ARCHITECT

SYSTEM

en

AFP

REF 7K67

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Read Highlighted Changes
Revised November, 2009

AFP

Customer Service: Contact your local representative or find country specific contact information on www.abbottdiagnostics.com

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

REF	List Number	CONTROL NO.	Control Number
IVD	*In Vitro* Diagnostic Medical Device	REAGENT LOT	Reagent Lot
LOT	Lot Number	REACTION VESSELS	Reaction Vessels
	Expiration Date	SEPTUM	Septum
2°C 8°C	Store at 2-8°C	SAMPLE CUPS	Sample Cups
	Caution	REPLACEMENT CAPS	Replacement Caps
SN	Serial Number		Consult instructions for use
	Manufacturer		

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

Abbott

WARNING: 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. 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. 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 serum, plasma, and amniotic fluids from pregnant women with confirmed gestational age.

NAME

ARCHITECT AFP (alpha-fetoprotein)

INTENDED USE

The ARCHITECT AFP assay is a Chemiluminescent Microparticle Immunoassay (CMIA) for the quantitative determination of alpha-fetoprotein (AFP) in:

1. Human serum or plasma to aid in the management of patients with nonseminomatous testicular cancer.
2. Human serum, plasma, and amniotic fluid at 15 to 21 weeks gestation to aid in the detection of fetal open neural tube defects (NTD). Test results when used in conjunction with ultrasonography or amniography are a safe and effective aid in the detection of fetal open NTD.

SUMMARY AND EXPLANATION OF TEST

The discovery of alpha-fetoprotein (AFP) in fetal serum was first recorded by Bergstrand and Czar in 1956.[1] Alpha-fetoprotein is a single polypeptide chain glycoprotein with a molecular weight of approximately 70,000 daltons. The physicochemical properties and amino acid composition are similar to those of albumin.[2,3] Synthesis of AFP occurs primarily in the liver and yolk sac of the fetus. It is secreted into fetal serum, reaching a peak at about 13 weeks gestation and gradually declining thereafter. Elevated serum AFP levels subsequently reappear during pregnancy and in conjunction with several malignant diseases.

Cancer Management

Alpha-fetoprotein (AFP) was first described as a human tumor-associated protein in 1964 by Tatarinov.[4] Since then it has been shown that elevation of serum AFP above values typically found in healthy individuals occurs in several malignant diseases,[5-8] most notably nonseminomatous testicular cancer and primary hepatocellular carcinoma. In the case of nonseminomatous testicular cancer, a direct relationship has been observed between the incidence of elevated AFP levels and the stage of disease.[9,10] Elevated AFP levels have also been observed in patients diagnosed as having seminoma with nonseminomatous elements but have not been observed in patients with pure seminoma.[7,9,11,12] Human chorionic gonadotropin (hCG) and AFP are also important prognostic indicators of survival rate among patients with advanced nonseminomatous germ cell testicular tumors.[13]

The usefulness of AFP measurements in the management of patients with nonseminomatous testicular cancers has been well documented.[7,11,14]

For patients in clinical remission following treatment, AFP levels generally decrease.[11] Post-operative AFP values which fail to return to normal strongly suggest the presence of residual tumor.[6,7,11] Tumor recurrence is often accompanied by a rise in AFP before progressive disease is clinically evident[7,9]

Greater than 70% of patients with primary hepatocellular carcinoma have been reported to have elevated levels of serum AFP.[5,6,15] Elevated AFP levels have occasionally been found in association with gastrointestinal tract cancers with and without liver metastases[16] and only rarely in other malignancies.[5,6] Serum AFP has been found to be elevated during pregnancy, in diseases such as ataxia telangiectasia, hereditary tyrosinemia, teratocarcinoma and in benign hepatic conditions, such as acute viral hepatitis, chronic active hepatitis and cirrhosis.[6,15,17] Elevation of serum AFP in benign hepatic diseases is usually transient.[5]

AFP testing is not recommended as a screening procedure to detect cancer in the general population.

Prenatal testing

Many studies have confirmed the utility of AFP in the early detection of fetal open neural tube defects (NTD).[18-20] In the US, NTD, primarily anencephaly and spina bifida, occur at the rate of between 1 and 2 per 1000 live births and are among the most common major congenital malformations.[21] The incidence of NTD varies geographically and across racial groups.[22-26]

Anencephaly is incompatible with life and accounts for one-third to one-half of all NTD. Open spina bifida can vary widely in severity.

Reports from the scientific literature suggest additional factors to be considered when assessing the risk of an NTD being present.[22-28] One is the effect of maternal weight. Maternal blood volume, as reflected by maternal weight, has been reported to affect maternal serum AFP (MSAFP) concentration in maternal circulation; the higher the maternal weight, the lower the MSAFP concentration.[26,29] Another factor to consider is maternal diabetes. Insulin dependent diabetic women reportedly have MSAFP levels significantly lower than non-diabetic women, and an increased incidence of NTD.[27,28,30] Maternal serum AFP levels in the black population average about 10% higher than MSAFP values in the non-black population. An adjustment factor or use of an appropriate normative data base have been suggested in the literature.[25,26]

Amniotic fluid AFP (AFAFP) levels peak at about 13 weeks gestation after which they rapidly decline until about 22 weeks gestation and then gradually decline until term. Transfer of AFP into maternal circulation is accomplished primarily through diffusion across the placenta.[31] If the fetus has an open neural tube defect, AFP is thought to leak directly into the amniotic fluid (AF) causing unexpectedly high levels of AFAFP. Subsequently, the AFAFP reaches the maternal circulation, thus producing abnormally elevated levels of MSAFP. Certain fetal abnormalities such as congenital renal disease and esophageal atresia also show AFAFP elevations.[32,33]

Other fetal distress situations such as omphalocele or gastroschisis, defective kidneys, threatened abortion, prematurity and sometimes fetal demise[34-37] may exhibit abnormally high levels of MSAFP. Increased MSAFP values are also seen in multiple pregnancies[38] and in normal singleton pregnancies in which the gestational age has been underestimated. Low MSAFP values have been associated with molar pregnancy, missed abortion, pseudocyesis, overestimated gestational age, and Down Syndrome.[39]

In a report on over 18,000 pregnancies, the U.K. Collaborative Study has established multiples of the median (MoM) as the preferred way to express AFP results.[18] The median AFP value for each gestational week is first determined; then individual AFP levels are reported as multiples of this value. This method of expression facilitates comparison of AFP test results across gestational weeks and between laboratories.

AFP testing during pregnancy is recommended as an effective way to determine those women potentially at risk of carrying a fetus affected with an open NTD. Used in conjunction with other confirmatory procedures such as ultrasonography or amniography measurement of AFP serves as an important tool in the care and management of these patients.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT AFP assay is a two-step immunoassay to determine the presence of AFP in human serum, plasma, and amniotic fluid, using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex.

In the first step, sample, assay diluent, and anti-AFP coated paramagnetic microparticles are combined. AFP present in the sample binds to the anti-AFP coated microparticles. After washing, anti-AFP acridinium-labeled conjugate is added in the second step. Pre-Trigger and Trigger Solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of AFP in the sample and the RLUs detected by the ARCHITECT *i** optical system.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

* *i* = immunoassay

REAGENTS

Reagent Kit, 100 Tests/500 Tests

NOTE: Some kit sizes are not available in all countries or for use on all ARCHITECT *i* Systems. Please contact your local distributor.

ARCHITECT AFP Reagent Kit (7K67)

- **MICROPARTICLES** 1 or 4 Bottle(s) (6.6 mL for 100 test bottle/27.0 mL for 500 test bottle) Anti-AFP (mouse, monoclonal) coated Microparticles in MES, TRIS buffer with protein (bovine) stabilizers. Preservatives: antimicrobial agents.
- **CONJUGATE** 1 or 4 Bottle(s) (5.9 mL for 100 test bottle/26.3 mL for 500 test bottle) Anti-AFP (mouse, monoclonal) acridinium-labeled Conjugate in MES buffer with protein (bovine) stabilizers. Minimum concentration: 20 ng/mL. Preservatives: antimicrobial agents.
- **ASSAY DILUENT** 1 or 4 Bottle(s) (10.4 mL for 100 test bottle/53.0 mL for 500 test bottle) AFP Assay Diluent containing TRIS buffer. Preservatives: antimicrobial agents.

Assay Diluent

ARCHITECT *i* Multi-Assay Manual Diluent (7D82-50)

- **MULTI-ASSAY MANUAL DILUENT** 1 bottle (100 mL) ARCHITECT *i* Multi-Assay Manual Diluent containing phosphate buffered saline solution. Preservative: antimicrobial agent.

Other Reagents

ARCHITECT *i* Pre-Trigger Solution

- **PRE-TRIGGER SOLUTION** Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

ARCHITECT *i* Trigger Solution

- **TRIGGER SOLUTION** Trigger Solution containing 0.35 N sodium hydroxide.

ARCHITECT *i* Wash Buffer

NOTE: Bottle and volume varies based on order.

- **WASH BUFFER** Wash Buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

WARNINGS AND PRECAUTIONS

- **IVD**
- For *In Vitro* Diagnostic Use

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

- **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens.[40] Biosafety Level 2[41] or other appropriate biosafety practices[42,43] should be used for materials that contain or are suspected of containing infectious agents.
- **For product not classified as dangerous per European Directive 1999/45/EC as amended - Safety data sheet available for professional user on request.**
- For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not mix reagents from different reagent kits.
- Prior to loading the ARCHITECT AFP Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
- To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
- Once a septum has been placed on an open reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts which have no effect on assay efficacy.
- For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Storage Instructions

- 2°C–8°C The ARCHITECT AFP Reagent Kit must be stored at 2-8°C and may be used immediately after removal from 2-8°C storage.
- When stored and handled as directed, reagents are stable until the expiration date.
- The ARCHITECT AFP Reagent Kit may be stored on board the ARCHITECT *i* System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.
- Reagents may be stored on or off the ARCHITECT *i* System. If reagents are removed from the system, store them at 2-8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded**. After reagents are removed from the system, a scan must be initiated to update the onboard stability timer.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and may require retesting. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

- The ARCHITECT AFP assay file must be installed on the ARCHITECT *i* System from the ARCHITECT *i* Assay CD-ROM, version 24.0 or higher prior to performing the assay. For detailed information on assay file installation and viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.
- For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.
- For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.
- The default result unit for the ARCHITECT AFP assay is ng/mL.

An alternate result unit, IU/mL, may be selected for reporting results by editing assay parameter “Result concentration units”, to IU/mL.

The conversion factor used by the system is 0.83.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Human Serum (including serum collected in serum separator tubes) or plasma (sodium heparin, lithium heparin, or EDTA) collected in glass or plastic tubes or amniotic fluid specimens may be used in the ARCHITECT AFP assay. Other anticoagulants have not been validated for use with the ARCHITECT AFP assay. Follow the tube manufacturers instructions for serum or plasma collection tubes.
- When serial specimens are being evaluated, the same type of specimen should be used throughout the study.
- Amniotic fluid should be collected aseptically with appropriate precautions relative to both fetal and maternal safety by appropriately trained personnel. Visibly bloodstained specimens should be examined for the presence of fetal blood cells by using the Kleihauer-Betke technique and/or fetal hemoglobin by electrophoresis, immunoelectrophoresis or other available techniques. Amniotic fluid specimens contaminated with fetal blood may exhibit abnormally high AFP values which may lead to misinterpretation of test results.
- The ARCHITECT *i* System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify the correct specimen types are used in the ARCHITECT AFP assay.
- Serum or plasma specimens should be collected aseptically in such a way as to avoid hemolysis. For maternal serum or plasma analysis, the blood specimen should be collected prior to the initiation of amniocentesis. It has been demonstrated that increased levels of AFP may occur in maternal serum or plasma following amniocentesis.[44]

- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- Do not use grossly hemolyzed specimens.
- **For optimal results, inspect all samples for bubbles. Remove bubbles with an applicator stick prior to analysis. Use a new applicator stick for each sample to prevent cross contamination.**
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.
- If testing will be delayed more than 24 hours, serum or plasma should be removed from the clot, serum separator, or red blood cells. Specimens may be stored for up to 7 days at 2-8°C prior to being tested.[45] If testing will be delayed more than 7 days, specimens should be stored frozen at - 20°C or colder.[50]
- For optimal results, specimens should be free of fibrin, red blood cells, or other particulate matter. **Centrifuge specimens containing fibrin, red blood cells, or particulate matter prior to use to ensure consistency in the results.**
- Multiple freeze-thaw cycles of specimens should be avoided. Specimens must be mixed THOROUGHLY after thawing, by vortexing. Thawed samples containing red blood cells or particulate matter, **or which are hazy or cloudy in appearance** must be centrifuged prior to use to ensure consistency in the results.
- Specimens with obvious microbial contamination should not be used.
- When shipped, specimens must be packaged and labeled in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances. Specimens may be shipped on wet or dry ice. Prior to shipment, it is recommended that specimens be removed from the clot, serum separator, or red blood cells.
- ARCHITECT AFP Calibrators and Controls should be mixed by gentle inversion prior to use.

PROCEDURE

Materials Provided:

- 7K67 ARCHITECT AFP Reagent Kit

Materials Required but not Provided:

- ARCHITECT *i* System
- ARCHITECT *i* Assay CD-ROM, version 24.0 or higher
- 7K67-02 ARCHITECT *i* AFP Calibrators
- 7D82-50 ARCHITECT *i* **MULTI-ASSAY MANUAL DILUENT**
- ARCHITECT *i* **PRE-TRIGGER SOLUTION**
- ARCHITECT *i* **TRIGGER SOLUTION**
- ARCHITECT *i* **WASH BUFFER**
- ARCHITECT *i* **REACTION VESSELS**
- ARCHITECT *i* **SAMPLE CUPS**
- ARCHITECT *i* **SEPTUM**
- ARCHITECT *i* **REPLACEMENT CAPS**
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.
- For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

Materials Available but not Provided:

- 7K67-12 ARCHITECT AFP Controls

Assay Procedure

- Before loading the ARCHITECT AFP Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment:
  - Invert the microparticle bottle 30 times.
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - Once the microparticles have been resuspended, remove and discard the cap. Wearing clean gloves, remove a septum from the bag. Carefully snap the septum onto the top of the bottle.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
- Order tests.
- Load the ARCHITECT AFP Reagent Kit on the ARCHITECT *i* System. Verify that all necessary assay reagents are present. Ensure that septums are present on all reagent bottles.
- The minimum sample cup volume is calculated by the system and is printed on the Orderlist report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation verify adequate sample cup volume is present prior to running the test.
  - Priority: 100 µL for the first AFP test plus 50 µL for each additional AFP test from the same sample cup.
  - < 3 hours on board: 150 µL for the first AFP test plus 50 µL for each additional AFP test from the same sample cup.
  - > 3 hours on board: additional sample volume is required. Refer to the ARCHITECT System Operations Manual, Section 5 for information on sample evaporation and volumes.
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
  - To obtain the recommended volume requirements for the ARCHITECT AFP Calibrators and Controls, hold the bottles **vertically** and dispense 4 drops of each calibrator or control into each respective sample cup.
- Load samples.
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN. The ARCHITECT *i* System performs the following functions:
  - Moves the sample carrier to the aspiration point
  - Loads a reaction vessel (RV) into the process path
  - Aspirates and transfers sample into the RV
  - Advances the RV one position and transfers microparticles into the RV
  - Mixes, incubates and washes the reaction mixture
  - Adds conjugate to the RV
  - Mixes, incubates and washes the reaction mixture
  - Adds Pre-Trigger and Trigger Solutions
  - Measures chemiluminescent emission to determine the quantity of AFP in the sample
  - Aspirates contents of RV to liquid waste and unloads RV to solid waste
  - Calculates the result
- For information on ordering patient specimens, calibrators and controls, and general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- For optimal performance, it is important to follow the routine maintenance procedures defined in the ARCHITECT System Operations Manual, Section 9. If your laboratory requires more frequent maintenance, follow those procedures.

Specimen Dilution Procedures

- Specimens with an AFP value exceeding 350 ng/mL are flagged with the code " >350.00" and may be diluted using either the Automated Dilution Protocol or the Manual Dilution Procedure.
- If the Automated Dilution Protocol is chosen, serum or plasma MUST USE ONLY 1:16.7 and amniotic fluid MUST USE ONLY 1:166.7 The system automatically calculates the concentration of the sample before dilution and reports the result.
- Dilutions other than automated 1:16.7 serum/plasma dilutions or 1:166.7 amniotic dilutions should be done manually.
  - For a 1:20 dilution, add 50 µL of the patient specimen to 950 µL of ARCHITECT *i* Multi-Assay Manual Diluent (7D82-50).
  - **NOTE:** Manual dilutions must use ARCHITECT *i* Multi-Assay Manual Diluent (7D82-50). ARCHITECT AFP Manual Diluent cannot be used with this assay.

- For a 1:101 dilution, add 10 µL of the patient specimen to 1 mL of ARCHITECT *i* Multi-Assay Manual Diluent.
- The operator must enter the dilution factor in the Patient or Control order screen. All assays selected for that order will be diluted. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution and report the result. The result (before the dilution factor is applied) should be greater than 15 ng/mL.

- For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- To perform an ARCHITECT AFP calibration, test calibrators 1 and 2 in duplicate. A single sample of all levels of AFP controls must be tested to evaluate the assay calibration. Ensure that assay control values are within the concentration ranges specified in the control package insert. Calibrators should be priority loaded.
- Calibration Range: 0 - 350 ng/mL.
- Once an ARCHITECT AFP calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - A reagent kit with a new lot number is used.
  - Controls are out of range.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

QUALITY CONTROL PROCEDURES

The recommended control requirement for the ARCHITECT AFP assay is a single sample of all control levels tested once every 24 hours each day of use. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific procedures. Ensure that assay control values are within the concentration ranges specified in the package insert.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B. The ARCHITECT AFP assay belongs to method group 1.

RESULTS

The ARCHITECT AFP assay utilizes a point to point data reduction method to generate a calibration curve.

Alternate Result Units

- The default result unit for the ARCHITECT AFP assay is ng/mL. When the alternate result unit, IU/mL, is selected, the conversion factor used by the system is 0.83.
- Conversion Formula: (Concentration in ng/mL) x (0.83) = IU/mL

Flags

- Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the ARCHITECT System Operations Manual, Section 5.

Measurement Range

- The analytical measurement range of the ARCHITECT AFP assay is 0.4 - 350 ng/mL. For patient specimens with an AFP assay value exceeding 350 ng/mL refer to the **Specimen Dilution Procedures** section of the package insert.

LIMITATIONS OF THE PROCEDURE

- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies.[46,47] ARCHITECT AFP reagents contain a component that reduces the effect of HAMA reactive specimens. Additional clinical or diagnostic information may be required to determine patient status.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[48] Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.
- The ARCHITECT AFP assay is a valuable aid in the management of nonseminomatous testicular cancer patients when used in conjunction with information available from the clinical evaluation and other diagnostic procedures. Increased serum AFP concentrations have also been observed in ataxia telangiectasia, hereditary tyrosinemia, primary hepatocellular carcinoma, teratocarcinoma, gastrointestinal tract cancers with and without liver metastases, and in benign hepatic conditions such as acute viral hepatitis, chronic active hepatitis, and cirrhosis.
- Valid measurements of AFP in maternal serum or plasma CANNOT be made after amniocentesis; therefore, maternal serum or plasma specimens MUST be drawn PRIOR to amniocentesis. For further information, refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section in this assay package insert.
- The reliability of MSAFP evaluation in prenatal testing is dependent upon the accurate determination of gestational age. An overestimated gestational age may result in the misinterpretation of MSAFP values, and thus, the underestimation of risk of NTD. An underestimated gestational age may also result in the misinterpretation of MSAFP values, that is, overestimation of risk of NTD which may lead to unnecessary additional testing. When gestational age is uncertain, a reliable ultrasound examination is important.
- While elevated levels of MSAFP indicate increased risk of NTD, they are not diagnostic. Increased serum AFP concentrations have been seen in some cancers and some nonmalignant diseases as described above and, thus, may be indicative of maternal conditions. Other conditions including placental malformations, open fetal malformations such as omphalocele or gastroschisis (ventral wall defects), fetal kidney abnormalities, threatened or imminent abortion, and fetal demise are associated with elevated levels of MSAFP. Elevated MSAFP levels have also been associated with premature deliveries and low birth weights and have been seen in multiple births. Rarely, singleton, viable, and unaffected pregnancies may exhibit elevated MSAFP levels. Confirmatory testing, such as amniocentesis for AFAFP evaluation, high resolution ultrasonography or amniography is an essential part of the AFP testing process.
- The ARCHITECT AFP assay should not be used as a cancer screening test.

EXPECTED VALUES

The distribution of ARCHITECT AFP values determined in 805 specimens from normal individuals and patients with nonmalignant or malignant diseases is shown in the following table.

Distribution of ARCHITECT AFP Values

		Distribution of Values (%)					
Group/ Category	Number of Subjects	0 - 8.04 (ng/mL)	>8.04 - 15.00 (ng/mL)	>15 - 20 (ng/mL)	>20 - 100 (ng/mL)	>100 - 350 (ng/mL)	>350 (ng/mL)
Healthy Subjects	400	97.3	2.5	0.3	0.0	0.0	0.0
Nonmalignant Disease							
Cirrhosis	87	92.0	5.7	0.0	2.3	0.0	0.0
Hepatitis	55	87.3	12.7	0.0	0.0	0.0	0.0
Pancreatitis	28	100.0	0.0	0.0	0.0	0.0	0.0
Genitourinary	23	91.3	8.7	0.0	0.0	0.0	0.0
Malignant Disease							
Gastrointestinal	82	91.5	7.3	0.0	0.0	0.0	1.2
Hepatocellular	49	34.7	10.2	2.0	8.2	10.2	34.7
Pancreatic	24	91.7	0.0	0.0	4.2	0.0	4.2
Testicular: Seminoma	27	92.6	3.7	0.0	0.0	0.0	3.7
Testicular: Nonseminoma	30	50.0	10.0	3.3	13.3	10.0	13.3

In this study of healthy subjects, the observed central 95% of the 400 normal individuals ranged from 1.09 - 8.04 ng/mL. It is recommended that each laboratory establish its own expected reference range for the population of interest.

AFP Values in Maternal Serum and Amniotic Fluid

It is important for each laboratory to establish its own reference ranges for maternal serum and amniotic fluid AFP. Due to potential variation in testing at different laboratories, it has proven useful to express AFP values as the median and multiples of the median (MoM)* for each gestational week. Each laboratory should attempt to gather 100 or more values for each gestational week in order to arrive at median values and then utilize a Cutoff Value (MoM) which most closely suits its needs for sensitivity and specificity.

$$*\text{MoM} = \frac{\text{AFP Specimen Concentration}}{\text{Median AFP Concentration for Gestational Week}}$$

AFP Values for Maternal Serum

Maternal serum AFP values from 749 unaffected, singleton pregnancies expressed as the regressed medians and multiples of the regressed medians (MoM) for gestational weeks 15 to 21 are shown in the following table. Due to variations in populations at different locations, it is important for each laboratory to establish its own reference ranges.

Maternal Serum AFP by ARCHITECT AFP
Results of Clinical Evaluations

Gest. Week	Number of Specimens	Regressed Medians* (ng/mL)	Multiples of Regressed Medians (ng/mL) 2.0	2.5	3.0
15	135	35.5	71.1	88.8	106.6
16	165	40.3	80.6	100.8	120.9
17	104	45.7	91.4	114.3	137.2
18	83	51.9	103.7	129.7	155.6
19	93	58.8	117.7	147.1	176.5
20	94	66.7	133.5	166.8	200.2
21	75	75.7	151.4	189.3	227.1

AFP Values for Amniotic Fluid

Amniotic fluid AFP values from 707 unaffected, singleton pregnancies expressed as regressed medians and multiples of the regressed medians (MoM) for gestational weeks 15 to 21 are shown in the following table. These results may serve as a guide until the laboratory has gathered sufficient data of its own.

Amniotic Fluid AFP by ARCHITECT AFP
Results of Clinical Evaluations

Gest. Week	Number of Specimens	Regressed Medians* (µg/mL)	Multiples of Regressed Medians (µg/mL) 2.0	2.5	3.0
15	75	18.7	37.5	46.8	56.2
16	185	15.7	31.3	39.1	47.0
17	179	13.1	26.1	32.7	39.2
18	122	10.9	21.8	27.3	32.8
19	54	9.1	18.2	22.8	27.4
20	49	7.6	15.2	19.0	22.9
21	43	6.4	12.7	15.9	19.1

Alpha-fetoprotein values have been assigned on the basis of COMPLETED gestational weeks. For example, a specimen obtained on gestational day 132 (week 18 day 6) is assigned week 18, because the gestation has only completed 18 gestational weeks, plus 6 days.

* The regressed median values were determined using a weighted log-linear regression analysis.[18]

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

The ARCHITECT AFP assay is designed to have a precision of ≤ 10% total CV. Precision was determined as described in the Clinical and Laboratory Standards Institute (CLSI) Protocol EP5-A2[49]. Seven samples consisting of four serum based panels and three AFP Controls were assayed at two different times per day for twenty days (N=320). The specimens were tested using two lots of reagents on two instruments in replicates of two using a single calibration for each reagent lot and instrument combination. Data from this study are summarized in the following table.

Precision (20 Day CLSI) Overall Precision

Precision Controls/ Panel Members	Total No. Reps	Grand Mean (ng/mL)	Within-run SD	%CV	Between-run SD	%CV	Between-day SD	%CV	Total[a] SD	%CV
Low Control	320	20.09	0.561	2.8	0.301	1.5	0.344	1.7	0.724	3.6
Medium Control	320	79.22	2.259	2.9	1.113	1.4	1.004	1.3	2.711	3.4
High Control	320	174.20	5.012	2.9	1.634	0.9	3.528	2.0	6.343	3.6
Panel 1	320	13.21	0.252	1.9	0.155	1.2	0.078	0.6	0.306	2.3
Panel 2	320	61.28	1.872	3.1	0.000	0.0	0.826	1.3	2.046	3.3
Panel 3	320	120.13	4.686	3.9	1.090	0.9	3.125	2.6	5.737	4.8
Panel 4	320	196.26	7.009	3.6	1.682	0.9	5.307	2.7	8.951	4.6

[a] Total variability contains within-run, between-run and between-day variance components.

Representative performance data are shown. Results obtained at individual laboratories may vary.

Analytical Sensitivity

The sensitivity of the ARCHITECT AFP assay was calculated to be less than 0.4 ng/mL. Sensitivity is defined as the concentration at two standard deviations above the mean RLU for the ARCHITECT AFP Calibrator 1 (0 ng/mL) and represents the lowest measurable concentration of AFP that can be distinguished from zero.

Specificity

The specificity of the ARCHITECT AFP assay is designed to have ≤ 10% cross reactivity when tested with the interfering compounds listed below. The specificity of the ARCHITECT AFP assay was determined by testing sera containing the compounds listed below. These compounds showed less than 10% interference in the ARCHITECT AFP assay at the levels indicated.

Test Compound	Test Concentration
Bilirubin	22 mg/dL
Hemoglobin	550 mg/dL
Total Protein	1.7 to 12.4 g/dL
Triglycerides	3,960 mg/dL
Acetaminophen	6.5 mg/mL
Albumin	160 mg/mL
Alpha-1-Acid Glycoprotein	2.0 mg/mL
Alpha-1-Antitrypsin	5 mg/mL
Alpha-Globulins	32 mg/mL
Aspirin	1 mg/mL
Bleomycin	1,000 µU/mL
Ceruloplasmin	2.5 mg/mL
Cisplatin	1,000 µg/mL
Human Chorionic Gonadotropin	1,000 IU/mL
Placental Lactogen	100 µg/mL
Prolactin	500 ng/mL
Transferrin	25 mg/mL
Vinblastine	500 µg/mL
Maternal Vitamin	N/A

Potentially Interfering Clinical Conditions

The ARCHITECT AFP assay is designed to minimize HAMA interference. An internal study was conducted by spiking up to 25 µg/mL of Purified Human Anti-Mouse Antibody (HAMA-PUR) from Bioreclamation, Inc. (Hicksville, NY) into samples containing 169.56 ng/mL AFP and exhibited <6% interference.

Carryover

No detectable carryover (less than 5 PPM) was observed when a sample containing 91,080 ng/mL of AFP was assayed. Under circumstances where AFP assays are performed on the same module as a high frequency of ARCHITECT B12 assays, carryover of analyte may occur causing falsely elevated results.

Note: To maintain optimum system performance and reduce the potential of carryover due to protein build up on the sample pipettor probe, it is important to follow the routine maintenance procedures defined in Section 9 of the ARCHITECT System Operations Manual, or, for troubleshooting information refer to the ARCHITECT System Operations Manual, Section 10.

High Dose Hook

High dose hook is a phenomenon whereby very high level specimens may read within the dynamic range of the assay. For the ARCHITECT AFP assay, no high dose hook effect was observed when samples containing up to 1,900,000 ng/mL of AFP were assayed.

WHO Recovery

The ARCHITECT AFP assay is designed to have a recovery range of between 90-110% at the control target concentrations using the World Health Organization (WHO) First International Standard 72/225 for AFP.

The World Health Organization (WHO) International Standard 72/225 for AFP was prepared in normal male serum at three WHO sample target levels (20 ng/mL, 80 ng/mL and 175 ng/mL) using the conversion factor of 0.83 International Units per nanogram of AFP and tested on four different instruments. The average percent recovery for each target level and overall grand mean percent recovery across all levels of AFP WHO from this study are displayed in the following table:

ARCHITECT AFP WHO Recovery

	Low Sample (20 ng/mL)	Medium Sample (80 ng/mL)	High Sample (175 ng/mL)
Average % recovery	104.04%	101.02%	100.94%
Overall % recovery	102.00%		

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