

ARCHITECT

SYSTEM



en

AFP

REF 3P36

B3P360

G6-2601/R06

Read Highlighted Changes
Revised October 2015

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	<i>In Vitro</i> Diagnostic Medical Device	REACTION VESSELS	Reaction Vessels
LOT	Lot Number	REAGENT LOT	Reagent Lot
	Expiration Date	REPLACEMENT CAPS	Replacement Caps
SN	Serial Number	SAMPLE CUPS	Sample Cups
SEPTUM	Septum	WARNING: SENSITIZER	Warning: May cause an allergic reaction
	Store at 2-8°C	CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
	Consult instructions for use	GTIN	Global Trade Item Number
	Manufacturer	PRODUCT OF IRELAND	Product of Ireland
		INFORMATION FOR USA ONLY	Information needed for United States of America only

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

WARNING: The concentration of alpha-fetoprotein (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 expected values for the new assay based on serum or plasma and amniotic fluid from pregnant women with confirmed gestational age.

Caution: United States 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.

NAME

ARCHITECT AFP

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 monitoring disease progression during the course of disease and treatment 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.^{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 metastases¹⁶ 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.^{21,31} 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 demise³⁴⁻³⁷ may exhibit abnormally high levels of MSAFP. Increased MSAFP values are also seen in multiple pregnancies³⁸ 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.^{29,39}

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 for the quantitative measurement of AFP in human serum, plasma and amniotic fluid using CMIA technology, with flexible assay protocols, referred to as Chemiflex.

In the first step, sample 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 to create a reaction mixture in the second step. Following another wash cycle, pre-trigger and trigger solutions are 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* System optics.

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

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 (3P36)

- **MICROPARTICLES** 1 or 4 Bottle(s) (6.6 mL/27.0 mL) Anti-AFP (mouse, monoclonal) coated microparticles in MES buffer with protein (bovine) stabilizer. Minimum concentration: 0.1% solids. Preservative: ProClin 300.
- **CONJUGATE** 1 or 4 Bottle(s) (5.9 mL/26.3 mL) Anti-AFP (mouse, monoclonal) acridinium-labeled conjugate in MES buffer with protein (bovine) stabilizer. Minimum concentration: 400 ng/mL. Preservatives: antimicrobial agents and sodium azide.

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

- **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 be handled in accordance with the OSHA Standard on Bloodborne Pathogens⁴⁰. Biosafety Level 2⁴¹ or other appropriate biosafety practices^{42,43} should be used for materials that contain or are suspected of containing infectious agents.
- The following warnings and precautions apply to this component:
 - Conjugate

Contains sodium azide.

EUH032 Contact with acids liberates very toxic gas.

This material and its container must be disposed of in a safe way.

- The following warnings and precautions apply to this component:
 - Microparticles



WARNING: Contains methylisothiazolones
H317 May cause an allergic skin reaction.

Prevention

P261 Avoid breathing mist/vapours/spray.
P272 Contaminated work clothing should not be allowed out of the workplace.
P280 Wear protective gloves/protective clothing/eye protection.

Response

P302+P352 IF ON SKIN: Wash with plenty of water.
P333+P313 If skin irritation or rash occurs: Get medical advice/attention.
P362+P364 Take off contaminated clothing and wash it before reuse.

This material and its container must be disposed of in a safe way.

- Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.
- For information on the safe disposal of sodium azide and 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 pool reagents within a kit or between reagent kits.**
- Before loading the ARCHITECT AFP Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may 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 the 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 and 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

-  The ARCHITECT AFP Reagent Kit must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
- When stored and handled as directed, the 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.** For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

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 are invalid and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

- The ARCHITECT AFP assay is designed for use on the ARCHITECT *i* System.
- The ARCHITECT AFP assay file (assay number 003) must be installed on the ARCHITECT *i* System before 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 as follows:
 - (Concentration in ng/mL) x (0.83) = IU/mL

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

- Serum, plasma, or amniotic fluid specimens may be used with the ARCHITECT AFP assay.
- The specimen collection tubes listed below were verified for use for serum and plasma with the ARCHITECT AFP assay. Other specimen collection tubes have not been tested with this assay.
 - Human serum, plastic (including serum collected in plastic serum separator tubes).
 - Human plasma collected in:
 - sodium heparin, plastic
 - dipotassium EDTA, plastic
 - lithium heparin, plastic
 - sodium EDTA, glass
- 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
- When serial specimens are being evaluated, the same type of specimen should be used.

- 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.
- Performance has not been established for the use of cadaveric specimens or body fluids other than human serum, plasma, or amniotic fluid.
- The ARCHITECT *i* System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the ARCHITECT AFP assay.

Specimen Conditions

- Do not use specimens with the following conditions:
 - heat-inactivated
 - pooled
 - grossly hemolyzed
 - obvious microbial contamination
- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- For optimal results, inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for serum and plasma collection tubes. Gravity separation is not sufficient for specimen preparation.
- Prepare frozen specimens as follows:
 - Frozen specimens must be completely thawed before mixing.
 - Mix thawed specimens thoroughly by inverting 10 times or by low speed vortexing. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous. If samples are not mixed thoroughly, inconsistent results may be obtained.
 - Centrifuge mixed specimens as described below.
- To ensure consistency in results, specimens must be transferred to a centrifuge tube and centrifuged at $\geq 10,000$ RCF (Relative Centrifugal Force) for 10 minutes before testing if
 - they contain fibrin, red blood cells, or other particulate matter or
 - they were frozen and thawed.
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or secondary tube. Care must be taken to transfer only the clarified specimen without the lipemic material.
- Transfer clarified specimen to a sample cup or secondary tube for testing.

Storage

Serum or Plasma

- Specimens may be stored on or off the clot, red blood cells, or separator gel for
 - up to 3 days at room temperature or
 - up to 7 days at 2-8°C.
- If testing will be delayed more than 7 days, remove serum or plasma from the clot, red blood cells, or separator gel and store at -20°C or colder.
- Avoid more than 5 freeze/thaw cycles.

Amniotic Fluid

- Specimens may be stored for
 - up to 2 days at room temperature or
 - up to 5 days at 2-8°C.
- If testing is delayed more than 5 days, store at -20°C or colder.
- Avoid more than 3 freeze/thaw cycles.

Shipping

- Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

- 3P36 ARCHITECT AFP Reagent Kit

Materials Required but not Provided

- ARCHITECT *i* System
- ARCHITECT AFP Assay file, may be obtained from:
 - ARCHITECT *i* System e-Assay CD-ROM found on www.abbottdiagnostics.com
 - ARCHITECT *i* System Assay CD-ROM
- 3P36-01 ARCHITECT AFP Calibrators
- 3P36-10 ARCHITECT AFP Controls or other control material
- 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 specified volumes.

For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

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 may have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
 - **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.
 - **If the microparticles do not resuspend, DO NOT USE. Contact your Abbott representative.**
 - Once the microparticles have been resuspended, place a septum on the bottle. For instructions about placing septums on bottles, refer to the **Handling Precautions** section of this package insert.
- Load the ARCHITECT AFP Reagent Kit on the ARCHITECT *i* System.
 - Verify that all necessary reagents are present.
 - Ensure that septums are present on all reagent bottles.
- Order calibration, if necessary.
 - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.
 - For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- 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 before running the test.
 - Priority: 75 μ L for the first AFP test plus 25 μ L for each additional AFP test from the same sample cup.
 - ≤ 3 hours on-board: 150 μ L for the first AFP test plus 25 μ L for each additional AFP test from the same sample cup.
 - > 3 hours on-board: replace with a fresh sample (patient specimens, controls and calibrators).
 - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.

- Prepare calibrators and controls.
 - Mix the ARCHITECT AFP Calibrators and Controls by gentle inversion before use.
 - 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.
- For additional information on principles of operation, refer to the ARCHITECT System Operations Manual, Section 3.
- For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Specimen Dilution Procedures

- Specimens with an AFP concentration greater than 2000 ng/mL will be flagged as “> 2000.00 ng/mL” and may be diluted using either the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol for Serum or Plasma Specimens

- If the Automated Dilution Protocol is chosen, use the 1:10 dilution for serum or plasma. The system automatically calculates the concentration of the sample before dilution and reports the result.
- Dilutions other than the automated 1:10 serum or plasma dilution should be done manually.

Automated Dilution Protocol for Amniotic Fluid Specimens

NOTE: Amniotic fluid specimens must be diluted.

- If the Automated Dilution Protocol is chosen, amniotic fluid **MUST ONLY USE** the 1:40 dilution. The system automatically calculates the concentration of the sample before dilution and reports the result.
- Dilutions other than the automated 1:40 amniotic fluid dilution should be done manually.

Manual Dilution Procedure for All Specimen Types

NOTE: The ARCHITECT *i* Multi-Assay Manual Diluent (7D82-50) must be used when performing the manual dilution procedure.

- For a 1:20 dilution, add 50 µL of the patient specimen to 950 µL of the ARCHITECT *i* Multi-Assay Manual Diluent (7D82-50). For a 1:101 dilution, add 10 µL of the patient specimen to 1 mL of the ARCHITECT *i* Multi-Assay Manual Diluent (7D82-50).
- The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution.
- For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- To perform an ARCHITECT AFP calibration, test calibrators A, B, C, D, E and F in replicates of 2. The calibrators should be priority loaded.
- Calibration Range: 0 to 2000 ng/mL.
- A single sample of each control level must be tested to evaluate the assay calibration.
 - Order controls as described in the **Assay Procedure** section.
 - Ensure that assay control values are within the ranges specified in the control package insert.
- 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 that a single sample of each control be tested once every 24 hours each day of use. If your laboratory quality control procedures require more frequent use of controls to verify test results, follow those procedures. Additional controls may be tested in conformance with local, state and/or federal regulations or accreditation requirements and your laboratory's quality control policy.

Each laboratory should establish control means and ranges to monitor the acceptable performance of the assay. If a control is out of its specified range, the associated test results are invalid and samples must be retested. Recalibration may be indicated.

After the median AFP values have been established for maternal serum/plasma and amniotic fluid, the control means should remain within acceptable limits set by the laboratory. The acceptability of each calibration should be closely monitored by the controls using guidance from CLSI I/LA25-A2,⁴⁵ the National Academy of Clinical Biochemistry (NACB),⁴⁶ and/or the laboratories internal operating procedures to detect any shifts which may require assay recalibration or re-evaluation of the maternal serum/plasma and amniotic fluid medians.

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 uses a 4 Parameter Logistic Curve fit (4PLC, Y-weighted) data reduction method to generate a calibration curve.

Alternate Result Unit

- 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
- To convert amniotic fluid values to µg/mL, divide the reported AFP concentration (ng/mL) by 1000, as this calculation is not performed automatically.

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.

Measuring Interval (Reportable Range)

Measuring interval is defined as the range of values in ng/mL which meets the limits of acceptable performance for both imprecision and bias for an undiluted sample. For the studies described in this package insert, the range was 2.00 ng/mL (Limit of Quantitation - LoQ) to 2000.00 ng/mL. When using the 1:10 automated dilution protocol, the assay can report values up to 20,000.00 ng/mL. When using the 1:40 automated dilution protocol, the assay can report values up to 80,000.00 ng/mL.

LIMITATIONS OF THE PROCEDURE

- If the AFP results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).^{47,48} Specimens containing HAMA may produce anomalous values when tested with assay kits such as ARCHITECT AFP that employ mouse monoclonal antibodies.⁴⁷
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.⁴⁹ Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous results may be observed. Additional information may be required for diagnosis.
- Although the ARCHITECT AFP assay is specifically designed to minimize the effects of HAMA and heterophilic antibodies, assay results that are not consistent with other clinical observations may require additional information 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.
- The ARCHITECT AFP assay should not be used as a cancer screening test.
- 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 package insert.

- Amniotic fluid specimens contaminated with fetal blood may exhibit abnormally high AFP values which may lead to misinterpretation of test results. 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. For further information, refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section in this package insert.
- The reliability of MSAFP evaluation in prenatal testing is dependent upon the accurate determination of gestational age. An inaccurate estimation of gestational age may result in an inaccurate estimation of risk of NTD. 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.
- Refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section of this package insert for specimen limitations.

EXPECTED VALUES

Data in the **EXPECTED VALUES** section were generated using the ARCHITECT *i* 2000/*i* 2000_{SR} Systems. The distribution of ARCHITECT AFP values was determined in 400 specimens from apparently healthy individuals (200 males and 200 females), in 238 patients with nonmalignant diseases and in 224 patients diagnosed with malignant diseases. The data are summarized in the following tables.

Distribution of ARCHITECT AFP Values

Group/Category	n	Distribution of Values (%) by AFP Concentration Range in ng/mL						
		0	>8.78	>15	>200	>500	>1000	>2000
		- 8.78	- 15.00	- 200	- 500	- 1000	- 2000	>2000
Apparently Healthy Subjects	400	97.5	2.0	0.5 ^a	0.0	0.0	0.0	0.0

^a These 2 samples had AFP concentrations of 25.16 and 27.81 ng/mL. The observed nonparametric central 95% of the 400 apparently healthy individuals ranged from 0.89 to 8.78 ng/mL. It is recommended that each laboratory establish its own expected reference range for the population of interest.

Distribution of ARCHITECT AFP Values

Group/Category	n	Distribution of Values (%) by AFP Concentration Range in ng/mL						
		0	>8.78	>15	>200	>500	>1000	>2000
		- 8.78	- 15.00	- 200	- 500	- 1000	- 2000	>2000
Nonmalignant Disease								
Cirrhosis	49	98.0	2.0	0.0	0.0	0.0	0.0	0.0
Genitourinary	26	100.0	0.0	0.0	0.0	0.0	0.0	0.0
Hepatitis	149	90.6	6.7	2.0	0.0	0.0	0.0	0.7
Pancreatitis	14	92.9	7.1	0.0	0.0	0.0	0.0	0.0
Malignant Disease^a								
Gastrointestinal	64	98.4	1.6	0.0	0.0	0.0	0.0	0.0
Hepatocellular	29	69.0	0.0	17.2	0.0	0.0	0.0	13.8
Pancreatic	34	85.3	0.0	5.9	2.9	0.0	2.9	2.9
Nonseminoma Testicular	72	87.5	1.4	9.7	1.4	0.0	0.0	0.0
Seminoma Testicular	25	100.0	0.0	0.0	0.0	0.0	0.0	0.0

^a The nonseminoma testicular samples were from treated patients. The disease status was unknown for specimens from the other malignant diseases.

AFP Values in Maternal Serum and Amniotic Fluid

Due to variations in populations at different locations, it is important for each laboratory to establish its own medians for each gestational week for maternal serum and amniotic fluid. AFP values should be expressed as multiples of the median (MoM) as shown in the calculation below:

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

Each laboratory should attempt to gather approximately 100 or more specimens for each gestational week in order to arrive at median values⁵⁰, then utilize a cutoff value (MoM) which most closely suits its needs for specificity and sensitivity.

A total of 685 maternal serum and 687 amniotic fluid specimens from unaffected or low-risk singleton pregnancies were evaluated with the ARCHITECT AFP assay. The AFP values expressed as the regressed medians and multiples of the regressed medians (MoM) for gestational weeks 15 to 21 are shown in the following tables.

Maternal Serum AFP

Gestational Week	n	Regressed Medians ^a (ng/mL)	Multiples of Regressed Medians (ng/mL)		
			2.0	2.5	3.0
15	101	32.17	64.35	80.44	96.52
16	95	36.86	73.73	92.16	110.59
17	102	42.24	84.48	105.60	126.72
18	103	48.40	96.79	120.99	145.19
19	101	55.45	110.90	138.63	166.35
20	106	63.53	127.07	158.84	190.60
21	77	72.80	145.59	181.99	218.39

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

Amniotic Fluid AFP

Gestational Week	n	Regressed Medians ^a (µg/mL)	Multiples of Regressed Medians (µg/mL)		
			2.0	2.5	3.0
15	104	16.41	32.82	41.02	49.22
16	108	13.38	26.76	33.45	40.14
17	105	10.91	21.82	27.27	32.72
18	109	8.89	17.79	22.23	26.68
19	102	7.25	14.50	18.13	21.75
20	97	5.91	11.83	14.78	17.74
21	62	4.82	9.64	12.05	14.46

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

Note: AFP values were assigned on the basis of completed gestational weeks. For example, a specimen obtained on gestational day 132 (week 18, day 6) was assigned week 18, because the gestation had only completed 18 gestational weeks, plus 6 days.

Clinical Specificity and Sensitivity

The specificity and sensitivity estimates (and associated 95% confidence intervals) of the ARCHITECT AFP assay were determined 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 open NTD and sensitivity is the probability that the test will be positive in the presence of open NTD.

The specificity table represents data gathered on unaffected singleton pregnancies from 15 to 21 weeks gestation using the ARCHITECT AFP assay. The data are summarized in the following table.

Specimen Type	n	Specificity (95% Confidence Interval) by Multiples of the Median (MoM)		
		2.0	2.5	3.0
Maternal Serum	682	95.45% (93.61%, 96.89%)	98.24% (96.95%, 99.09%)	99.71% (98.94%, 99.96%)
Amniotic Fluid	222	98.65% (96.10%, 99.72%)	99.10% (96.78%, 99.89%)	99.55% (97.52%, 99.99%)

The sensitivity table represents data gathered on confirmed affected, singleton pregnancies using the ARCHITECT AFP assay. The data are summarized in the following table.

Specimen Type	n	Sensitivity (95% Confidence interval) by Multiples of the Median (MoM)		
		2.0	2.5	3.0
Maternal Serum	21	95.24% (76.18%, 99.88%)	80.95% (58.09%, 94.55%)	71.43% (47.82%, 88.72%)
Amniotic Fluid	19	100.00% (82.35%, 100.00%)	94.74% (73.97%, 99.87%)	94.74% (73.97%, 99.87%)

AFP Serial Monitoring Performance

In conjunction with physical examination, histology/pathology, and other clinical evaluation procedures, changes observed in serial AFP assay values should be evaluated when monitoring nonseminomatous testicular cancer.

The reference change value (RCV) was used to determine if a significant change in AFP occurred.⁵¹ For this calculation, the RCV for each assay (ARCHITECT AFP and the comparator) was derived by taking into account the published biological variation for AFP⁵² and the total imprecision of the specific assay. The RCV for the ARCHITECT AFP method was calculated to be 39.22% and that of the comparator to be 39.98%. A minimum of 3 serial samples were obtained from each of 72 subjects and were analyzed to determine the change in AFP concentration per sequential pair (n=207). The data are summarized in the following tables.

% Change in AFP	Change in Disease Status				
	R n (%)	S n (%)	NED n (%)	P n (%)	Total n (%)
> RCV Increase	7 (3.38)	3 (1.45)	9 (4.35)	8 (3.86)	27 (13.04)
No Significant Change	20 (9.66)	38 (18.36)	70 (33.82)	18 (8.70)	146 (70.53)
> RCV Decrease	8 (3.86)	12 (5.80)	5 (2.42)	9 (4.35)	34 (16.43)
Total	35 (16.91)	53 (25.60)	84 (40.58)	35 (16.91)	207 (100.00)

R = Responding; S = Stable; NED = No Evidence of Disease; P = Progressing.

SPECIFIC PERFORMANCE CHARACTERISTICS

All performance studies were conducted using the ARCHITECT *i* 2000/*i* 2000_{SR} Systems. Assay results obtained in individual laboratories may vary from data presented.

Precision

The ARCHITECT AFP assay is designed to have an imprecision of ≤ 7.5% within-laboratory (Total) %CV for samples between 10 and 2000 ng/mL and an SD of ≤ 0.75 for samples less than 10 ng/mL down to the LoQ (*i.e.*, 2.0 ng/mL).

System Reproducibility

A 5-day precision study was performed for the ARCHITECT AFP assay based on guidance from the National Committee for Clinical Laboratory Standards (NCCLS) document EP5-A2⁵³ and the Clinical and Laboratory Standards Institute (CLSI) document EP15-A2.⁵⁴ Testing was conducted at 3 clinical sites using 3 lots each of ARCHITECT AFP Reagents, Calibrators and Controls and 1 ARCHITECT *i* 2000/*i* 2000_{SR} instrument per site. Three controls and 5 human serum panels were assayed in replicates of 4 at 2 separate times of day for 5 days. The results are summarized in the following table.

Sample	n	Grand Mean (ng/mL)	Within-Run		Within-Day		Within-Laboratory Precision (Total) ^a		Precision with Additional Component of Between-Site		Precision with Additional Component of Between-Lot		Precision with Additional Components of Site and Lot (Overall) ^b	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Control	360	19.46	0.685	3.5	0.747	3.8	0.747	3.8	0.797	4.1	0.813	4.2	0.830	4.3
Medium Control	360	203.65	7.502	3.7	7.987	3.9	7.987	3.9	13.984	6.9	17.293	8.5	17.293	8.5
High Control	360	973.10	45.461	4.7	46.393	4.8	46.450	4.8	63.066	6.5	65.166	6.7	65.891	6.8
Panel 1	360	2.95	0.114	3.9	0.127	4.3	0.128	4.4	0.239	8.1	0.278	9.4	0.278	9.4
Panel 2	360	9.47	0.355	3.7	0.359	3.8	0.377	4.0	0.506	5.3	0.486	5.1	0.527	5.6
Panel 3	360	591.26	25.303	4.3	25.620	4.3	26.503	4.5	42.717	7.2	50.677	8.6	50.677	8.6
Panel 4	360	1511.80	70.406	4.7	75.992	5.0	81.050	5.4	90.629	6.0	90.629	6.0	90.629	6.0
Panel 5	360	1743.35	79.509	4.6	86.121	4.9	89.956	5.2	100.632	5.8	100.632	5.8	100.632	5.8

^a Within-Laboratory (Total) variability contains within-run, within-day and between-day variance components.

^b Overall variability contains within-run, within-day, between-day, between-lot, between-site and lot-site interaction variance components.

Within-Laboratory Precision

A study was performed based on guidance from the NCCLS document EP5-A2.⁵³ Testing was conducted using 3 lots of ARCHITECT AFP Reagents and Calibrators, 1 lot of ARCHITECT AFP Controls and 4 instruments. Three controls and 5 human serum panels were assayed in a minimum of 2 replicates at 2 separate times per day for 20 different days. Each reagent lot used a single calibration curve throughout the study. The data are summarized in the following table.

% Change in AFP	Change in Disease Status		
	Progression	No Progression	Total
> 39.22% Increase	8 (A)	19 (B)	27 (A+B)
≤ 39.22% Increase	27 (C)	153 (D)	180 (C+D)
Total	35 (A+C)	172 (B+D)	207 (A+B+C+D)

Specificity = $D / (B+D) \times 100\% = 88.95\%$; 95% CI = 84.35% to 93.55%

Sensitivity = $A / (A+C) \times 100\% = 22.86\%$; 95% CI = 9.38% to 40.00%

Negative Predictive Value =

$D / (C+D) \times 100\% = 85.00\%$; 95% CI = 78.34% to 90.68%

Positive Predictive Value =

$A / (A+B) \times 100\% = 29.63\%$; 95% CI = 12.00% to 52.17%

In addition, samples were analyzed on a per subject basis. Efficacy is demonstrated when the sum of sensitivity and specificity are greater than one. In this study, the RCV efficacy for monitoring testicular cancer was determined to be 1.12 with a 95% CI of 0.98 to 1.25.

The change in AFP concentration results available on both the ARCHITECT AFP assay and the comparator AFP assay were analyzed for agreement using their respective RCV.

ARCHITECT AFP	Comparator AFP		
	> 39.98% Increase	≤ 39.98% Increase	Total
> 39.22% Increase	18 (A)	9 (B)	27 (A+B)
≤ 39.22% Increase	12 (C)	166 (D)	178 (C+D)
Total	30 (A+C)	175 (B+D)	205 (A+B+C+D)

Overall Agreement =

$(A+D) / (A+B+C+D) \times 100\% = 89.76\%$; 95% CI = 84.77% to 93.55%

Positive Agreement =

$A / (A+C) \times 100\% = 60.00\%$; 95% CI = 40.60% to 77.34%

Negative Agreement =

$D / (B+D) \times 100\% = 94.86\%$; 95% CI = 90.46% to 97.62%

Instrument	Reagent Lot	Sample	n	Mean (ng/mL)	Within-Run		Within-Laboratory Precision (Total) ^a	
					SD	%CV	SD	%CV
<i>i</i> 2000 _{SR} (1)	1	Low Control	120	19.81	0.317	1.6	0.327	1.6
		Medium Control	120	199.11	3.165	1.6	3.263	1.6
		High Control	120	950.53	16.411	1.7	17.200	1.8
	2	Low Control	120	20.02	0.349	1.7	0.349	1.7
		Medium Control	120	195.29	2.725	1.4	3.043	1.6
		High Control	120	928.69	16.340	1.8	17.628	1.9
	3	Low Control	120	20.23	0.248	1.2	0.286	1.4
		Medium Control	120	198.45	2.743	1.4	3.058	1.5
		High Control	120	955.96	17.389	1.8	17.389	1.8
<i>i</i> 2000 _{SR} (2)	1	Panel 1	120	3.01	0.070	2.3	0.082	2.7
		Panel 2	120	9.54	0.191	2.0	0.201	2.1
		Panel 3	120	577.58	13.137	2.3	13.977	2.4
		Panel 4	120	1514.74	41.437	2.7	47.765	3.2
		Panel 5	120	1763.53	43.353	2.5	51.115	2.9
	2	Panel 1	120	3.10	0.060	1.9	0.065	2.1
		Panel 2	120	9.67	0.188	1.9	0.202	2.1
		Panel 3	120	564.10	13.445	2.4	14.358	2.5
		Panel 4	120	1489.93	43.567	2.9	44.077	3.0
		Panel 5	120	1729.19	50.297	2.9	54.344	3.1
	3	Panel 1	120	3.15	0.061	1.9	0.068	2.2
		Panel 2	120	9.73	0.190	2.0	0.197	2.0
		Panel 3	120	559.72	12.053	2.2	12.053	2.2
		Panel 4	120	1490.94	43.967	2.9	45.619	3.1
		Panel 5	120	1743.06	53.149	3.0	55.158	3.2
<i>i</i> 2000 (1)	1	Low Control	120	19.53	0.403	2.1	0.419	2.1
		Medium Control	120	192.55	3.896	2.0	4.161	2.2
		High Control	120	925.64	20.138	2.2	22.571	2.4
	2	Low Control	120	19.60	0.460	2.3	0.476	2.4
		Medium Control	120	192.92	4.090	2.1	4.233	2.2
		High Control	120	917.39	25.481	2.8	26.696	2.9
	3	Low Control	120	19.46	0.412	2.1	0.438	2.2
		Medium Control	120	194.92	3.602	1.8	3.602	1.8
		High Control	120	942.88	25.442	2.7	26.752	2.8
<i>i</i> 2000 (2)	1	Panel 1	120	3.05	0.075	2.5	0.082	2.7
		Panel 2	120	9.63	0.204	2.1	0.205	2.1
		Panel 3	120	569.00	15.652	2.8	16.041	2.8
		Panel 4	120	1530.79	56.561	3.7	60.769	4.0
		Panel 5	120	1796.54	63.911	3.6	70.192	3.9
	2	Panel 1	120	3.10	0.070	2.3	0.074	2.4
		Panel 2	120	9.68	0.186	1.9	0.212	2.2
		Panel 3	120	553.43	13.334	2.4	13.972	2.5
		Panel 4	120	1454.43	46.282	3.2	49.527	3.4
		Panel 5	120	1693.81	53.646	3.2	56.319	3.3
	3	Panel 1	120	2.87	0.071	2.5	0.077	2.7
		Panel 2	120	9.31	0.237	2.6	0.262	2.8
		Panel 3	120	583.73	14.561	2.5	16.269	2.8
		Panel 4	120	1456.89	44.111	3.0	45.569	3.1
		Panel 5	120	1666.40	46.687	2.8	49.738	3.0

^a Within-Laboratory (Total) variability contains within-run, within-day and between-day variance components.

WHO Recovery

The ARCHITECT AFP assay is designed to have a recovery range of 100 ± 10% when analyzing samples spiked with known amounts of AFP using the WHO 1st International Standard 72/225.

A study was performed with 16 low-level AFP serum specimens and 14 amniotic fluid specimens. The serum specimens were spiked with the WHO 1st International Standard 72/225 to create test samples across the measuring interval of the assay. The amniotic fluid specimens were diluted 1:40 using the ARCHITECT *i* Multi-Assay Manual Diluent and spiked with the WHO 1st International Standard 72/225 to create test samples with AFP concentrations within the range of 312.5 to 1250 ng/mL.* The samples were tested using the ARCHITECT AFP assay on 1 instrument and the resulting percent recovery was calculated. For serum specimens, the mean percent recovery was 103.1% (range: 99.5% to 108.6%). For amniotic fluid specimens, the mean percent recovery was 101.2% (range: 95.1% to 107.3%).

* A 1:40 dilution of specimens in the range of 12.5 to 50 µg/mL equates to a range of 312.5 to 1250 ng/mL within the measuring interval of the assay.

Linearity

The ARCHITECT AFP assay is designed to have a deviation from linearity within ± 1 ng/mL for samples less than 10 ng/mL and within ± 10% for samples between 10 ng/mL and 2000 ng/mL.

A study was performed based on guidance from the NCCLS document EP6-A.⁵⁵ Three dilution series were prepared as follows: a high AFP sample (> 2000 ng/mL) was combined in specific ratios with a low AFP sample (< 2.0 ng/mL). The 3 dilution series, including the low-level and high-level samples, were tested using the ARCHITECT AFP assay. The ARCHITECT AFP assay demonstrated linearity from 0.91 ng/mL to 2487.76 ng/mL.

Sensitivity

Limit of Detection and Limit of Quantitation

The ARCHITECT AFP assay is designed to have a Limit of Detection (LoD) of ≤ 1.0 ng/mL and a Limit of Quantitation (LoQ) of ≤ 2.0 ng/mL. The LoQ is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total analytical error of ≤ 2.5 ng/mL.

Based on guidance from the NCCLS document EP17-A,⁵⁶ a study was performed with 4 zero-level samples (Calibrator A) and 8 low-level AFP samples (2 samples at each of 4 unique target concentration levels of approximately 0.50, 1.00, 1.50 and 2.00 ng/mL). These samples were tested in 5 separate runs over a minimum of 3 days using 3 reagent lots and 2 instruments. The observed LoD was 0.04 ng/mL and the observed LoQ was 0.5 ng/mL.

Limit of Blank

In the same study, the Limit of Blank (LoB) was determined to be 0.0 ng/mL.

Interference

Potentially Interfering Endogenous Substances

The ARCHITECT AFP assay is designed to have a difference in AFP concentration within or equal to ± 10% when comparing samples containing elevated levels of endogenous substances to reference samples.

A study was performed based on guidance from the CLSI document EP7-A2.⁵⁷ Potentially interfering endogenous substances were evaluated to determine whether AFP concentrations were affected when using the ARCHITECT AFP assay. The endogenous substances listed below were spiked into samples with 2 levels of AFP (approximately 10 and 1000 ng/mL). The samples were assayed (n = 20) and the AFP concentrations of the spiked samples were compared to reference samples. The data are summarized in the following table.

Potentially Interfering Endogenous Substance	High Test Level	% Interference ^a	
		10 ng/mL	1000 ng/mL
Bilirubin (Unconjugated)	20 mg/dL	-0.5	0.3
Bilirubin (Conjugated)	20 mg/dL	-0.9	-0.8
Hemoglobin	500 mg/dL	-0.2	-1.3
Total Protein	12 g/dL	2.9	-0.2
Triglycerides	3000 mg/dL	-1.0	-1.9

$$\text{Mean/Median Test Result} - \frac{\text{Mean/Median Reference Result}}{\text{Mean/Median Reference Result}} \times 100$$

^a % Interference = $\frac{\text{Mean/Median Test Result} - \text{Mean/Median Reference Result}}{\text{Mean/Median Reference Result}} \times 100$

Potentially Interfering Substances

The ARCHITECT AFP assay is designed to have a mean % recovery of 100% ± 10% when analyzing Rheumatoid Factor (RF) and Human Anti-Mouse Antibodies (HAMA) samples spiked with known amounts of AFP.

A study was performed based on guidance from the CLSI document EP7-A2.⁵⁷ Potentially interfering substances were evaluated to determine whether AFP concentrations were affected when using the ARCHITECT AFP assay. Specimens from individuals with the substances listed below were divided into 3 samples. Two of the samples were spiked to 2 levels of AFP (approximately 10 and 1000 ng/mL). The samples were assayed and the AFP concentrations of the spiked samples were compared to the samples that were not spiked with AFP. The data are summarized in the following table.

Potentially Interfering Substances	n	% Recovery ^a	
		10 ng/mL	1000 ng/mL
Human Anti-Mouse Antibodies	13	104.7	105.9
Rheumatoid Factor	13	104.6	102.3

$$^a \quad \% \text{ Recovery} = \frac{\text{Mean/Median Spiked Result} - \text{Mean/Median Unspiked Result}}{\text{Mean/Median Amount AFP Added}} \times 100$$

Analytical Specificity

The ARCHITECT AFP assay is designed to have a difference in AFP concentration within or equal to ± 10% when comparing samples containing potential interferents to reference samples.

A study was performed based on guidance from the CLSI document EP7-A2.⁵⁷ Potential interferents were evaluated to determine whether AFP concentrations were affected when using the ARCHITECT AFP assay. The potential interferents were spiked into samples with 2 levels of AFP (approximately 10 and 1000 ng/mL). The samples were assayed and the AFP concentrations of the spiked samples were compared to the reference samples. The data are summarized in the following table.

Potential Interferent	High Test Level	% Interference ^a	
		10 ng/mL	1000 ng/mL
5-Fluorouracil	3 mmol/L	-0.4	0.3
Acetaminophen	6.5 mg/mL	-3.1	-3.1
Albumin	160 mg/mL	2.4	-4.1
Alpha-1-Acid Glycoprotein	2 mg/mL	0.2	-1.1
Alpha-1-Antitrypsin	5 mg/mL	8.1	0.3
Alpha-2-Macroglobulin	9 mg/mL	0.1	-0.1
Aspirin	10 mg/mL	-4.7	-4.9
Bleomycin	1000 µU/mL	-2.5	-4.3
Carboplatin	0.432 mg/mL	0.3	1.3
Ceruloplasmin	2.5 mg/mL	-0.3	-0.6
Chorionic Gonadotropin	1000 IU/mL	-1.1	-2.2
Cisplatin	1000 µg/mL	-0.6	-1.2
Cyclophosphamide	1437 µmol/L	0.3	-0.7

Potential Interferent	High Test Level	% Interference ^a	
		10 ng/mL	1000 ng/mL
Etoposide	30 µg/mL	-0.8	0.3
Gamma-Globulins	30 mg/mL	-2.7	-2.4
Haptoglobin	6 mg/mL	0.7	-1.0
Ifosfamide	249 µg/mL	-3.1	-2.7
Methotrexate	2 mmol/L	-0.6	-0.5
Placental Lactogen	100 µg/mL	-3.3	-3.6
Prolactin	500 ng/mL	-4.8	-5.0
Transferrin	25 mg/mL	-1.6	-3.4
Vinblastine	500 µg/mL	-3.4	-3.5
Vincristine	1000 ng/mL	-3.1	-4.4

$$^a \quad \% \text{ Interference} = \frac{\text{Mean/Median Test Result} - \text{Mean/Median Reference Result}}{\text{Mean/Median Reference Result}} \times 100$$

Autodilution Verification

The ARCHITECT AFP assay is designed to have a mean difference in concentration within ± 10% when comparing the autodilution method to the manual dilution method for samples with values > 2000 ng/mL.

Twenty-one serum samples were evaluated with the 1:10 autodilution method versus a 1:10 manual dilution method. Fifteen amniotic fluid samples were evaluated with the 1:40 autodilution method versus a 1:40 manual dilution method.

The manually diluted samples and the undiluted samples designated for autodilution were assayed in replicates of 2 using the ARCHITECT AFP assay. For serum samples, the mean percent difference was 2.9% (range: -5.8% to 10.9%) and for amniotic fluid samples, the mean percent difference was 4.6% (range: -1.1% to 11.2%).

High Dose Hook

High dose hook is a phenomenon whereby very high level specimens may read within the measuring interval of the assay. For the ARCHITECT AFP assay, no high dose hook effect was observed when samples containing up to 10,000,000 ng/mL of AFP were assayed.

ARCHITECT *i*1000_{SR} SYSTEM SPECIFIC STUDIES

The following studies were conducted using the ARCHITECT *i*1000_{SR} System. Assay results obtained in individual laboratories may vary from data presented.

Precision

The ARCHITECT AFP assay is designed to have an imprecision of ≤ 7.5% within-laboratory (Total) %CV for samples between 10 and 2000 ng/mL and an SD of ≤ 0.75 for samples less than 10 ng/mL down to the LoQ (*i.e.*, 2.0 ng/mL).

System Reproducibility

A 5-day precision study was performed for the ARCHITECT AFP assay based on guidance from the National Committee for Clinical Laboratory Standards (NCCLS) document EP5-A2⁵³ and the Clinical and Laboratory Standards Institute (CLSI) document EP15-A2.⁵⁴ Testing was conducted at 3 clinical sites using 1 lot of ARCHITECT AFP Reagents, Calibrators, and Controls and 1 ARCHITECT *i*1000_{SR} instrument per site. Three controls and 5 human serum panels were assayed in replicates of 4 at 2 separate times of day for 5 days. The results are summarized in the following table.

Sample	n	Grand Mean (ng/mL)	Within-Run		Within-Day		Within-Laboratory Precision (Total) ^a		Precision with Additional Component of Between-Site (Overall)	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Control	120	20.02	0.319	1.6	0.373	1.9	0.522	2.6	0.522	2.6
Medium Control	120	200.45	2.835	1.4	2.901	1.4	4.662	2.3	5.350	2.7
High Control	120	959.62	16.945	1.8	20.440	2.1	26.446	2.8	26.446	2.8
Panel 1	120	3.05	0.049	1.6	0.084	2.8	0.121	4.0	0.144	4.7
Panel 2	120	9.69	0.149	1.5	0.198	2.0	0.264	2.7	0.290	3.0
Panel 3	120	578.85	9.449	1.6	11.025	1.9	14.212	2.5	15.580	2.7
Panel 4	120	1494.96	36.675	2.5	38.393	2.6	48.435	3.2	49.885	3.3
Panel 5	120	1723.05	37.272	2.2	44.664	2.6	50.517	2.9	59.295	3.4

^a Within-Laboratory (Total) variability contains within-run, within-day, and between-day variance components.

Within-Laboratory Precision

A study was performed based on guidance from the NCCLS document EP5-A2.⁵³ Testing was conducted using 3 lots of ARCHITECT AFP Reagents and Calibrators, 1 lot of ARCHITECT AFP Controls, and 2 instruments. Three controls and 5 human serum panels were assayed in a minimum of 2 replicates at 2 separate times per day for 20 different days. Each reagent lot used a single calibration curve throughout the study. The data are summarized in the following table.

Instrument	Reagent Lot	Sample	n	Mean (ng/mL)	Within-Run		Within-Laboratory Precision (Total) ^a	
					SD	%CV	SD	%CV
<i>i</i> 1000 _{SR} (1)	1	Low Control	120	19.91	0.431	2.2	0.495	2.5
		Medium Control	120	195.96	3.684	1.9	4.274	2.2
		High Control	120	953.56	24.911	2.6	27.613	2.9
	2	Low Control	120	19.61	0.373	1.9	0.429	2.2
		Medium Control	120	194.04	3.712	1.9	4.630	2.4
		High Control	120	941.16	25.317	2.7	28.814	3.1
	3	Low Control	120	19.68	0.407	2.1	0.491	2.5
		Medium Control	120	195.83	3.767	1.9	4.335	2.2
		High Control	120	939.12	23.829	2.5	24.118	2.6
<i>i</i> 1000 _{SR} (2)	1	Panel 1	120	3.05	0.057	1.9	0.063	2.1
		Panel 2	119	9.67	0.143	1.5	0.212	2.2
		Panel 3	120	573.62	11.594	2.0	14.622	2.5
		Panel 4	120	1492.07	35.498	2.4	45.627	3.1
		Panel 5	120	1731.59	44.660	2.6	51.398	3.0
	2	Panel 1	119	3.14	0.056	1.8	0.072	2.3
		Panel 2	120	9.80	0.170	1.7	0.217	2.2
		Panel 3	120	557.31	10.350	1.9	12.635	2.3
		Panel 4	120	1456.72	34.841	2.4	41.001	2.8
		Panel 5	120	1702.52	46.419	2.7	53.955	3.2
	3	Panel 1	120	3.09	0.059	1.9	0.070	2.3
		Panel 2	120	9.72	0.155	1.6	0.190	2.0
		Panel 3	120	570.03	11.540	2.0	13.176	2.3
		Panel 4	119	1471.66	37.128	2.5	50.591	3.4
		Panel 5	120	1698.64	39.124	2.3	48.897	2.9

^a Within-Laboratory (Total) variability contains within-run, within-day, and between-day variance components.

Comparison Between the ARCHITECT *i* 1000_{SR} System and the ARCHITECT *i* 2000/*i* 2000_{SR} System

The comparison between the ARCHITECT *i* 1000_{SR} and the ARCHITECT *i* 2000/*i* 2000_{SR} was evaluated by testing 205 serum panel members and 205 manually diluted amniotic fluid panel members using 1 lot each of ARCHITECT AFP Reagents, Calibrators, and Controls. Testing of each sample type was performed on 1 ARCHITECT *i* 1000_{SR} instrument at each of 3 clinical testing sites and on 1 ARCHITECT *i* 2000/*i* 2000_{SR} instrument at 1 clinical testing site.

Regression

The panel members were evaluated using the Deming regression method. The data are summarized in the following table.

Sample Type	Concentration Range (ng/mL)		Correlation Coefficient (r)		Intercept	95% CI ^a	Slope	95% CI ^a
	<i>i</i> 1000 _{SR}	<i>i</i> 2000/ <i>i</i> 2000 _{SR}	r	95% CI ^a				
Diluted Amniotic Fluid	5.29 - 1929.41	4.65 - 1918.58	0.999	(0.998, 0.999)	-8.18	(-11.88, -4.49)	0.98	(0.97, 0.99)
Serum	2.93 - 1949.81	2.84 - 1955.56	0.999	(0.999, 0.999)	4.71	(3.00, 6.43)	0.96	(0.95, 0.97)

^a 95% CI = Confidence Interval

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The following U.S. Patents are relevant to the ARCHITECT System or its components. There are other such patents and patent applications in the United States and worldwide.

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