ARCHITECT SYSTEM

AFP Calibrators

en

AFP

REF 3P36-01

S3P360

G4-5528/R02

Read Highlighted Changes
Revised November 2013

INTENDED USE

The ARCHITECT AFP Calibrators are for the calibration of the ARCHITECT *i* System when used for the quantitative determination of alpha-fetoprotein (AFP) in human serum, plasma and amniotic fluid. Refer to the ARCHITECT AFP reagent package insert and the ARCHITECT System Operations Manual for additional information.

CONTENTS

6 Bottles (4.0 mL each) of ARCHITECT AFP Calibrators A-F (CAL A - CAL F). Calibrator A contains buffer solution with protein (bovine) stabilizer. Calibrators B-F contain purified AFP (from human cord serum) prepared in buffer solution with protein (bovine) stabilizer. Preservatives: ProClin 300 and ProClin 950.

The calibrators are at the following concentrations:

	Concentration	
Calibrator	ng/mL	IU/mL
CAL A	0	0
CAL B	15	12.45
CAL C	45	37.35
CAL D	300	249
CAL E	1500	1245
CAL F	2000	1660

STANDARDIZATION

The ARCHITECT AFP calibrators are manufactured gravimetrically and are referenced to the World Health Organization (WHO) First International Standard 72/225 for Alpha-fetoprotein at each concentration level. The conversion factor is 0.83 International Units per nanogram of AFP.

PRECAUTIONS

- IVD
- For *In Vitro* Diagnostic Use
- ⚠ **CAUTION:** This product contains human sourced and/or potentially infectious components. Refer to the **CONTENTS** section of this insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens[1]. Biosafety Level 2[2] or other appropriate biosafety practices[3,4] should be used for materials that contain or are suspected of containing infectious agents.
- Calibrators B-F contain purified AFP from human cord serum tested and found to be nonreactive for HIV 1 & 2, HCV, and HBV.
- WARNING: SENSITIZER Warning: May cause an allergic reaction.
- The following warnings and precautions apply to these components
  - Calibrators A-F

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.

STORAGE

- ARCHITECT AFP Calibrators are stable until the expiration date when stored and handled as directed.
- Do not use past expiration date.
- ARCHITECT AFP Calibrators must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
- z°C — 8°C

QUALITY CONTROL PROCEDURES

A single sample of each control level must be tested to evaluate the assay calibration. For information on ordering controls, refer to the ARCHITECT System Operations Manual, Section 5.

- 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

PREPARATION FOR USE

- ARCHITECT AFP Calibrators must be mixed by gentle inversion before use.
- To perform a calibration, test the calibrators in duplicate. The calibrators should be priority loaded.
- To obtain the recommended volume requirements for the calibrators, hold the bottles **vertically** and dispense a minimum of 4 drops of each calibrator into each respective sample cup.
- After each use, tightly close the caps and return the calibrators to 2-8°C storage.
- For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.

BIBLIOGRAPHY

1. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030. Bloodborne pathogens.
2. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009.
3. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva: World Health Organization; 2004.
4. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline - Third Edition*. CLSI Document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.

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Key to symbols used

GTIN	Global Trade Item Number
PRODUCT OF IRELAND	Product of Ireland
INFORMATION FOR USA ONLY	Information needed for United States of America only

ARCHITECT SYSTEM

AFP Controls

en

AFP

REF 3P36-10

C3P360

G4-5558/R03

Read Highlighted Changes
Revised November 2013

INTENDED USE

The ARCHITECT AFP Controls are for the estimation of test precision and the detection of systematic analytical deviations of the ARCHITECT *i* System when used for the quantitative determination of alpha-fetoprotein (AFP) in human serum, plasma and amniotic fluid.

Refer to the ARCHITECT AFP reagent package insert and the ARCHITECT System Operations Manual for additional information.

CONTENTS

3 Bottles (8.0 mL each) of ARCHITECT AFP Controls, Low (CONTROL L), Medium (CONTROL M), and High (CONTROL H) ARCHITECT AFP Controls contain purified AFP (from human cord serum) prepared in buffer solution with protein (bovine) stabilizer. Preservatives: ProClin 300 and ProClin 950.

The controls are at the following concentrations:

Control	Target Conc. CONC (ng/mL)	Control Range RANGE (ng/mL)	Target Conc. (IU/mL)	Control Range (IU/mL)
CONTROL L	20	13.50 - 26.50	16.6	11.21 - 22.00
CONTROL M	200	136.00 - 265.00	166	112.05 - 219.95
CONTROL H	1000	675.00 - 1325.00	830	560.25 - 1099.75

Each laboratory should establish its own concentration ranges for new control lots at each control level. This can be accomplished by assaying a minimum of 20 replicates over several (3-5) days. Sources of variation that can be expected should be included in this study in order to be representative of future system performance. These may include:

- Multiple stored calibrations
- Multiple reagent lots
- Multiple calibrator lots
- Multiple processing modules
- Data points collected at different times of the day

These results should be applied to your laboratory's quality control practices.

PRECAUTIONS

- IVD
- For *In Vitro* Diagnostic Use
- **CAUTION:** This product contains human sourced and/or potentially infectious components. Refer to the **CONTENTS** section of this insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens[1]. Biosafety Level 2[2] or other appropriate biosafety practices[2-4] should be used for materials that contain or are suspected of containing infectious agents.
- Low, Medium, and High Controls contain purified AFP from human cord serum tested and found to be nonreactive for HIV 1 & 2, HCV, and HBV.
- WARNING: SENSITIZER Warning: May cause an allergic reaction.
- The following warnings and precautions apply to these components:
  - Controls

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.

STORAGE

- ARCHITECT AFP Controls are stable until the expiration date when stored and handled as directed.
- Do not use past expiration date.
- ARCHITECT AFP Controls must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
- 2°C – 8°C

QUALITY CONTROL PROCEDURES

Refer to the ARCHITECT AFP assay reagent package insert and ARCHITECT System Operations Manual for additional information.

The recommended control requirement for the ARCHITECT AFP assay is that a single sample of each control level 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.

The control values must be within the acceptable ranges specified in the control package insert. If a control is out of its specified range, the associated test results are invalid and must be retested. Recalibration may be indicated.

PREPARATION FOR USE

- ARCHITECT AFP Controls must be mixed by gentle inversion before use.
- To obtain the recommended volume requirements for the controls, hold the bottles **vertically** and dispense a minimum of 4 drops of each control into each respective sample cup.
- After each use, tightly close the caps and return the controls to 2-8°C storage.
- For information on ordering controls, refer to the ARCHITECT System Operations Manual, Section 5.

BIBLIOGRAPHY

1. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne pathogens.
2. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009.
3. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva: World Health Organization; 2004.
4. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline – Third Edition*. CLSI Document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.

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Key to symbols used

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INFORMATION FOR USA ONLY	Information needed for United States of America only

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	*In Vitro* 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
2°C 8°C	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.

Abbott

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.[5,7,11] 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.[21,31] 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.[27-39] 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.[28-29] 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.[27,28,39] 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.[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 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.
- 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

- 2°C–8°C 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 ≥ 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 µL for the first AFP test plus 25 µL for each additional AFP test from the same sample cup.
  - ≤ 3 hours on-board: 150 µL for the first AFP test plus 25 µL 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	>8.78 - 15.00	>15 - 200	>200 - 500	>500 - 1000	>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	>8.78 - 15.00	>15 - 200	>200 - 500	>500 - 1000	>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 values[50], 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%, 99.85%)	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 AFP[52] 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.

% 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) × 100% = 88.95%; 95% CI = 84.35% to 93.55%

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

Negative Predictive Value =
D / (C+D) × 100% = 85.00%; 95% CI = 78.34% to 90.68%

Positive Predictive Value =
A / (A+B) × 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) × 100% = 89.76%; 95% CI = 84.77% to 93.55%

Positive Agreement =
A / (A+C) × 100% = 60.00%; 95% CI = 40.60% to 77.34%

Negative Agreement =
D / (B+D) × 100% = 94.86%; 95% CI = 90.46% to 97.62%

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-A2[53] 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.

Instrument	Reagent Lot	Sample	n	Mean (ng/mL)	Within-Run		Within-Laboratory Precision (Total)[a]	
					SD	%CV	SD	%CV
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	950.55	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 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	1729.13	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 2000 (1)	1	Low Control	120	19.53	0.403	2.1	0.419	2.1
		Medium Control	120	192.55	3.996	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	192.02	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 2000 (2)	1	Panel 1	120	3.05	0.075	2.5	0.082	2.7
		Panel 2	120	9.83	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	49.736	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 0.81 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

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

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

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
Etoposide	30 µg/mL	-0.6	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] $$\% \text{ Interference} = \frac{\text{Mean/Median Test Result} - \text{Mean/Median Reference Result}}{\text{Mean/Median Reference Result}} \times 100$$

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-A2[33] and the Clinical and Laboratory Standards Institute (CLSI) document EP15-A2.[34] 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 SD	Within-Run %CV	Within-Laboratory Precision (Total)[a] SD	Within-Laboratory Precision (Total)[a] %CV
*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	28.614	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*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	11.584	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) *i*1000$_{SR}$	Concentration Range (ng/mL) *i*2000/*i*2000$_{SR}$	Correlation Coefficient (r) r	Correlation Coefficient (r) 95% CI[a]	Intercept	95% CI[a]	Slope	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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Read Highlighted Changes: Revised November 2015.

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.

WARNING: The concentration of CEA 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 CEA 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 CEA levels serially is changed, additional sequential testing should be carried out. Before changing assays, the laboratory MUST confirm baseline values for patients being serially monitored.

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 CEA (carcinoembryonic antigen)

INTENDED USE

The ARCHITECT CEA assay is a Chemiluminescent Microparticle Immunoassay (CMIA) for the quantitative determination of Carcinoembryonic Antigen (CEA) in human serum and plasma. The ARCHITECT CEA assay is to be used as an aid in the prognosis and management of cancer patients in whom changing concentrations of CEA are observed.

SUMMARY AND EXPLANATION OF THE TEST

Carcinoembryonic antigen (CEA), first described in 1965 by Gold and Freedman,[1] is a tumor associated antigen. CEA was characterized as a glycoprotein of approximately 200,000 molecular weight with a β-electrophoretic mobility.[2, 3] Subsequent development of a radioimmunoassay (RIA) by Thomson, et al[4] made it possible to detect the very low concentrations of CEA in blood, other body fluids, and also in normal and diseased tissues.[5-7] Two years later, Hansen, et al[8] developed a modified RIA for CEA.

The result of clinical studies to date indicate that CEA, although originally thought to be specific for digestive tract cancers, may also be elevated in other malignancies and in some nonmalignant disorders.[9-15]

CEA testing can have significant value in the monitoring of patients with diagnosed malignancies in whom changing concentrations of CEA are observed. A persistent elevation in circulating CEA following treatment is strongly indicative of occult metastatic and/or residual disease.[16-20]

A persistently rising CEA value may be associated with progressive malignant disease and a poor therapeutic response.[21-23] A declining CEA value is generally indicative of a favorable prognosis and a good response to treatment.[21, 23, 24] Patients who have low pretherapy CEA levels may later show elevations in the CEA level as an indication of progressive disease.[25]

Clinical relevance of the CEA assay has been shown in the follow-up management of patients with colorectal, gastric, breast, lung, prostatic, pancreatic, and ovarian carcinoma.[18, 24, 26-31] Follow-up studies of patients with colorectal, breast, and lung carcinoma suggest that the preoperative CEA level has prognostic significance.[32-35]

CEA testing is not recommended as a screening procedure to detect cancer in the general population; however, use of the CEA test as an adjunctive test in predicting prognosis and as an aid in the management of cancer patients has been widely accepted.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT CEA assay is a two-step immunoassay to determine the presence of CEA in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex.

1. Sample and anti-CEA coated paramagnetic microparticles are combined. The CEA present in the sample binds to the anti-CEA coated microparticles.
2. After washing, anti-CEA acridinium-labeled conjugate is added to create a reaction mixture.
3. Following another wash cycle, Pre-Trigger and Trigger Solutions are added to the reaction mixture.
4. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of CEA in the sample and the RLUs detected by the ARCHITECT iSystem optics.

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

REAGENTS

Kit Contents

ARCHITECT CEA 7K68

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

REF	7K68-27	7K68-22	7K68-35	7K68-32
Σ	100	400	500	2000
MICROPARTICLES	1 x 6.6 mL	4 x 6.6 mL	1 x 27.0 mL	4 x 27.0 mL
CONJUGATE	1 x 5.9 mL	4 x 5.9 mL	1 x 26.3 mL	4 x 26.3 mL

MICROPARTICLES anti-CEA (mouse, monoclonal) coated Microparticles in TRIS buffer with protein (bovine) stabilizer. Minimum concentration: 0.1% solids. Preservative: Antimicrobial Agents.

CONJUGATE anti-CEA (mouse, monoclonal) acridinium-labeled Conjugate in phosphate buffer with protein (bovine) stabilizer. Minimum concentration: 0.8 µg/mL. Preservative: Antimicrobial Agents.

Other Reagents

MULTI-ASSAY MANUAL DILUENT 1 x 100 mL ARCHITECT Multi-Assay Manual Diluent, REF 7D82-50, containing phosphate buffered saline solution. Preservative: antimicrobial agent.

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

TRIGGER SOLUTION ARCHITECT Trigger Solution containing 0.35 N sodium hydroxide.

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

NOTE: Bottle and volume varies based on order.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use

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. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[29,33]

Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Reagent Handling

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a kit or between kits.**
- Before loading the 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 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 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.

Reagent Storage

- When stored and handled as directed, reagents are stable until the expiration date.

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened/ Opened*	2-8°C	Until expiration date	May be used immediately after removal from 2-8°C storage.
On board	System temperature	30 days	Discard after 30 days. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.

* Reagents may be stored on or off the ARCHITECT iSystem. 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 CEA assay file must be installed on the ARCHITECT iSystem from an ARCHITECT iSystem Assay CD-ROM 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.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

Verified specimen types to be used with this assay:

Specimen Types	Collection Tubes
Human serum	Serum
	Serum separator tubes
Human plasma	Heparin (sodium and lithium)
	Potassium EDTA

- Other specimen collection tube types have not been tested with this assay.
- Plasma specimens collected in lithium or sodium heparin have been shown to exhibit an average of 7% to 8% higher results compared to corresponding serum results.
- When serial specimens are being evaluated, the same type of specimen should be used throughout the study.
- The instrument 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 assay.

Specimen Conditions

- Do not use specimens with the following conditions:
  - grossly hemolyzed
  - obvious microbial 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.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for specimen collection tubes.
- ARCHITECT CEA Calibrators and Controls should be mixed by gentle inversion prior to use.
- For optimal results, specimens should be free of fibrin, red blood cells, or other particulate matter. **Centrifuge serum and plasma specimens containing fibrin, red blood cells, or particulate matter prior to use to ensure consistency in the results.**
- 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.

- 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.

Specimen Storage

Specimen Type	Storage Temperature	Maximum Storage Time
Serum/Plasma	2-8°C	≤ 7 days

If testing will be delayed more than 24 hours, serum or plasma should be removed from the clot, serum separator, or red blood cells.

If testing will be delayed more than 7 days, specimens should be stored/frozen at - 20°C or colder.

Avoid multiple freeze/thaw cycles.

Specimen 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

7K68 ARCHITECT CEA Reagent Kit

Materials Required but not Provided

- ARCHITECT CEA Assay file obtained from the ARCHITECT iSystem e-Assay CD-ROM found on www.abbottdiagnostics.com.
- 7K68-02 ARCHITECT CEA Calibrators
- 7D82-50 ARCHITECT Multi-Assay Manual Diluent
- ARCHITECT Pre-Trigger Solution
- ARCHITECT Trigger Solution
- ARCHITECT Wash Buffer
- ARCHITECT Reaction Vessels
- ARCHITECT Sample Cups
- ARCHITECT Septum
- ARCHITECT 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:

- 7K68-12 ARCHITECT CEA Controls

Assay Procedure

- Before loading the 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 local Abbott representative.**
  - Once the microparticles have been resuspended, place a septum on the bottle. For instructions about placing septums on bottles, refer to the **Reagent Handling** section of this package insert.
- Load the reagent kit on the ARCHITECT iSystem.
  - Verify that all necessary reagents are present.
  - Ensure that septums are present on all reagent bottles.
- Order tests.
  - For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- Minimum sample cup volume is calculated by the system and printed on the Orderlist report. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

  Maximum number of replicates sampled from the same sample cup: 10
  - Priority:

    Sample volume for first test: 60 µL

    Sample volume for each additional test from same sample cup: 10 µL
  - ≤ 3 hours on board:

    Sample volume for first test: 150 µL

    Sample volume for each additional test from same sample cup: 10 µL
  - > 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.
- Prepare ARCHITECT CEA Calibrators and Controls.
  - Mix calibrator(s) and controls by gentle inversion before use.
  - Hold bottles **vertically** and dispense recommended volumes into each respective sample cup.
  - Recommended volumes:

    for each calibrator: 5 drops

    for each control: 4 drops
- 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 a CEA value exceeding 1500 ng/mL are flagged with the code ">1500.00" and may be diluted using either the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

The system performs a 1:10 dilution of the specimen and automatically calculates the concentration of the specimen before dilution and reports the result.

Manual Dilution Procedure

Suggested dilution: 1:100

An additional 1:10 dilution may be made if needed. It is recommended that dilutions not exceed 1:1000.

1. Add 20 µL of the patient specimen to 1980 µL of ARCHITECT Multi-Assay Manual Diluent.
2. 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 dilution should be performed so that the diluted result reads > 4 ng/mL.

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

A comparison of the Automated Dilution Protocol to the Manual Dilution Procedure yielded recoveries between 86% and 97%.

Calibration

- Test Calibrators 1 and 2 in duplicate. The calibrators should be priority loaded.

  A single sample of each control level must be tested to evaluate the assay calibration. Ensure that assay control values are within the ranges specified in the respective control package insert.
- Calibration Range: 0 - 500 ng/mL.
- The assay protocol allows for the range to be extended to 1500 ng/mL.
- Once an ARCHITECT CEA 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 or
  - 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 CEA assay is that a single sample of each control level be 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 CEA assay belongs to method group 1.

RESULTS

Calculation

The ARCHITECT CEA assay utilizes a 4 Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) to generate a calibration curve.

Flags

- The default result unit for the ARCHITECT CEA assay is ng/mL.
- 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.

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 such as ARCHITECT CEA that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.[40, 41]

  ARCHITECT CEA 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. Patients routinely exposed to animals or to animal serum products can be prone to this interference, and anomalous values may be observed. Additional information may be required for diagnosis.[42]
- The ARCHITECT CEA assay should not be used as a cancer screening test.
- Patients with confirmed carcinoma frequently have a pretreatment CEA level in the same range as healthy individuals. Elevations in circulating CEA levels may be observed in smokers as well as patients with nonmalignant disease. For these reasons, a serum or plasma CEA level, regardless of value, should not be interpreted as absolute evidence for the presence or absence of malignant disease. The CEA level should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

EXPECTED VALUES

The distribution of ARCHITECT CEA values determined in 1,141 specimens is shown in the following table.*

Distribution of ARCHITECT CEA Values

		Percent (%)			
	Number of Subjects	0 - 3 (ng/mL)	>3 - 5 (ng/mL)	>5 - 10 (ng/mL)	>10 (ng/mL)
Healthy Subjects					
Smokers	159	74.2	18.2	6.9	0.6
Non-smokers	149	83.2	11.4	5.4	0.0
Total	308	78.6	14.9	6.2	0.3
Nonmalignant Disease					
Ulcerative Colitis	50	72.0	20.0	4.0	4.0
Rectal Polyps	78	83.3	10.3	5.1	1.3
Pulmonary	60	61.7	20.0	13.3	5.0
Cirrhosis	110	47.3	30.0	15.5	7.3
Hepatitis	60	70.0	16.7	11.7	1.7
Renal	20	60.0	15.0	15.0	10.0
Malignant Disease					
Colorectal	150	24.0	10.7	10.7	54.7
Gastric	37	62.2	5.4	10.8	21.6
Pulmonary	110	47.3	19.1	9.1	24.5
Mammary	117	62.4	11.1	10.3	16.2
Ovarian	41	78.0	7.3	2.4	12.2

* Representative data; results in individual laboratories may vary.

In this study, 93.5% of healthy subjects (n=308) had CEA values of 5.00 ng/mL or less.

It is expected that each laboratory establish its own expected reference range for the population of interest.

The distribution table above for malignant disease is derived primarily from patients representing both active (clinical evidence of disease progression) and inactive (no clinical evidence of disease progression) disease states. When changing CEA assay methods in the course of monitoring a patient, additional sequential testing should be carried out to confirm baseline values.

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

The Architect CEA assay precision is ≤ 8%. Precision was determined as described in the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) Protocol EP5-T2.[43] Five samples, consisting of two serum based panels and three CEA controls, were assayed at three laboratories in replicates of two at two separate times per day for twenty days (n=80 for each sample), using a single lot of reagents and a single calibration. Data from this study are summarized in the following table.*

Reproducibility of ARCHITECT CEA

Sample	Lab	Mean CEA (ng/mL)	Within Run SD	Within Run %CV	Total SD	Total %CV
Low Control	1	5.05	0.180	3.6	0.202	4.0
	2	4.79	0.098	2.1	0.178	3.7
	3	4.86	0.110	2.3	0.162	3.3
Medium Control	1	20.17	0.512	2.5	0.641	3.2
	2	19.08	0.515	2.7	0.629	3.3
	3	19.99	0.605	3.0	0.685	3.4
High Control	1	99.45	3.074	3.1	3.182	3.2
	2	93.97	2.082	2.2	2.559	2.7
	3	99.51	2.898	2.9	3.072	3.1
Panel 1	1	417.34	9.587	2.3	10.483	2.5
	2	395.70	11.313	2.9	13.995	3.5
	3	419.93	11.591	2.8	13.870	3.3
Panel 2	1	1294.72	40.660	3.1	46.508	3.6
	2	1185.03	21.570	1.8	26.285	2.2
	3	1309.28	29.760	2.3	38.030	2.9

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

Recovery

Known amounts of CEA were added to normal human serum and plasma samples. The concentration of CEA was determined using the ARCHITECT CEA assay and the resulting percent recovery was calculated.*

Recovery

Sample Type	Endogenous Level (ng/mL)	CEA Added (ng/mL)	CEA Observed (ng/mL)	Percent Recovery
Serum				
1	0.86	94.76	91.00	95.1
2	1.07	4.49	5.56	100.0
3	0.94	94.76	94.53	98.8
4	1.11	4.49	5.85	105.6
			Average % Recovery:	99.9%
EDTA				
1	0.81	94.76	91.77	96.0
2	0.70	94.76	92.29	96.7
3	1.10	4.49	5.77	104.0
4	1.72	4.49	6.21	100.0
			Average % Recovery:	99.2%
Heparin				
1	0.93	94.76	94.59	98.8
2	1.26	4.49	6.10	107.8
3	0.92	94.76	95.24	99.5
4	1.17	4.49	5.92	105.8
			Average % Recovery:	103.0%

* Representative data; results in individual laboratories may vary.

$$\% \text{ Recovery} = \frac{\text{Observed (ng/mL)} - \text{Endogenous Level (ng/mL)}}{\text{CEA Added (ng/mL)}} \times 100$$

Analytical Sensitivity

The sensitivity of the ARCHITECT CEA assay was calculated to be better than 0.5 ng/mL at the 95% level of confidence (n = 18 runs). Sensitivity is defined as the concentration at two standard deviations above the mean RLU for the ARCHITECT CEA MasterCheck Level 0 and represents the lowest measurable concentration of CEA that can be distinguished from zero.

Specificity

The specificity of the ARCHITECT CEA assay was determined by testing sera containing the compounds listed below. These compounds showed less than 10% interference in the ARCHITECT CEA assay at the levels indicated.

Test Compound	Test Concentration
Bilirubin	22 mg/dL
Hemoglobin	550 mg/dL
Total Protein	1.8 to 13.2 g/dL
Triglycerides	3300 mg/dL

Carryover

No detectable carryover (less than 12 PPM) was observed when a sample containing 43,630 ng/mL of CEA was assayed.

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 CEA assay, no high dose hook effect was observed when samples containing up to approximately 60,000 ng/mL of CEA were assayed.

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Key to Symbols

	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
CONJUGATE	Conjugate
CONTROL NO.	Control Number
DISTRIBUTED IN THE USA BY	Distributed in the USA by
GTIN	Global Trade Item Number
INFORMATION FOR USA ONLY	Information needed for United States of America only
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
MICROPARTICLES	Microparticles
MULTI-ASSAY MANUAL DILUENT	Multi-Assay Manual Diluent
PRE-TRIGGER SOLUTION	Pre-Trigger Solution
PRODUCT OF IRELAND	Product of Ireland
REACTION VESSELS	Reaction Vessels
REAGENT LOT	Reagent Lot
REF	List Number
REPLACEMENT CAPS	Replacement Caps
SAMPLE CUPS	Sample Cups
SEPTUM	Septum
SN	Serial number
TRIGGER SOLUTION	Trigger Solution
WASH BUFFER	Wash Buffer

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Abbott

ARCHITECT CA 19-9XR

REF 2K91-24
REF 2K91-32
REF 2K91-39

en
CA 19-9XR
2K91
613-031 11/16/R03
B2K9Y0

Read Highlighted Changes: Revised November 2016.

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.

WARNING: The Abbott ARCHITECT CA 19-9XR CMIA assay utilizes an antibody/antigen system based on the 1116-NS-19-9 antibody. The unique reagent formulation employed in the ARCHITECT CA 19-9XR assay may return elevated concentrations when compared to other methods for samples expressing high levels of 1116-NS-19-9 reactive determinants.[1, 2] Additionally, there is no internationally recognized standard for CA 19-9, which can contribute to differences between assay methods. The ARCHITECT CA 19-9XR assay is standardized to a reference standard prepared by Fujirebio Diagnostics, Inc. Performance characteristics of the Abbott ARCHITECT CA 19-9XR assay are NOT transferable to other diagnostic kits.

The concentration of 1116-NS-19-9 reactive determinants obtained with different assay methods cannot be used interchangeably due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the CA 19-9 assay used. If, in the course of monitoring a patient, the assay method used for determining serial 1116-NS-19-9 reactive determinant levels is changed, additional sequential testing should be carried out. Prior to changing assays, the laboratory MUST confirm baseline values for patients being serially monitored.

WARNING: 1116-NS-19-9 reactive determinants are shed naturally in saliva and other body fluids.[3] Contamination of the samples or the ARCHITECT iSystem disposables with saliva or aerosols (e.g., as a result of sneezing) may cause falsely elevated CA 19-9 assay values. It is recommended that all elevated values be reviewed and testing repeated as appropriate. Gloves should always be worn when handling samples, sample cups, reaction vessels, and septums. Face masks are also recommended.

NAME

ARCHITECT CA 19-9XR

INTENDED USE

The ARCHITECT CA 19-9XR assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of 1116-NS-19-9 reactive determinants in human serum or plasma on the ARCHITECT iSystem. The ARCHITECT CA 19-9XR assay is to be used as an aid in the management of pancreatic cancer patients in conjunction with other clinical methods.

SUMMARY AND EXPLANATION OF THE TEST

The ARCHITECT CA 19-9XR assay detects a tumor-associated antigen, which occurs in tissue as a monosialoganglioside and in serum as a high molecular weight, carbohydrate-rich glycoprotein known as a mucin.[4-7]

The ARCHITECT CA 19-9XR assay is based upon a monoclonal antibody, 1116-NS-19-9, which reacts with a carbohydrate antigenic determinant expressed on the circulating antigen.[4-6]

The results of published research studies[8-14] indicate that the CA 19-9 assay value is frequently elevated in the serum of subjects with various gastrointestinal conditions, such as pancreatic, colorectal, gastric, and hepatic carcinomas. No data exist to support the use of CA 19-9 in screening for malignancies.[15, 16] The role of CA 19-9 is to be used as an adjunct with other diagnostic information in the management of patients with pancreatic cancer.[15] Increased serum CA 19-9 assay values have also been observed in patients with metastases and in nonmalignant conditions such as hepatitis, cirrhosis, pancreatitis, and other gastrointestinal disease.[8-11, 17-20] Elevated levels have also been seen in cystic fibrosis.[21-24] Research studies demonstrate that CA 19-9 assay values may have utility in monitoring subjects with the above-mentioned diagnosed gastrointestinal malignancies.[25-28] It has been shown that a persistent elevation in CA 19-9 assay value following treatment may be indicative of occult metastatic and/or residual disease. A persistently rising CA 19-9 assay value may be associated with progressive malignant disease and poor therapeutic response. A declining CA 19-9 assay value may be indicative of a favorable prognosis and a good response to treatment.[29-35]

Testing for 1116-NS-19-9 reactive determinants must not be used as a screening procedure for malignancy. 1116-NS-19-9 reactive determinants are present as a normal constituent in serum and plasma of individuals without gastrointestinal carcinomas or having certain aforementioned non-cancer related conditions.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT CA 19-9XR assay is a two-step immunoassay for the quantitative determination of 1116-NS-19-9 reactive determinants in human serum or plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex.

1. Sample and 1116-NS-19-9 coated paramagnetic microparticles are combined. The 1116-NS-19-9 reactive determinants present in the sample bind to the 1116-NS-19-9 coated microparticles.
2. After washing, 1116-NS-19-9 acridinium-labeled conjugate is added to create a reaction mixture.
3. Following another wash cycle, Pre-Trigger and Trigger Solutions are added to the reaction mixture.
4. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of 1116-NS-19-9 reactive determinants in the sample and the RLUs detected by the ARCHITECT iSystem optics.

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

REAGENTS

Kit Contents

ARCHITECT CA 19-9XR 2K91

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

REF	2K91-32	2K91-24	2K91-39
Σ	100	400	500
MICROPARTICLES	1 x 6.6 mL	4 x 6.6 mL	1 x 27.0 mL
CONJUGATE	1 x 5.9 mL	4 x 5.9 mL	1 x 26.3 mL

MICROPARTICLES 1116-NS-19-9 (mouse, monoclonal) coated microparticles in citrate buffer with protein (bovine) stabilizer. Minimum concentration: 0.09% solids. Preservatives: sodium azide and ProClin 300.

CONJUGATE 1116-NS-19-9 (mouse, monoclonal) acridinium-labeled conjugate in phosphate buffer with protein (bovine) stabilizer. Minimum concentration: 0.5 µg/mL. Preservatives: sodium azide and ProClin 300.

Other Reagents

MULTI-ASSAY MANUAL DILUENT 1 x 100 mL ARCHITECT Multi-Assay Manual Diluent, REF 7D82-50, containing phosphate buffered saline solution. Preservative: antimicrobial agent.

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

TRIGGER SOLUTION ARCHITECT Trigger Solution containing 0.35 N sodium hydroxide.

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

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use

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. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[36-39]

The following warnings and precautions apply to: MICROPARTICLES	
	
WARNING	Contains methylisothiazolones and sodium azide.
H317	May cause an allergic skin reaction.
EUH032	Contact with acids liberates very toxic gas.
Prevention	
P261	Avoid breathing mist / vapors / 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.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: CONJUGATE	
	
DANGER	Contains polyethylene glycol octylphenyl ether, methylisothiazolones and sodium azide.
H317	May cause an allergic skin reaction.
H318	Causes serious eye damage.
H412	Harmful to aquatic life with long lasting effects.
EUH032	Contact with acids liberates very toxic gas.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P280	Wear protective gloves / protective clothing / eye protection.
P272	Contaminated work clothing should not be allowed out of the workplace.
P273	Avoid release to the environment.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER or doctor / physician.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Reagent Handling

- **1116-NS-19-9 reactive determinants are shed naturally in saliva and other body fluids.[3] Contamination of the samples or the ARCHITECT iSystem disposables with saliva or aerosols (e.g., as a result of sneezing) may cause falsely elevated CA 19-9 assay values. It is recommended that all elevated values be reviewed and testing repeated as appropriate. Gloves should always be worn when handling samples, sample cups, reaction vessels, and septums. Face masks are also recommended.**
- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a kit or between kits.**
- Before loading the 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 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 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.

Reagent Storage

When stored and handled as directed, reagents are stable until the expiration date.

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened/ Opened*	2-8°C	Until expiration date	May be used immediately after removal from 2-8°C storage. Store in upright position.
On board	System temperature	30 days	Discard after 30 days. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.

* Reagents may be stored on or off the ARCHITECT iSystem. 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 any reagent 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 CA 19-9XR assay file must be installed on the ARCHITECT iSystem 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.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

Verified specimen types to be used with this assay:

Specimen Types	Collection Tubes
Serum	Serum Serum separator tubes
Plasma	Tripotassium EDTA Sodium Heparin Lithium Heparin

- Other specimen collection tube types have not been tested with this assay.
- The instrument 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 assay.
- Follow the tube manufacturer's processing instructions for serum and plasma collection tubes.
- When serial specimens are being evaluated, the same type of specimen should be used throughout the study.

Specimen Conditions

- Do not use specimens with the following conditions:
  - grossly hemolyzed
  - obvious microbial 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.
- Performance has not been established using body fluids other than human serum or plasma.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for specimen collection tubes.
- Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.
- To ensure consistency in results, centrifuge specimens before testing if
  - they contain fibrin, red blood cells, or other particulate matter,
  - they require repeat testing, or
  - they were frozen and thawed.
- Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.
- 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.

Specimen Storage

Specimen Type	Storage Temperature	Maximum Storage Time
Serum/Plasma	2-8°C	≤ 7 days
	-20°C or colder	>7 days

- If testing will be delayed more than 24 hours, remove serum or plasma 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.
- If testing will be delayed more than 7 days, serum or plasma should be stored frozen at -20°C or colder.
- Avoid multiple freeze/thaw cycles.

Specimen 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

2K91 ARCHITECT CA 19-9XR Reagent Kit

Materials Required but not Provided

- ARCHITECT CA 19-9XR Assay file obtained from the ARCHITECT iSystem e-Assay CD-ROM found on www.abbottdiagnostics.com.
- 2K91 ARCHITECT CA 19-9XR Calibrators
- 2K91 ARCHITECT CA 19-9XR Controls
- 7D82-50 ARCHITECT Multi-Assay Manual Diluent
- ARCHITECT Pre-Trigger Solution
- ARCHITECT Trigger Solution
- ARCHITECT Wash Buffer
- ARCHITECT Reaction Vessels
- ARCHITECT Sample Cups
- ARCHITECT Septum
- ARCHITECT 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.

Assay Procedure

- Before loading the 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 local Abbott representative.**
  - Once the microparticles have been resuspended, place a septum on the bottle. For instructions about placing septums on bottles, refer to the **Reagent Handling** section of this package insert.
- Load the reagent kit on the ARCHITECT iSystem.
  - 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.
- Minimum sample cup volume is calculated by the system and printed on the Orderlist report. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

  Maximum number of replicates sampled from the same sample cup: 10
  - Priority:

    Sample volume for first test: 80 µL

    Sample volume for each additional test from same sample cup: 30 µL
  - ≤ 3 hours on board:

    Sample volume for first test: 150 µL

    Sample volume for each additional test from same sample cup: 30 µL
  - > 3 hours on board: Additional sample volume required
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- Prepare ARCHITECT CA 19-9XR Calibrators and Controls.
  - Mix calibrator(s) and controls by gentle inversion before use.
  - Hold bottles **vertically** and dispense recommended volumes into each respective sample cup.
  - Recommended volumes:

    for each calibrator: 4 drops

    for each control: 4 drops
- 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 ARCHITECT CA 19-9XR value exceeding 1200 U/mL are flagged with the code "> 1200.00" and may be diluted using either the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

The system performs a 1:10 dilution of the specimen and automatically calculates the concentration of the specimen before dilution and reports the result.

Manual Dilution Procedure

Suggested dilution: 1:10

An additional 1:10 dilution may be made if needed.

1. Add 50 µL of the patient specimen to 450 µL of ARCHITECT Multi-Assay Manual Diluent (7D82).
2. 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 and report the result. The dilution should be performed so that the diluted result reads greater than 30 U/mL.

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

Calibration

- Test Calibrators A-F in duplicate. The calibrators should be priority loaded.

  A single sample of each control level must be tested to evaluate the assay calibration. Ensure that assay control values are within the ranges specified in the respective control package insert.
- Calibration Range: 0 - 1200 U/mL.
- Once an ARCHITECT CA 19-9XR 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 or
  - 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 CA 19-9XR assay is that a single sample of each control level be 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.

The ARCHITECT CA 19-9XR values must be within the acceptable ranges specified in the control package insert. If a control is out of the specified range, the associated test results are invalid and samples must be retested. Recalibration may be indicated.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B.

The ARCHITECT CA 19-9XR assay belongs to method group 1.

RESULTS

Calculation

The ARCHITECT CA 19-9XR assay utilizes a Linear Regression data reduction method to generate a calibration curve.

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.

LIMITATIONS OF THE PROCEDURE

- The ARCHITECT CA 19-9XR assay value must be used in conjunction with information available from clinical evaluation and other diagnostic procedures.
- If the ARCHITECT CA 19-9XR results are inconsistent with clinical evidence, additional testing is recommended.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.[40]
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Specimens containing HAMA may produce anomalous values when tested with assay kits that employ mouse monoclonal antibodies. ARCHITECT CA 19-9XR reagents contain a component that reduces the effect of HAMA reactive specimens. Additional clinical or diagnostic information may be required to determine patient status.[41, 42]
- Patients with confirmed carcinoma may have pretreatment CA 19-9 assay values in the same range as healthy individuals. Elevations in circulating 1116-NS-19-9 reactive determinants may be observed in patients with metastases and in nonmalignant conditions such as hepatitis, cirrhosis, pancreatitis, and other gastrointestinal disease. Elevated levels have also been seen in cystic fibrosis.[21] For these reasons, a CA 19-9 assay value, regardless of level, should not be interpreted as absolute evidence for the presence or absence of malignant disease. **The ARCHITECT CA 19-9XR assay must not be used as a cancer screening test.**
- Patients with the Le^{a-b-} phenotype may not express the 1116-NS-19-9 reactive determinant.[43]
- Representative performance data are given in the **EXPECTED VALUES and SPECIFIC PERFORMANCE CHARACTERISTICS** sections. Results obtained in individual laboratories may vary.

EXPECTED VALUES

APPARENTLY HEALTHY SUBJECTS

A study was performed with three hundred sixty (360) serum specimens from apparently healthy individuals. The distribution of ARCHITECT CA 19-9XR assay values from these specimens is shown in the table below.*

Distribution of ARCHITECT CA 19-9XR Values

		Percent (%)				
	Number of Subjects	0-37.0 (U/mL)	37.1-100 (U/mL)	100.1-500 (U/mL)	500.1-1200 (U/mL)	>1200 (U/mL)
Apparently Healthy Subjects	360	94.4	5.6	0.0	0.0	0.0

In this study, 94.4% of the specimens from apparently healthy subjects (n=360) had values of 37 U/mL or less.

* Representative data; results in individual laboratories may vary from these data.

NONMALIGNANT DISEASE

A study was performed with four hundred forty one (441) samples from patients with nonmalignant disease to determine the distribution of serum ARCHITECT CA 19-9XR assay values. The distribution of values determined in this study is shown in the table below.*

Distribution of ARCHITECT CA 19-9XR Values

		Percent (%)				
Nonmalignant Disease	Number of Subjects	0-37.0 (U/mL)	37.1-100 (U/mL)	100.1-500 (U/mL)	500.1-1200 (U/mL)	>1200 (U/mL)
Rectal Polyps	33	97.0	3.0	0.0	0.0	0.0
Pancreatitis	3	100.0	0.0	0.0	0.0	0.0
Gallbladder	21	95.2	0.0	0.0	0.0	4.8
Diabetes	38	94.7	5.3	0.0	0.0	0.0
Pulmonary	40	100.0	0.0	0.0	0.0	0.0
Cirrhosis	153	92.8	4.6	0.7	0.7	1.3
Hepatitis	68	92.6	7.4	0.0	0.0	0.0
Renal	34	91.2	8.8	0.0	0.0	0.0
Other Gastrointestinal	51	96.1	3.9	0.0	0.0	0.0

The ARCHITECT CA 19-9XR assay is used in conjunction with other clinical methods in the management of cancer patients.

It is recommended that each laboratory establish its own reference value for the population of interest.

* Representative data; results in individual laboratories may vary from these data.

Monitoring of Disease State in Patients Diagnosed with Pancreatic Cancer

Changes observed in serial CA 19-9 assay values when monitoring pancreatic cancer patients must be evaluated in conjunction with other clinical methods.

The effectiveness of the ARCHITECT CA 19-9XR assay as an aid in monitoring of disease state in pancreatic cancer patients was determined by assessing changes in levels of 1116-NS-19-9 reactive determinants in serial serum samples from 74 patients compared to changes in disease state. A study involving a total of 261 observations was performed with an average number of 3.5 observations per patient. In this study a significant change in levels of 1116-NS-19-9 reactive determinants was defined as at least a 14.0% increase in assay value (i.e., 2.5 times greater than the average of the assay's observed total %CV [5.6%]). A 14.0% change represents the minimum magnitude change between two serial ARCHITECT CA 19-9XR measurements that could not be attributed to assay variation or noise. Positive concordance between serial samples with at least a 14.0% increase in assay value and disease progression was found to be 48% (16/33). Negative concordance between serial samples with less than a 14.0% increase in assay value and no disease progression was found to be 64% (98/154). The overall concordance was found to be 61% (114/187). The following table presents the data in a 2 x 2 classification scheme*.

Change in Disease State per Sequential Pair

Change in the Level of 1116-NS-19-9 Reactive Determinants	Progression	No Progression	Total
≥14.0%	16	56	72
<14.0%	17	98	115
Total	33	154	187

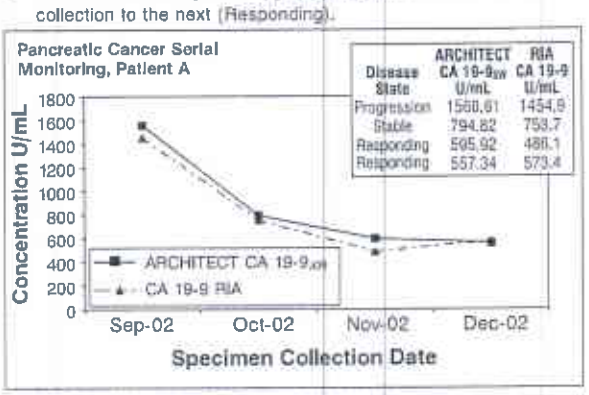
The following table provides the per patient distribution*. Positive concordance between serial samples with at least a 14.0% increase in assay value and disease progression was found to be 68% (15/22). Negative concordance between serial samples with less than a 14.0% increase in assay value and no disease progression was found to be 69% (36/52). The overall concordance was found to be 69% (51/74).

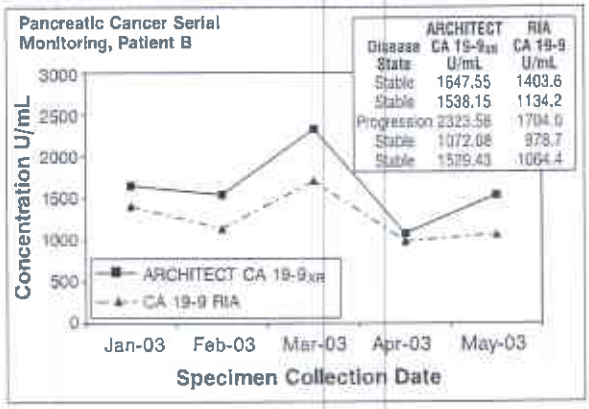
Change in Disease State per Patient			
Change in the Level of 1116-NS-19-9 Reactive Determinants	Progression	No Progression	Total
≥14.0%	15	16	31
<14.0%	7	36	43
Total	22	52	74

* Representative data; results in individual laboratories may vary from these data.

Below are examples of serial monitoring profiles for two patients with the disease state, ARCHITECT CA 19-9XR assay values, and the CA 19-9 RIA values.* The disease states are:

- Progression from one collection to the next collection (Progression).
- No Change in disease state (Stable).
- Reduction in the signs and symptoms of the disease from one collection to the next (Responding).





* Representative data; results in individual laboratories may vary from these data.

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

The ARCHITECT CA 19-9XR assay is designed to have an assay precision of ≤10% total CV.

A study was performed as described per the National Committee for Clinical Laboratory Standards (NCCLS) Protocol EP5-A2.[44] Six samples were tested consisting of two panels of pooled serum (panels 1 and 2), one panel of serum to which 1116-NS-19-9 reactive determinants were added (panel 3), and the three ARCHITECT CA 19-9XR Controls. Testing was performed using two lots of reagents, in replicates of two at two separate times per day for 20 days on two separate instruments. Each reagent lot used a single calibration curve throughout the study. Data from this study are summarized below.*

Sample	Reagent Lot	Instrument	n	Mean Conc. (U/mL)	Within Run SD	Within Run %CV	Total SD	Total %CV
Panel 1	1	1	80	56.52	1.69	3.0	2.19	3.9
	2	2	80	51.20	1.80	3.5	2.10	4.1
Panel 2	1	1	80	311.49	7.22	2.3	10.72	3.4
	2	2	80	288.82	9.14	3.2	11.23	3.9
Panel 3	1	1	80	744.81	27.82	3.7	36.85	5.0
	2	2	80	728.82	42.53	5.8	47.66	6.5
Low Control	1	1	80	45.03	2.59	5.8	2.98	6.6
	2	2	80	42.33	2.94	6.9	3.60	8.5
Medium Control	1	1	80	157.66	5.99	3.8	8.52	5.4
	2	2	80	146.93	6.26	4.3	8.14	5.5
High Control	1	1	80	781.68	44.76	5.7	49.87	6.4
	2	2	80	781.42	62.10	8.0	65.28	8.4

* Representative data; results in individual laboratories may vary from these data.

Recovery

The ARCHITECT CA 19-9XR assay is designed to have a mean recovery of 100 ± 15% when 1116-NS-19-9 reactive determinants are added to serum samples.

A study was performed for the ARCHITECT CA 19-9XR assay based on guidance from Tietz Textbook of Clinical Chemistry.[45] Known concentrations of 1116-NS-19-9 reactive determinants were added to human serum samples. The concentration of 1116-NS-19-9 reactive determinants was determined using the ARCHITECT CA 19-9XR assay, and the resulting percent recovery was calculated. Representative data from this study are summarized in the table below.*

Sample	Endogenous Assay Value (U/mL)	1116-NS-19-9 Reactive Determinants Added (U/mL)	Observed ARCHITECT CA 19-9XR Assay Value (U/mL)	% Recovery**
1	46.58	124.21	152.42	89
		629.91	645.00	95
2	26.36	124.21	145.73	96
		629.91	598.93	91
3	38.42	124.21	175.18	108
		629.91	652.12	98

Mean recovery across two separate spiked concentrations shown above = 96 %

$$\text{** \% Recovery} = \frac{\text{Observed (U/mL)}}{\text{Endogenous Level (U/mL)} + \text{1116-NS-19-9 Reactive Determinants Added (U/mL)}} \times 100$$

* Representative data; results in individual laboratories may vary from these data.

Dilution Linearity

The ARCHITECT CA 19-9XR assay is designed to have a mean recovery of 100 ± 15% of the expected result for diluted specimens.

A study was performed for the ARCHITECT CA 19-9XR assay modeled after the National Committee for Clinical Laboratory Standards (NCCLS) Protocol EP6-P2.[46] Samples with known elevated 1116-NS-19-9 reactive determinant concentrations were diluted with ARCHITECT Multi-Assay Manual Diluent. The 1116-NS-19-9 reactive determinants concentration was determined for each dilution and the percent recovery was calculated. Representative data from this study are summarized below.*

Sample	Final Dilution Factor	Expected Value (U/mL)	Value Obtained (U/mL)	% Recovery**
1	Undiluted	1024.55	1024.55	-
	1:2	512.27	472.46	92
	1:4	256.14	264.26	103
	1:5	204.91	208.57	102
	1:10	102.45	108.94	106
	1:20	51.23	54.33	106
2	Undiluted	1150.50	1150.50	-
	1:2	575.25	551.62	96
	1:4	287.63	291.06	101
	1:5	230.10	253.65	110
	1:10	115.05	125.97	109
	1:20	57.53	62.57	109
3	Undiluted	1028.25	1028.25	-
	1:2	514.12	492.39	96
	1:4	257.06	290.24	113
	1:5	205.65	204.03	99
	1:10	102.82	120.76	117
	1:20	51.41	57.25	111

Mean recovery across the three diluted samples shown above = 105%

$$** \% \text{ Recovery} = \frac{\text{Values Obtained x Dilution Factor}}{\text{Undiluted Expected Value}} \times 100$$

* Representative data; results in individual laboratories may vary from these data.

Analytical Sensitivity

The analytical sensitivity of the ARCHITECT CA 19-9XR assay was calculated to be better than 2.00 U/mL (n = 18 runs, in replicates of 10).

Analytical sensitivity is defined as the concentration at two standard deviations from the ARCHITECT CA 19-9XR Calibrator A (0 U/mL), and represents the lowest measurable concentration of 1116-NS-19-9 reactive determinants that can be distinguished from zero.

Interference

The ARCHITECT CA 19-9XR assay is designed to have a mean recovery of 100 ± 12% in the presence of the chemotherapeutic agents listed below and elevated levels of bilirubin, hemoglobin, triglycerides, and total protein at the levels indicated.

A study based on guidance from the NCCLS Protocol EP7-A[47] was performed for the ARCHITECT CA 19-9XR assay. Specimens with 1116-NS-19-9 reactive determinant levels between 49.6 and 509.4 U/mL were supplemented with the following potentially interfering substances and chemotherapeutic agents.

POTENTIALLY INTERFERING SUBSTANCES

The average recovery observed during the study ranged from 91% to 102%.*

Substance	Concentration
Bilirubin	22 mg/dL
Hemoglobin	600 mg/dL
Total Protein	10 g/dL
Triglycerides	5100 mg/dL

* Representative data; results in individual laboratories may vary from these data.

CHEMOTHERAPEUTIC AGENTS

The average recovery observed during the study ranged from 95% to 104%.*

Substance	Concentration
5-Fluorouracil	0.390 mg/mL
Cisplatin	0.057 mg/mL
Cyclophosphamide	0.375 mg/mL
Cytarabine	30 µg/mL
Doxorubicin	40 µg/mL
Gemcitabine	0.382 mg/mL
Leucovorin	0.114 mg/mL
Methotrexate	0.909 mg/mL
Paclitaxel	0.067 mg/mL
Streptozotocin	0.28 mg/mL
Tamoxifen	2.28 µg/dL

* Representative data; results in individual laboratories may vary from these data.

EVALUATION OF POTENTIALLY INTERFERING CLINICAL CONDITIONS

The ARCHITECT CA 19-9XR assay is designed to have a mean recovery of 100 ± 12% in the presence of HAMA and rheumatoid factor (RF).

The ARCHITECT CA 19-9XR assay was evaluated using specimens with HAMA and RF to further assess the clinical specificity. Five specimens positive for HAMA and five specimens positive for RF were evaluated for % recovery with 1116-NS-19-9 reactive determinants spiked into each specimen at 35 and 250 U/mL. Mean percent recovery results are summarized in the following table.*

Clinical Condition	Number of Specimens	Mean % Recovery
HAMA	10	93
RF	10	93

* Representative data; results in individual laboratories may vary from these data.

Carryover

No significant carryover (less than 2.00 U/mL in CA19-9XR Calibrator A*) was observed for the ARCHITECT CA 19-9XR assay when a sample containing up to 320,000 U/mL of 1116-NS-19-9 reactive determinants was assayed.

* Representative data; results in individual laboratories may vary from these data.

High Dose Hook

No high dose hook effect was observed for the ARCHITECT CA 19-9XR assay when samples containing up to 1,750,000 U/mL* of 1116-NS-19-9 reactive determinants were assayed. High dose hook is a phenomenon whereby very high level specimens may falsely read within the dynamic range of the assay.

* Representative data; results in individual laboratories may vary from these data.

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Key to Symbols

	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
CONJUGATE	Conjugate
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
CONTROL NO.	Control Number
ECO HAZARD	Ecological hazard
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
MICROPARTICLES	Microparticles
MULTI-ASSAY MANUAL DILUENT	Multi-Assay Manual Diluent
PRE-TRIGGER SOLUTION	Pre-Trigger Solution
PRODUCED FOR ABBOTT BY	Produced for Abbott by
PRODUCT OF USA	Product of USA
REACTION VESSELS	Reaction Vessels
REAGENT LOT	Reagent Lot
REF	List Number
REPLACEMENT CAPS	Replacement Caps
SAMPLE CUPS	Sample Cups
SEPTUM	Septum
SN	Serial number
TRIGGER SOLUTION	Trigger Solution
WARNING: SENSITIZER	Warning: May cause an allergic reaction.
WASH BUFFER	Wash Buffer

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Revised November 2016.

Abbott

ARCHITECT
CA 125 II Calibrators

REF 2K45-01

en
CA 125 II
2K45
602-001 8/14/R11
S2K450

Read Highlighted Changes: Revised November 2014.

INTENDED USE

The ARCHITECT CA 125 II Calibrators are for the calibration of the ARCHITECT iSystem when used for the quantitative determination of OC 125 defined antigen in human serum and plasma.

Refer to the ARCHITECT CA 125 II reagent package insert for additional information.

CONTENTS

6 Bottles (4 mL each) of ARCHITECT CA 125 II Calibrators. Calibrator A contains TRIS buffer with protein (bovine) stabilizers. Calibrators B - F contain OC125 defined antigen (human) prepared in TRIS buffer with protein (bovine) stabilizers. Preservatives: Sodium Azide and ProClin 300.

The calibrators yield the following concentrations:

	CA 125 II Concentration
Calibrators	U/mL
CAL A	0
CAL B	20
CAL C	75
CAL D	225
CAL E	500
CAL F	1000

STANDARDIZATION

CA 125 assay values are expressed as U/mL. A unit is a value related to a Fujirebio Diagnostics, Inc. maintained reference preparation. The calibrators for the ARCHITECT CA 125 II assay are manufactured volumetrically and are referenced to this standard prepared by Fujirebio Diagnostics, Inc. There is no internationally recognized CA 125 standard available at this time.

PRECAUTIONS

- IVD
- For *In Vitro* Diagnostic Use
- **CAUTION:** This product contains human-sourced and/or potentially infectious components. Calibrators B-F contain antigen derived from a human cell line. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human-sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens, Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[1-4]

The following warnings and precautions apply to: CAL A - CAL F	
WARNING	Contains methylisothiazolones and sodium azide.
H317	May cause an allergic skin reaction.
EUH032	Contact with acids liberates very toxic gas.
Prevention	
P261	Avoid breathing mist / vapors / 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.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

STORAGE

- Calibrators are stable until the expiration date when stored and handled as directed.
- Do not use past expiration date.
- 2°C – 8°C

PREPARATION FOR ANALYSIS

- Calibrators may be used immediately after removal from 2-8°C storage.
- Prior to use, mix by gentle inversion (5-10 times).
- After each use, tightly close the caps and return the calibrators to 2-8°C storage.

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Key to Symbols

	Caution
	Consult instructions for use
	Manufacturer
	Temperature limitation
	Use by/Expiration date
CAL | A	Calibrator (A,B,C,D,E or F)
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
EC REP	Authorized Representative in the European Community
INFORMATION FOR USA ONLY	Information needed for United States of America only
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
PRODUCED FOR ABBOTT BY	Produced for Abbott by
PRODUCT OF USA	Product of USA
REF	List Number
WARNING: SENSITIZER	Warning: May cause an allergic reaction

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Revised November 2014.

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