| **Ferritin Reagent** |
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| **Purpose** | This procedure provides instructions for performing FERRITIN on ABBOTT INSTRUMENTATION. The Alinity i Ferritin assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of ferritin in human serum and plasma on the Alinity i analyzer.  |
| **Policy Statements** | This procedure applies to all personnel responsible for operating the Abbott Architect c4000 or Alinity c at Children’s Minnesota Laboratory. |
| **Principle** | This assay is a two-step immunoassay for the quantitative determination of ferritin in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology. Sample and anti-ferritin coated paramagnetic microparticles are combined and incubated. The ferritin present in the sample binds to the anti-ferritin coated microparticles. The mixture is washed. Antiferritin 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 ferritin 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.**Methodology:** The Alinity i Ferritin assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of ferritin in human serum and plasma on the Alinity i analyzer. |
| **Clinical Significance** | Ferritin is a high-molecular weight iron-containing protein that functions in the body as an iron storage compound. Each ferritin molecule is thought to consist of a spherical protein shell ofmolecular weight about 460 000 daltons made up of 24 subunits with a variable amount of iron as a core of ferricoxide-phosphate It has been demonstrated that the ferritin molecule, when fully saturated, may consist of over 20% iron by weight. Approximately 25% of the iron in a normal adult is present in various storage forms. About two-thirds of the iron stores in the human body exist in the form of ferritin. The remaining iron stores are contained in insoluble hemosiderin, which most likely represents a form of denatured ferritin.The availability of sensitive methods for measuring serum ferritin have significantly advanced the ability to detect iron deficiency and overload. Since iron deficiency is present before the onset of anemia, detection of an iron depleted state is important for the control of nutritional anemia. The clinical assessment of iron stores has historically relied on the determination of serum iron, total iron binding capacity (TIBC) and percent transferrin (ratio of serum iron and TIBC) or direct examination of bone marrow.The estimation of stainable iron in the bone marrow is the traditional method for assessing body iron stores. This biopsy method provides a sensitive index of iron deficiency but has the disadvantage of being subjective and semiquantitative. Low hemoglobin concentration is the most readily available sign of anemia, but a significant fall in circulating hemoglobin cannot be detected until the final stage of iron deficiency anemia. Serum iron, TIBC and percent transferrin saturation do not distinguish iron deficiency as a progressive disease. Also, these measurements are affected by diurnal variation and may not discriminate between depleted iron stores and conditions associated with defective reticuloendothelial release of iron (e.g., anemia of chronic disease). Recent literature suggests that ferritin provides a more sensitive, specific and reliable measurement for determining iron deficiency at an early stage. In patients being given iron orally, serum ferritin measurements have been shown to be useful for monitoring the reaccumulation of iron stores and determining when therapy can be discontinued. In chronic inflammatory disorders, infections, and in chronic renal failure, there is a disproportionate increase in serum ferritin levels in relation to iron stores. The correlation of serum ferritin to body iron stores still exists, however, it is set at a higher level of serum ferritin. |
| **Analyzer** | **Minneapolis: Abbott Alinity i (Sunquest method code: MACI)****Backup:** Minneapolis Abbott Architect i1000SR (Sunquest Method Code: AI1) |
| **Sunquest Test Codes** | **FERI** |
| **Specimen** | Sample: Serum or Plasma (with or without gel barrier)**Preferred:** Serum**Alternative:** Lithium Heparin, EDTA Plasma* Individual plasma concentration values may differ from serum by more than 10%.
* Samples in tripotassium EDTA may give values below those of serum, while samples collected in lithium heparin may give values greater than serum values.
* When serial specimens are being evaluated, the same type of specimen should be used throughout the study.

**Minimum sample volume:** 0.75 mL blood, 0.25 mL serum/plasma**Maximum number of replicates sampled from the same sample cup**: 10**Run the sample in the priority lane on Alinity ci RSM.****Priority loaded:** **◦** Sample volume for first test: 70 μL **◦** Sample volume for each additional test from same sample cup: 20 μLRoutinely loaded: **◦** Sample volume for first test: 150 μL **◦** Sample volume for each additional test from same sample cup: 20 μL**Run the sample in the priority lane on Abbott Architect i1000 RSH**Maximum number of replicates sampled from the same sample cup: 10• **Priority loaded:**Sample volume for first test: 70 μLSample volume for each additional test from same sample cup: 20 μL• Routinely loaded:Sample volume for first test: 150 μLSample volume for each additional test from same sample cup: 20 μL**Stability when separated from cells/gel:** **20 to 25°C** Not specified. Move to storage at least once per shift.**2 to 8°C:** 7 days**-20°C:** 12 months **Rejection criteria:** Unlabeled tube, sample type other than serum or acceptable plasma**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 directly to a properly labeled pilot tube.
5. Architect and Alinity systems utilize a specimen level detect mechanism, so special racks specific to tube-type are not required.
6. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.
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| **Reagents** | **Reagent Handling** **Abbott Alinity i**• 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.• If a reagent cartridge is dropped, place 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. Use a pipette to remove all bubbles prior to loading on the Alinity or Architect system.* 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.

**Abbott Architect i1000:****•** 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. Microparticle mixing instructions:Do not remove the cap of the reagent with the pink microparticle label. Ensure it is tight. 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. AVOID bubbles and foam! 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.• 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. |
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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Alinity i Ferritin Reagent Kit | 07P6520 | **Store at:** 2 to 8°C**Unopened:** Until expiration 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**: 30 days**Stored off the system:** Until expiration date. 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 i Ferritin Calibrators | 07P6501 | **Store at:** 2 to 8°C * This product is liquid ready-to-use.
* This product may be used immediately after removal from 2 to8°C storage.
* Prior to each use, mix by gentle inversion.

**Unopened:** Until expiration date**Opened:** Until manufacturer’s printed expiration date.Store tightly capped with new replacement cap. Return to refrigerated storage after use. |
| Abbott Architect Ferritin Calibrators | 07K59-01 | **Store at:** 2 to 8°C • This product is liquid ready-to-use.• This product may be used immediately after removal from 2 to 8°C storage.• Prior to each use, mix by gentle inversion.**Unopened:** Until expiration date**Opened:** Until manufacturer’s printed expiration date. Store tightly capped with new replacement cap. Return to refrigerated storage after use. |
| Abbott Architect Ferritin Reagent | 07K59-25 | **Store at:** 2 to 8°C**Unopened:** Until expiration date. May be used immediately after removal from 2-8°C storage. Store in upright position.**On-board:** 30 days. Discard after 30 days. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.**Stored off the system:** Until expiration date. 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. |
| Abbott Alinity Multi-Assay Manual Diluent | 09P1540 | **Store at 15 to 30°C, tightly capped** until printed expiration date.  |
| Abbott Architect Multiassay Diluent | 07D8250 | **Store at 15 to 30°C, tightly capped until printed expiration date.**  |

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| **Risk and Safety** | **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. *For a detailed discussion of safety precautions during system operation, refer to the Alinity ci-series Operations Manual, Section 8.***The following warnings and precautions apply to:** *Cal 1 and Cal 2*C:\Users\CE154502\AppData\Local\Temp\SNAGHTML1b55202c.PNG **CAUTION:** This product contains human-sourced and/or potentially infectious components. Refer to the CONTENTS section of this package 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 this product 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. Contains sodium azide. Contact with acids liberates very toxic gas. Dispose of contents / container in accordance with local regulations.No special reagent disposal is indicated.Safety data sheets (MSDS/SDS) available on [Children’s Intranet](https://starnet.childrenshc.org/emergency-and-safety/) |
| **Calibration** |

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| Assay Range: | 2 – 1676 ng/mL |
| Reference Material: | Alinity i Ferritin calibrator kit, Architect Ferritin calibrator kit |
| Suggested Calibration Levels: | CAL 1: 10CAL 2: 1000 ng/mL |
| Calibration Scheme: | 2 Levels |
| Calibration Frequency: | With every new lot number, after maintenance or service of major instrument parts, as indicated by quality controls, and as directed by field service representatives. |
| AMR | AMR is verified twice annually using the AUDIT K833M-5 by running all applicable levels in triplicate. Assay results are submitted to Audit Microcontrols and/or entered in EP Evaluator for compilation. Results are reviewed and approved by the Technical Specialist. Questionable results are investigated and corrective actions documented. |

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| **Quality Control** | **QC Material:** BioRad Liquichek Immunoassay Plus Levels 1,2 and 3 **Frequency:** Three levels each day of use. **Stability:** 5 Days at 2-8°C (due to the inclusion and use of Estradiol in this control) **Preparation**: Let vials thaw for 30 minutes at room temperature and gently swirl to ensure homogeneity. Do not allow to stand at room temperature longer than 20 minutes after completely thawed. **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** | The testing demonstrated ≤ 10% mean interference at the levels indicated below.Hemoglobin: ≤ 200 mg/dLBilirubin: ≤ 20 mg/dLTriglycerides: ≤ 3000 mg/dLProtein: ≤ 12 g/dLSamples with moderate to gross hemolysis may yield unpredictable results. Append –HP (hemolysis present) to samples with visible moderate to marked hemolysis.  |
| **Reference Intervals** | **Male and Female**:0 to 14 Days: 99.6-717 15 Days to < 6 Months: 14-647.2 6 Months to < 1 Year: 8.4-181.9 1 to < 5 Years: 5.3-99.95 to < 14 Years: 13.7-78.8  **Female**: 14 to < 19 Years: 5.5-67.4  Adult: 5 - 204  **Male**: 14 to < 16 Years: 12.7-82.8 16 to < 19 Years: 11.1-171.9  Adult: 22 – 275 |
| **Critical Values** | None specified |
| **Limitations** | For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.If the Ferritin results are inconsistent with clinical evidence, additional testing is recommended.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 Alinity i Ferritin that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.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. |
| **Dilutions** |

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| Max Auto Dilution: | 1:20. Utilize the automated instrument dilution.  |
| Maximum Manual Dilution: | 1:100Carefully pipette 450 µL of Abbott Architect or Alinity Multi-Assay Manual Diluent into a pilot tube. Add exactly 50.0 µL patient sample to make a 1:10 dilution. Mix well; do not create bubbles or foam. In another pilot tube, pipette 450 µL of Multi-Assay Manual diluent, this time adding exactly 50.0 µL of the 1:10 dilution to make a 1:100 dilution. Mix well; avoid bubbles or foam. Manually program the 1:100 dilution on the analyzer. |   |
| Diluent: | Onboard diluent (automated) or Alinity/Architect specific Multi-Assay Manual Diluent (manual) |
| Automated dilution: | Follow Abbott [Alinity Operator’s Manual](https://starnet.childrenshc.org/References/labsop/chem/operator/alinity-ci-series-operations-manual.pdf) instructions for programming automated and manual dilutions. The system will automatically calculate the concentration of the sample and report the result when the dilutions are programmed correctly.If a diluted sample result is less than the lower value of the measuring interval of 2 ng/mL, do not report the result. Rerun and/or investigate for other possible causes of error. |

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| **Result Reporting** | * Results between 2 and 1676 ng/mL without error messages are released
* Results below 2 without error messages are reported as < 2 ng/mL.
* Results > 1676 should be diluted using the onboard automated 1:20 dilution. Release results without error messages following this dilution.
* Results > 33520 following automated dilution should be manually diluted 1:100 using the serial dilution technique described above. Release results without error messages following this dilution.
* Results >167600 following this dilution are reported as >167600 ng/mL.
* Append –HP to all samples that have moderate to gross visible hemolysis.
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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 Ferritin Reagent Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised February 2018
2. Abbott Alinity i Ferritin Calibrator Kit Package Insert, Abbott Diagnostics Division, Abbott Park, IL USA. Revised February 2018
3. Abbott ARCHITECT Ferritin Reagent Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised November 2015
4. Abbott ARCHITECT Ferritin Calibrator Kit Package Insert, Abbott Diagnostics Division, Abbott Park, IL USA. Revised November 2015
5. [CALIPER Reference Interval Studies](https://caliper.research.sickkids.ca/#/login;next=search;queryParams=%7B%7D), accessed 10/27/2020.
6. BioRad Liquichek Immunoassay Plus Quality Control Package Insert, BioRad Laboratories, Irvine, CA. Revised April 2020
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Elauteria Earnhardt | May 6, 2020 | New Procedure for Abbott analyzers |
| 1 | Erin Bartos | October 28, 2020 | Corrected instrument, added AMR, dilution, calibrator, reference intervals, corrected QC product, added references, etc for new procedure. |
|  | 2 | Erin Bartos | January 27, 2021 | Added Architect i1000SR analyzer information and manual dilution up to 1:100 |