| **AFP** |
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| **Purpose** | This procedure provides instructions Alpha-fetoprotein ON ABBOTT INSTRUMENTATION. The Alinity i AFP assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of alpha-fetoprotein (AFP) on the Alinity i analyzer in human serum or plasma to aid in monitoring disease progression during the course of disease and treatment of patients with nonseminomatous testicular cancer. |
| **Policy Statements** | This procedure applies to all personnel responsible for operating the Abbott Alinity i at Children’s Minnesota Laboratory. |
| **Principle** | This assay is a two-step immunoassay for the quantitative determination of AFP in human serum, plasma and amniotic fluid using chemiluminescent microparticle immunoassay (CMIA) technology.Sample and anti-AFP coated paramagnetic microparticles are combined and incubated. The AFP present in the sample binds to the anti-AFP coated microparticles. The mixture is washed. Anti-AFP acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of AFP in the sample and the RLUs detected by the system optics. For additional information on system and assay technology, refer to the Alinity ci-series Operations Manual, Section 3. |
| **Clinical Significance** | 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. 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.

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|  **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.  |

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| **Analyzer** | **Minneapolis: Abbott Alinity i (Sunquest method code: MACI)****Backup: MML** |
| **Sunquest Test Codes** | **AFPR** |
| **Specimen** | Sample: Serum or SST**Alternative: Lithium heparin or sodium heparin****Minimum sample volume:** 0.6 mL blood, 0.2 mL serum**Stability when separated from cells/gel:** **20 to 25°C- 3 days** **2 to 8°C- 7 days** **-20°C**- 7 days. Avoid more than 5 freeze/thaw cycles **Rejection criteria:** Unlabeled tube, sample type other than serum or acceptable plasma. Heat-inactivated specimensPooled specimensGrossly hemolyzed specimensSpecimens with obvious microbial contamination**Preparation:** 1. Whole blood specimens should be centrifuged following complete clot formation according to Specimen Processing procedures prior to analysis.
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. Specimens should be free of particulate matter.
4. Transfer serum or plasma to a properly labeled pilot tube. Alinity systems utilize a specimen level detect mechanism, so special racks specific to tube-type are not required.
5. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.
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| **Reagents** | **Reagent Handling** • Upon receipt, gently invert the unopened reagent kit by rotating it over and back for a full 180 degrees, 5 times with green label stripe facing up and then 5 times with green label stripe facing down. This ensures that liquid covers all sides of the bottles within the cartridges. During reagent shipment, microparticles can settle on the reagent septum.**–– Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.**• After mixing, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate. Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results. * Do not use reagents beyond the expiration date.
* Do not pool reagents within a kit or between kits.
* Do not use components from one lot with components from another lot.
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|  | **Alinity c:**

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Alinity AFP Reagent Kit | 07P9020 | **Store at:** 2 to 8 °C **Unopened:** 2 to 8 °C till Manufacturer’s Date. Store in upright position. If cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 1 hour before use. **On-board**: System temperature for 30 days.**Opened:** Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright. Store in upright position.If cartridge does not remain upright during storage, discard the cartridge.Do not reuse original reagent caps or replacement caps due to the risk of contamination and potential to compromise reagent performance.  |
| Alinity AFP Calibrators | 07P9001 | **Store at:** 2 to 8°C until expiration date**Opened expiration:** 2 to 8°C until expiration date. Return to refrigerated storage after use. Store tightly capped with new replacement cap. The analyzer will track In-use Stability, which is the time the calibrator is outside of refrigerated storage while on the analyzer. The analyzer will not allow the use of the calibrator if the In-use Stability has been exceeded. Maximum In-use Stability can be found in the Assay Parameter Report. For additional information on calibrator In-use Stability, refer to the Alinity ci-series Operations Manual, Section 5. |

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| **Risk and Safety** | Safety data sheets (MSDS/SDS) available on [Children’s Intranet](https://starnet.childrenshc.org/emergency-and-safety/) |
| **Calibration** | **Alinity c:**

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| Assay Range: | 2.0 to 2000.0 ng/mL |
| Reference Material: | Alinity AFP Calibrators |
| Suggested Calibration Levels: | A: 0B: 15C: 45D: 300E: 1500F: 2000 |
| Calibration Scheme: | The Alinity i AFP assay utilizes a 4 Parameter Logistic Curve fit datareduction method (4PLC, Y-weighted) |
| Calibration Frequency: | Every 6 months, every new lot of reagent, every time a critical part of the instrument is replaced, and as indicated by QC results |
| AMR | AMR is verified with each reagent calibration and at least once every 6 months. |

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| **Quality Control** | **QC Material:** BioRad Liquichek Immunoassay Plus Control, # 361/362/363. Levels 1, 2, 3**Frequency:** Three levels every 24 hours**Stability:** 5 day open stability when stored at 2 to 8 degrees C**Preparation**: Let stand at room temperature until thawed. Mix will by gently swirling and by inversion, taking care not to shake or induce bubbles or foaming. Ensure product is returned to refrigerated storage immediately after aliquoting. **Acceptable ranges:** * Non-Bio-Rad controls will utilize manufacturer ranges and 2 SD Westgard rules.
* New lots of Bio-Rad controls should be run for 20 days in parallel with the current lot whenever possible prior to switching to the new lot.
* Refer to the Westgard Rules in Chemistry procedure for current Westgard rules in place for each analyte.
* **Acceptable ranges are current in Unity Real Time only.** Quality Control results must be rejected in Sunquest when the results cross the interface.
* In the event of a QC failure, refer to the [Unity Real Time QC Review, General User](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.17-unity-real-time-qc-review-general-user.pdf) and navigate to the QC Troubleshooting section.
* Do not load or release patients until QC is acceptable in Unity Real Time.
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| **Interferences** | See package insert for substances tested that do not interfere with the reagent.  |
| **Reference Intervals** |

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| **Age** | **Reference Range** |
| 0-1 month | >2000.0 ng/mL |
| 1-6 months | 9.8-1359.0 ng/mL |
| 6-12 months | 0.4 – 103.1 ng/mL |
| 1-18 years | 0.8 to 34.8 ng/mL |
| 19 years and up | < 8.8 ng/mL |

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| **Critical Values** | None |
| **Limitations** | **•** 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). Specimens containing HAMA may produce anomalous values when tested with assay kits such as Alinity i 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 Alinity i 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 Alinity i 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 Alinity i AFP assay should not be used as a cancer screening test |
| **Dilutions** |

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| Max Auto Dilution: | 1:10 |
| Maximum Manual Dilution: | For a 1:20 dilution: Add exactly 50.0 μL of patient specimen to 950.0 μL of the Alinity i Multi-Assay Manual Diluent. For a 1:101 dilution using Alinity i Multi-Assay Manual Diluent:While adding the 10.0 uL of patient specimen to exactly 1.0 mL diluent in a properly labeled pilot tube, do not insert the tip of the pipette more than 2 mm below the surface of the diluent. Do not wet the tip with patient sample. Mix gently but thoroughly using a transfer pipette, ensuring no loss of patient specimen or diluent occurs. The operator must enter the dilution factor in the Specimen or Control tab of the Create Order screen. The system will use this dilution factor to automatically calculate the concentration of the sample and report the result. |
| Diluent: | Alinity i Multi-Assay Manual Diluent |
| Manual Dilution:  | Follow Abbott Operating instructions for programming manual dilutions. The system will automatically calculate the concentration of the sample and report the result. If a diluted sample result is less than the lower value of the measuring interval of 2.0 do not report the result. Rerun and/or investigate for other possible causes of error. |
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| **Result Reporting** | * Results between 2.0 and 2000.0 ng/mL without error messages are released
* Results below 2.0 without error messages are reported as < 2.0 ng/mL.
* Results > 2000.0 should be diluted using the onboard automated 1:10. Release results without error messages following this dilution.
* Results greater than 20000 following instrument dilution, perform 1:20 manual dilution. Results without error messages following this dilution are reported.
* Results > 40000.0 following the 1:20 manual dilution are further diluted using the 1:101 manual dilution. Results without error messages are released, and those greater than 202000.0 following the 1:101 dilution are credited and automatically reflexed to Mayo Medical Laboratories for testing.
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| **Specimen Storage** | Promptly stopper tested specimen and store upright in a specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 14 days in specimen storage freezer. |
| **References** | 1. Abbott Alinity i AFP Package Insert. Abbott Diagnostics Division, Abbott Park IL, USA. August 2019
2. Abbott Alinity i AFP Calibrator Package Insert. Abbott Diagnostics Division, Abbott Park, IL, USA. August 2019
3. [CALIPER reference Interval Study,](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CChemistry%5CReview%202020%5CAll%20Complete%5Caliper.research.sickkids.ca%5C#/login;next=search;queryParams=%7B%7D) accessed October 27, 2020
4. Blohm ME, Vesterling-Horner D, Calaminus G, et al: Alpha-1-fetoprotein (AFP) reference values in infants up to 2 years of age. Pediatr Hematol Onco 1998 Mar-April;15(2):135-142
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Erin Bartos | 10/28/2020 | New Procedure for Alinity |
| 2 | Erin Bartos | 11/9/2020 | Corrected Age ranges for reference intervals and added references to adult and 0-1 month reference intervals. |
|  | 3 | Erin Bartos | 11/23/2020 | Added 1:101 dilution, updated reflex value. Adult ref interval changed to <8.8  |