| **Alpha-Fetoprotein (AFP)** |
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| **Purpose** | This procedure provides instructions for performing AFP on the Abbott Architect i1000SR |
| **Policy Statements** | This procedure applies to all personnel responsible for performing testing on the Abbott Architect i1000SR. |
| **Principle** | 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. |
| **Clinical Significance** | The discovery of alpha-fetoprotein (AFP) in fetal serum was first recorded by Bergstrand and Czar in 1956. 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. 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. Since then, it has been shown that elevation of serum AFP above values typically found in healthy individuals occurs in several malignant diseases, 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. 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. Human chorionic gonadotropin (hCG) and AFP are also important prognostic indicators of survival rate among patients with advanced nonseminomatous germ cell testicular tumors. The usefulness of AFP measurements in the management of patients with nonseminomatous testicular cancers has been well documented. For patients in clinical remission following treatment, AFP levels generally decrease. Post-operative AFP values which fail to return to normal strongly suggest the presence of residual tumor. Tumor recurrence is often accompanied by a rise in AFP before progressive disease is clinically evident. Greater than 70% of patients with primary hepatocellular carcinoma have been reported to have elevated levels of serum AFP. Elevated AFP levels have occasionally been found in association with gastrointestinal tract cancers with and without liver metastases and only rarely in other malignancies. 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. Elevation of serum AFP in benign hepatic diseases is usually transient. AFP testing is not recommended as a screening procedure to detect cancer in the general population. |
| **Instrument** | **PRIMARY METHOD: Abbott Architect i1000SR**Backup Method**: Mayo Medical Laboratories** |
| **Sunquest Test Code** | AFPR |
| **Specimen** | **Sample type:** Serum SST preferred. Also acceptable: Lithium Heparin plasma, Sodium Heparin plasma, EDTA plasma**Preferred Draw Volume: 600 uL****Minimum Processed volume:** 75 µL of serum or plasma (does not allow for dilutions or repeat)**Stability:** 3 days at room temperature, 7 days at 2-8°C, 2 years at -20°C**Rejection criteria:** Unlabeled specimens, incorrect sample type, amniocentesis fluid (has not been verified for use at Children’s)**Preparation:** 1. Serum specimens should be centrifuged following complete clot formation, according to Specimen Processing procedures prior to analysis. Plasma specimens can be centrifuged immediately
2. Serum or plasma should be physically separated from cells as soon as possible with a maximum limit of two hours from the time of collection.
3. Lipemic samples should be ultrafuged.
4. Specimens should be free of particulate matter. Recentrifuge an aliquot if necessary.
5. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.
6. Transfer sample into an Architect sample cup, labeled with a foot label, and placed on top of an appropriately labeled sendout tube. You must use Architect sample cups for this test due to low volume sample.
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| **Reagents** |

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| AFP Reagent | 03P36-25 | **Store at:** 2 – 8 °C**Unopened/Opened:** Manufacturer expiration date.**On-board:** 30 Days |
| AFP Calibrator | 03P36-01 | **Store at:**  2-8°C**Unopened**: Manufacturer expiration date.**Opened**: Store at 2 – 8 °C, stable until expiration date when stored and handled as directed.  |
| Multiassay Diluent | 07D82-50 | Refer to Supply Status on Analyzer |
| Pre-Trigger Solution | 06E23-65 | Refer to Supply Status on Analyzer |
| Trigger Solution | 06C55-60 | Refer to Supply Status on Analyzer |
| Wash Buffer | 06C54-58 | Refer to Supply Status on Analyzer |
| Reaction Vessels  | 07C15 (-02 or -03) | N/A |

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| **Risk and Safety:** | Contains sodium azide. Avoid contact with skin and eye. Causes serious eye irritation. Wear gloves. Contact with acids liberates very toxic gas. Recap and dispose of in appropriate Hazardous Waste Container. |
| Calibration/ Verification/AMR |

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| Analytical Measuring Range: | 2.0 – 2000.0 ng/mL |
| Reference Material: | AFP Calibrator |
| Suggested Calibration Levels | A – 0.0 ng/mLB – 15 ng/mLC – 45 ng/mLD – 300 ng/mLE – 1500 ng/mLF – 2000 ng/mL |
| Verification Scheme: | n=6 |
| Verification Frequency: | * For each new lot of reagent
* After major maintenance or service, if indicated by quality control results
* As indicated in laboratory quality control procedures
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| AMR | Verification of AMR is accomplished with each calibration.* Cal Verification and AMR verification are performed at least once every six (6) months.
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| **Quality Control** | Bio-Rad Lyphochek Immunoassay Plus Controls Levels 1,2 and 3**Frequency:** Three levels each day of use.**Stability:** 3 Days at 2-8°C.**Preparation**: Reconstitute with exactly 5.0 mL of DI water. Let vials sit for 15 minutes and gently swirl to ensure homogeneity. Do not allow to stand at room temperature longer than 20 minutes. **Sunquest Control names:** Level 1 = C-LYIP1, Level 2 = C-LYIP2, Level 3= C-LYIP3**Acceptable ranges:** * Ranges are current in Sunquest and the instrument. Refer to the Quality Control in Chemistry procedure for QC exception codes.
* If a control value is outside the confidence interval, the determination must be repeated. If the repeat determination confirms the deviation, a new reference curve should be established.
* Do not release patient results until the cause of deviation has been identified and corrected
* When a new lot of assayed control is received, validate the manufacturer’s insert range by running the new lot in parallel with the current lot, and confirming that the results obtained are within the stated range
* When a new lot of unassayed control is received, verify new ranges by running the new lot in parallel with the current lot 30 times, and calculate a new range using the method mean ± 3 SD. Ranges are current in Sunquest and the instrument. Refer to the Quality Control Procedure for QC exception codes.
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| **Interferences** | * 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 such as ARCHITECT AFP that employ mouse monoclonal antibodies.
* 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 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.
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| **Reference Range** |

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| **Age** | **Reference Range** |
| 0-1 month | >2000 ng/mL |
| 1-3 months | 10-1359 ng/mL |
| 3-6 months | 4-275 ng/mL |
| 6-12 months | 3-148 ng/mL |
| 1-3 years | 3-21 ng/mL |
| >3 years | <5 ng/mL |

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| **Critical Values** | None specified |
| **Limitations** | * The instrument reporting system contains error messages to warn the operator of specific malfunctions. Refer to Operator’s Manual for troubleshooting specific error messages.
* 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.
* This assay is intended only as an adjunct in the diagnosis and monitoring of AFP-producing tumors. The diagnosis should be confirmed by other tests or procedures. AFP is not recommended as a screening procedure for cancer detection in the general population.
* Amniotic fluid should not be sent, because this test is only used as a tumor marker. This test is not the correct AFP test for pregnant patients.
* This test is not intended for detection of neural tube defects. Higher values are found in newborns and pregnant women. Not useful in patients with pure seminoma or dysgerminoma.
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| **Dilutions** | 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 and/or the Manual Dilution Procedure. **Automated Dilution:**1. Maximum autodilution factor is 1:10 for serum and plasma.
2. 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. Do not use 1:40 instrument dilution; this is for amniocentesis fluid only, which is not an acceptable sample type at Children’s Minnesota.**Manual Dilution:**1. For a 1:20 dilution, add 50 μL of the patient specimen to 950 μL of the ARCHITECT *i* Multi-Assay Manual Diluent. Mix well.
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.
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| **Result Reporting** | * Results between 2 ng/mL and 2000 ng/mL will autofile
* Results <2 ng/mL should be reported as <2 ng/mL rather than the numerical value
* Results >2000 ng/mL should trigger an auto dilution of 1:10 on the analyzer.
* Results >20000 ng/mL will require a 1:20 manual dilution by tech.
* Results >40000 ng/mL will be reported as >40000 ng/mL rather than the numerical value after performing a 1:20 manual dilution
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| **Specimen Storage** | Promptly stopper tested specimen and store upright in specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 14 days in specimen storage freezer. |
| **References** | 1. Abbott Architect AFP reagent package insert, Abbott Laboratories, Abbott Park, IL, 60064. Revised Date May 2016
2. Abbott Architect AFP calibrator package insert Abbott Laboratories, Abbott Park, IL 60064. Revised November 2015.
3. Abbott Architect Safety Data Sheet, Abbott Diagnostics, Abbott Park, IL 60064. Revised July 2015.
4. Bio-Rad Lyphochek Specialty Immunoassay Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618 January 2018
5. [CALIPER Reference Studies](http://www.sickkids.ca/Caliperproject/index.html), accessed 4/20/2018.
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| **Historical Record** |

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| **Version** | **Written/Revised By** | **Effective Date** | **Summary of Revisions** |
| 1 | Kelsi Brown/Erin Bartos | May 15, 2018 | New Procedure |
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