BECKMAN COULTER

ACCESS
Immunoassay Systems

Instructions For Use

Access AFP (300 and 600) Test Kit
Alpha-fetoprotein

REF 33211
C28649

FOR PROFESSIONAL USE ONLY

For *in vitro* diagnostic use

Rx Only

For use on DxI Access Immunoassay Analyzers

PRINCIPLE

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, open neural tube defects (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. 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.

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.

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 1,000 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]

METHODOLOGY

Assay type: one-step, sandwich

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, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate 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 analyte in the sample. Analyte concentration is automatically determined from a stored calibration.

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.

SPECIMEN

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:[29]
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - Physically separate serum from contact with cells as soon as possible.
   - Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than 8 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 1,800 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.

REAGENTS

CONTENTS

Access AFP Reagent and Calibrator kit

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

Ref. No. C28649: 600 determinations, 6 reagent packs, 100 tests/packs; 1 set of seven calibrators, S0-S6, 2.5 mL/vial

The same reagent formulation is used on all Access Immunoassay Systems.

- All antisera are polyclonal unless otherwise indicated.

Well	Contents	Ingredients
R1a:	3.10 mL	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:	2.98 mL	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 3,000 ng/mL, respectively (2.1, 4.1, 20, 82, 413, and 2,478 IU/mL), in buffered BSA matrix with surfactant, < 0.1% sodium azide, and 0.1% ProClin 300.
Calibration Card:	1

*ProClin is a trademark of LANXESS Corp.

WARNING 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.[30]

- For hazards presented by the product refer to the following sections: REACTIVE INGREDIENTS and GHS HAZARD CLASSIFICATION.
- The Access AFP reagents and calibrators are packaged as a matched set. DO NOT mix materials from different Kit lot numbers.

REACTIVE INGREDIENTS

> ⚠ **CAUTION**
>
> **Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).**
> **To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.**

GHS HAZARD CLASSIFICATION

PMP (Compartment R1a)	WARNING	
		
	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
Conjugate (Compartment R1b)	WARNING	
		
	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.

		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
AFP Calibrators S0	WARNING	
		
	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
AFP Calibrators S1, S2, S3, S4, S5, S6	WARNING	
		
	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

1. Quality Control (QC) materials: FDA approved or cleared commercial control material.
2. Lumi-Phos PRO
   Ref. No. B96000
3. UniCel DxI Wash Buffer II

Ref. No. A16793

4. Optional materials for dilution:
   - Access Wash Buffer II
     Ref. No. A16792
   - Access AFP Sample Diluent
     Ref. No. 33216

REAGENT PREPARATION

Provided ready to use.

REAGENT STORAGE AND STABILITY

Stability	
Stability	Up to stated expiration date
Reagent: After opening at 2 to 10°C	28 days
Calibrators: After opening at 2 to 10°C	Typically until the expiration date stated on the vial label when properly stored and handled

- Store upright.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- Signs of possible deterioration are a broken elastomeric layer on the pack or quality control values out of range.
- If the reagent pack is damaged (e.g., broken elastomer), discard the pack.
- Mix calibrators well by gently inverting before use. Avoid bubble formation.

CALIBRATION

CALIBRATION INFORMATION

An active calibration is required for all tests. Calibration is required every 28 days. See calibrator Instructions For Use (IFU) for additional calibration information. Refer to the appropriate system manuals and/or Help system for information on calibration method, 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 3,000 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 are essential for monitoring the system performance. Quality controls with varying concentration ranges should be run individually at least once every 24 hours when the assay is being performed.[31] Quality control ranges should be determined by each laboratory's individual requirements. Follow applicable regulations and guidelines for quality control.

TESTING PROCEDURE(S)

PROCEDURE

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. Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.
3. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
4. Use 10 µL of sample for each determination in addition to the sample container and system dead volumes. Use 4 µL of sample in addition to the sample container and system dead volumes for each determination run with the automated dilution feature. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.
5. 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.

LIMITATIONS

1. 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 human anti-animal antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other antibodies such as human anti-goat antibodies may be present in patient samples.[32,33] Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
2. Other potential interferences in the sample could be present and may cause erroneous results in immunoassays. Some examples that have been documented in literature include rheumatoid factor, fibrin, endogenous alkaline phosphatase, exogenous alkaline phosphatase (e.g. asfotase alfa, Strensiq), and proteins capable of binding to alkaline phosphatase. Carefully evaluate results if the sample is suspected of having these types of interferences.[34,35]
3. The 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.
4. The Access AFP assay does not demonstrate any “hook” effect up to 500,000 ng/mL (413,000 IU/mL).
5. 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.
6. Valid measurements of AFP in maternal serum CANNOT be made after amniocentesis. Maternal serum samples MUST be drawn PRIOR to amniocentesis.
7. 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.
8. 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.

9. 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.

RESULTS INTERPRETATION

Test results are determined automatically by the system software. 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.

REPORTING RESULTS

MEASURING INTERVAL

0.50-3,000 ng/mL (0.41-2,478 IU/mL)

Automated dilution: Up to 303,000 ng/mL (250,278 IU/mL)

Samples can be accurately measured within the measuring interval defined as the lower Limit of Quantitation (LoQ) and the highest calibrator value. The measuring interval was verified using serum samples.

1. If a sample contains less than the lower limit for the assay, report the result as less than that value (i.e. < 0.50 ng/mL (< 0.41 IU/mL)).
2. If a sample contains more than the stated value of the highest calibrator, report the result as greater than that value (e.g. > 3,000 ng/mL (> 2,478 IU/mL)). Alternatively, the sample may be diluted to obtain a result.
   - For automated dilutions, the system dilutes one volume of sample with 100 volumes of Wash Buffer II. Refer to the appropriate system manuals and/or Help system for instructions.
   - For manual dilutions:
     - Dilute one volume of sample with 10 or 100 volumes of Wash Buffer II or Access AFP Sample Diluent. The pre-dilution factors are 11 and 101 respectively. 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.
     - If the system reports a pre-diluted AFP result as < 0.50 ng/mL then re-dilute with a lesser dilution.

EXPECTED VALUES

Cancer

1. Each laboratory should validate or establish its own reference intervals to assure proper representation of specific populations.
2. The AFP level was measured, using the Access AFP assay, in 1,126 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 (%)	301-1,000 ng/mL (%)	> 1,000 ng/mL (%)
Apparently Healthy	177	98.9	1.1	0.0	0.0	0.0
Testicular Carcinoma						

Clinical Category	n	0-9.0 ng/mL (%)	9.1-100 ng/mL (%)	101-300 ng/mL (%)	301-1,000 ng/mL (%)	> 1,000 ng/mL (%)
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 2,539 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

Gestational Week††	Number of Samples	Median Concentration (µg/mL)	Multiples of Median Concentration (µg/mL)		
			2.0	2.5	3.0
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% 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

Sample Type	Number of Samples	Multiples of the Median (MoM)		
		≥ 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% N/A	100% N/A	100% N/A

PERFORMANCE CHARACTERISTICS

ASSAY CRITERIA AND REPRESENTATIVE DATA

Representative data is provided for illustration only. Performance obtained in individual laboratories may vary.

METHODS COMPARISON

A study based on CLSI EP09c, 3rd Edition[36] using Weighted Deming regression and Pearson's correlation compared serum samples on the Access 2 Immunoassay System and the DxI 9000 Access Immunoassay Analyzer.

N	Concentration Range* (ng/mL)	Slope	Slope 95% CI	Intercept	Intercept 95% CI	Correlation Coefficient R
642	1.2 - 2,976	1.04	1.03 - 1.05	-0.12	-0.15 - (-0.08)	0.99

*Range is Access 2 values

LINEARITY

A study based on CLSI EP06-Ed2[37] performed on the DxI 9000 Access Immunoassay Analyzer determined the assay demonstrated linearity across the measuring interval.

IMPRECISION

The assay was designed to have within-laboratory imprecision as listed below:

- ≤ 0.08 ng/mL (0.07 IU/mL) SD at concentrations ≤ 1.0 ng/mL (0.8 IU/mL)
- ≤ 8.0% CV at concentrations > 1.0 ng/mL (0.8 IU/mL)

  A study based on CLSI EP05-A3[38] performed on the DxI 9000 Access Immunoassay Analyzer tested multiple serum samples in triplicate in 2 runs per day for a minimum of 20 days.

Concentration (ng/mL)			Repeatability (Within-Run)		Between-Run		Between-Day		Within-Laboratory (Total)	
Sample	N	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	120	8.5	0.23	2.7	0.10	1.1	0.36	4.2	0.43	5.1
Sample 2	120	25	1.1	4.6	0.5	2.1	0.4	1.7	1.3	5.3
Sample 3	120	88	2.5	2.9	1.8	2.0	2.0	2.3	3.7	4.2
Sample 4	120	180	6.3	3.5	3.8	2.1	3.7	2.1	8.2	4.6
Sample 5	120	677	15.9	2.4	11.8	1.7	18.2	2.7	26.9	4.0
Sample 6	120	1,700	73.1	4.3	23.4	1.4	46.9	2.8	90.0	5.3
Sample 7	120	2,867	100.0	3.5	41.0	1.4	135.1	4.7	173.0	6.0

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	1,500 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	1,000 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	1,000 µg/mL	Prednisone	0.3 mg/dL
Cisplatin	1,000 µg/mL	Reserpine	100 µg/mL
Cobalamine	500 µg/mL	Retinoic acid	500 µg/mL

Substance	Analyte Added	Substance	Analyte Added
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

DETECTION CAPABILITY

Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) studies were conducted using serum samples on the DxI 9000 Access Immunoassay Analyzer following CLSI guideline EP17-A2.[39] The LoB study included multiple reagent lots and 3 instruments over a minimum of 3 days. The LoD and LoQ studies included multiple reagent lots and 3 instruments over a minimum of 5 days.

	ng/mL	IU/mL
Limit of Blank (LoB)	0.2	0.2
Limit of Detection (LoD)	0.50	0.41
Limit of Quantitation (LoQ) ≤ 20% within-lab CV	0.50	0.41

ADDITIONAL INFORMATION

REVISION HISTORY

Revision A

New release of DxI Access Immunoassay Analyzer reagent IFU.

SYMBOLS KEY

Glossary of Symbols is available at beckmancoulter.com/techdocs (document number C02724).

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