Intercept	Slope	Correlation Coefficient (r)
Cancer Serum	170	0.80–2277.84 ng/mL	3.86 ng/mL	0.91	0.988
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

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)	Determined 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
		Mean % Recovery	**105.6**

Sample 2 Maternal Serum	Expected Concentration (ng/mL)	Determined 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
		Mean % Recovery	**102.8**

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)	Determined 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)	Determined 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

Imprecision

This assay exhibits total imprecision of less than or equal to 8% across the assay range. Reproducibility was determined for three levels of serum-based controls by performing triplicate measurements in 2 assays per day for 20 days on the Access Immunoassay System. The data were analyzed via analysis of variance (ANOVA) [35,36] and are as follows:

Sample	Grand Mean (n=120) (ng/mL)	Within Run (%CV)	Between Run (%CV)	Total Imprecision (%CV)
Level 1	6.53	3.22	3.22	4.44
Level 2	72.10	2.88	2.04	3.54
Level 3	1672.88	2.71	2.07	3.41

The Dil-AFP assay exhibits total imprecision of less than or equal to 12% across the range of the Dil-AFP assay.

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	Analyte Added	Substance	Analyte Added
Acetaminophen	1500 µg/mL	Hemoglobin	1.2 g/dL
Acetylsalicylic acid	10 mg/mL	hFSH	2 IU/mL
Alpha-1 acid glycoprotein	4.54 mg/mL	hLH	2 IU/mL
Alpha-1 anti-trypsin	14.8 mg/mL	hTSH	6 µg/mL
Ascorbic acid	1000 µg/mL	Human placental lactogen	100 µg/mL
Azathioprine	3.0 mg/dL	Lipemia	520 mg/dL
Bleomycin	100 µU/mL	Phenacetin	500 µg/mL
Bilirubin	25 mg/dL	Phenothiazine	150 µg/mL
CEA	375 µg/mL	Prednisolone	3.0 mg/dL
Chlorothiozide	1000 µg/mL	Prednisone	0.3 mg/dL
Cisplatin	1000 µg/mL	Reserpine	100 µg/mL
Cobalamine	500 µg/mL	Retinoic acid	500 µg/mL
Cyclosporine	20.4 mg/dL	Rheumatoid factor	600 IU/mL
Diazepam	50 µg/mL	Riboflavin	50 µg/mL
Ethanol	1.90%	Serum Albumin (BSA)	6 mg/mL
Fetal hemoglobin	500 µg/mL	Spironolactone	15 µg/mL
Haptoglobin	20.0 mg/mL	Thiamine	50 µg/mL
hCG	200 µg/mL	Transferrin	23.7 mg/mL
		Vinblastine	500 µg/mL

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 calibration curve, controls and 25 replicates of the zero calibrator in multiple assays. The analytical sensitivity value is interpolated from the curve at the point that is two standard deviations from the mean measured zero calibrator signal.

Comparison of Access Immunoassay Systems

The following table provides the regression statistics for the Access Immunoassay Systems over the reportable and/or clinical range of the AFP assay.

Access Systems	N	Range of Observations (ng/mL)	Intercept (ng/mL)	Slope	Correlation Coefficient (r)
Access 2 v. Access	139	0.8–2683.9	-2.48 95% CI (-7.42 to 2.47)	1.089 95% CI (1.082 to 1.097)	0.998
Synchron LXi 725 v. Access 2	61	0.02–2921.9	-1.14 95% CI (-34.02 to 31.73)	0.966 95% CI (0.943 to 0.989)	0.991
UniCel DxI 800 v. Access 2	119	1.6–2786.0	-14.00 95% CI (-24.62 to -3.39)	1.080 95% CI (1.068 to 1.096)	0.994
	75	1.6–232.3	0.49 95% CI (-0.64 to 1.63)	1.007 95% CI (0.994 to 1.021)	0.996
UniCel DxC 600i v. Access 2	75	1.23–2644.26	0.926 95% CI (-2.28 to 4.13)	0.981 95% CI (0.975 to 0.989)	0.9995
	58	1.23–244.06	0.136 95% CI (-0.232 to 0.504)	0.988 95% CI (0.978 to 0.999)	0.9992
UniCel DxI 600 v. UniCel DxI 800	220	1.4–2683.3	-2.70 95% CI (-12.30 to 6.91)	1.025 95% CI (1.013 to 1.038)	0.996
	164	1.4–282.1	0.87 95% CI (-0.06 to 1.79)	0.971 95% CI (0.957 to 0.985)	0.996

Access
Immunoassay Systems

AFP SAMPLE DILUENT
REF 33216

Intended Use
The Access AFP Sample Diluent is intended for use with the Access AFP assay to dilute patient samples containing AFP concentrations greater than the S6 calibrator.

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

Product Information
Access AFP Sample Diluent
Cat. No. 33216: 14 mL/vial

- Provided ready to use.
- Allow the contents to stand for 10 minutes at room temperature.
- Mix gently by inverting before use. Avoid bubble formation.
- Stable until the expiration date stated on the vial label when stored at 2 to 10°C.

Diluent:	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
- For *in vitro* diagnostic use.
- 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.
- 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.[30]
- Xi. Irritant: 0.1% 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.

- The Material Safety Data Sheet (MSDS) is available upon request.

Procedure
Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value (approximately 0.50 to 3000 ng/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. Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution.

All amniotic fluid samples require a pre-dilution. Dilute one 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 Immunoassay System 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 (e.g. Other or Amniotic) and enter the dilution factor 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.

Limitations of the Procedure

If there is evidence of microbial contamination or excessive turbidity in the reagent, discard the vial.

References

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

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

3 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.

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

5 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.

6 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.

7 Tatarinov YS. Finding of an embryospecific alpha-globulin in blood stream in a patient with primary hepatic cancer (Russian). Vopr. Med Khim 1964; 10: 90.

8 Javadpour N, McIntire KR, Waldmann TA. Human chorionic gonadotropin (HCG) and alpha-fetoprotein (AFP) in sera and tumor cells of patients with testicular seminoma: A prospective study. Cancer 1978; 42: 2768.

9 Waldmann TA, McIntire KR. The use of radioimmunoassay for alpha-fetoprotein in the diagnosis of malignancy. Cancer 1974; 34: 1510.

10 Silver HKB, Gold P, Feder S, Freedman SO, Shuster J. Radioimmunoassay for human alpha-fetoprotein. Proc Natl Acad Sci U.S.A. 1973; 70: 526.

11 Kohn J, Orr AH, McElwain TJ, Bentall M, Peckham MJ. Serum alpha1-fetoprotein in patients with testicular tumours. Lancet 1976; 1(7982): 433.

12 Abelev GI. Alpha-fetoprotein in ontogenesis and its association with malignant tumors. Adv Cancer Res 1971; 14: 295.

13 Lange PH, McIntire KR, Waldman TA, Hakala TR, Fraley EE. Serum alpha-fetoprotein and human chorionic gonadotropin in the diagnosis and management of non-seminomatous germ-cell testicular cancer. N Engl J Med 1976; 295: 1237.

14 Report from the Medical Research Council Working Party on Testicular Tumours. Prognostic factors in advanced non-seminomatous germ-cell testicular tumours: Results of a multicentre study. Lancet 1985; 1(8419): 8.

15 Bos GJ, Lange PH, Fraley EE, Goldman A, Nochomovitz LE, Rosai J, Waldmann TA, Johnson K, Kennedy BJ. Human chorionic gonadotropin and alpha-fetoprotein in the staging of non-seminomatous testicular cancer. Cancer 1981; 47: 328.

16 Vogelzang NJ, Lange, PH, Goldman A, Vessela RH, Fraley EE, Kennedy BJ. Acute changes of alpha-fetoprotein and human chorionic gonadotropin during induction chemotherapy of germ cell tumors. Cancer Res 1982; 42: 4855.

17 Perlin E, Engeler JE, Edson M, Karp D, McIntire KR, Waldmann TA. The value of serial measurement of both human chorionic gonadotropin and alpha-fetoprotein for monitoring germinal cell tumors. Cancer 1976; 37: 215.

18 Waldmann TA, McIntire KR. Serum alpha-fetoprotein levels in patients with ataxia-telangiectasia. Lancet 1972; 2(7787): 1112.

19 Kew MC, Purves LR, Bersohn I. Serum alpha-fetoprotein levels in acute viral hepatitis. Gut 1973; 14: 939.

20 Endo Y, Kanai K, Oda T, Mitamura K, Iino S, Suzuki H. Clinical significance of alpha-fetoprotein in hepatitis and liver cirrhosis. Ann NY Acad Sci 1975; 259: 234.

21 McIntire KR, Waldmann TA, Moertel C, Go VLW. Serum alpha-fetoprotein in patients with neoplasms of the gastrointestinal tract. Cancer Res 1975; 35: 991.

22 Chen DS, Sung JL. Relationship of Hepatitis B surface antigen to serum alpha-fetoprotein in nonmalignant diseases of the liver. Cancer 1979; 44: 984.

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

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

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

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

27 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.

28 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.

29 HHS Publication, 4th ed., April 1999. Biosafety in Microbiological and Biomedical Laboratories. Available http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

30 DHHS (NIOSH) Publication No. 78-127, August 1976. Current Intelligence Bulletin 13 - Explosive Azide Hazard. Available http://www.cdc.gov/niosh.

31 Approved Guideline – Procedures for the Handling and Processing of Blood Specimens, H18-A3. 2004. Clinical and Laboratory Standards Institute.

32 Cembrowski GS, Carey RN. Laboratory quality management: QC≠QA. ASCP Press, Chicago, IL, 1989.
33 Kricka L. Interferences in immunoassays – still a threat. Clin Chem 2000; 46: 1037–1038.
34 Bjerner J, et al. Immunometric assay interference: incidence and prevention. Clin Chem 2002; 48: 613–621.
35 Approved Guideline – Evaluation of Precision Performance of Clinical Chemistry Devices, EP5-A. 1999. National Committee for Clinical Laboratory Standards.
36 Krouwer JS, Rabinowitz R. How to improve estimates of imprecision. Clin Chem 1984; 30: 290.

Manufactured by:
Beckman Coulter, Inc.
250 S. Kraemer Blvd.
Brea, CA 92821 U.S.A.

EC REP
Beckman Coulter Ireland Inc.
Mervue Business Park,
Mervue, Galway,
Ireland 353 91 774068

Printed in U.S.A.
Made in U.S.A.
Revised November 2013