| **Free Thyroxine (FT4)** |
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| **Purpose** | This procedure provides instructions for performing FREE THYROXINE (FT4) on ABBOTT INSTRUMENTATION. The Alinity and Architect Free T4 assays are a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of free thyroxine (free T4) in human serum and plasma on the Alinity and Architect analyzers. |
| **Policy Statements** | This procedure applies to all personnel responsible for operating Alinity ci and Architect i1000 SR at Children’s Minnesota Laboratory in Minneapolis. |
| **Principle** | This assay is a two-step immunoassay for the quantitative determination of free thyroxine (free T4) in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology. Sample and anti-T4 coated paramagnetic microparticles are combined and incubated. The free T4 present in the sample binds to the anti-T4 coated microparticles. The mixture is washed. T3 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 an inverse relationship between the amount of free T4 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** | Thyroxine (T4) circulates in the blood as an equilibrium mixture of free and serum protein bound hormone. Thyroxine binding globulin (TBG), albumin and pre-albumin bind approximately 75%, 10% and 15% of the total circulating T4 respectively. The binding of T4 by these proteins is such that less than 0.03% is present in the circulation as unbound, free T4. This small percentage of the total T4 represents the physiologically available hormone which is biologically active. Once the free T4 is absorbed by the target cells, the equilibrium reestablishes circulating free T4 levels. The equilibrium results in the maintenance of a constant level of free T4 when alterations occur in either the concentration or affinity of the serum binding proteins. Therefore, in a variety of normal (pregnancy) and abnormal (Familial Dysalbuminemic Hyperthyroxinemia, FDH) states, or as a result of the administration of certain drugs (e.g., furosemide and fenclofenac), the target tissues are assured of receiving the required amount of hormone. Free T4 values may, therefore, provide the best indication of thyroid dysfunction, since free T4 is less sensitive to changes in the serum binding proteins.Historically, the diagnosis of thyroid function has involved performing a total T4 assay in addition to a Thyroxine Uptake (TU) assay of the same sample. The mathematical combination of these two assays produces a Free Thyroxine Index (FTI) which provides an indirect proportional estimate for free T4. Alternatively, direct assays have been developed using equilibrium dialysis, ultrafiltration, RIA, and solid-phase EIA technology to measure free T4. In these methods, separation of free and bound tracer is achieved either with a membrane, or by binding free T4 to a solid phase antibody. This extraction step removes an amount of T4 which is proportional to the original amount of free T4 present in the patient sample. Provided that the extracted T4 is less than approximately 5% of the T4 in the sample, a true estimation of the free T4 content can be obtained. |
| **Analyzer** | **Primary: Abbott Alinity ci (Sunquest method code: MACI)****Backup: Abbott Architect i1000SR (Sunquest method code: AI1**) |
| **Sunquest Test Codes** | **FT4** Free Thyroxine**LFT4** Free T4 Low, reflex to Free T4 by dialysis at Mayo |
| **Specimen** | Sample: Plasma or Serum (with or without gel barrier)**Preferred:** Lithium Heparin**Alternative:** SST, Sodium Heparin, EDTA When serial specimens are being evaluated, the same type of specimen should be used throughout the study.**Minimum sample volume:** 0.6 mL blood, 0.2 mL serum/plasma**LFT4:** 2.6 mL Serum (1.2 mL minimum serum)/7.0 mL whole blood (3.1 mL whole blood minimum). (Gold Top, Marble Top or Red Top). Serum must be separated from gel within 4 to 6 hours of draw. Reject samples with gross hemolysis, icterus, or lipids. Stability: 2-8°C / 28 days. This sample reflexes to Mayo if less than 0.8Maximum number of replicates sampled from the same sample cup: 10**Alinity ci****Load samples in a priority lane on the RSH to minimize sample requirements.****Priority loaded:**  Sample volume for first test: 84 μL Sample volume for each additional test from same sample cup: 34 μLRoutinely loaded: Sample volume for first test: 150 μL Sample volume for each additional test from same sample cup: 34 μL**Architect i1000SR** **Load samples in a priority lane on the RSM to minimize sample requirements.****Priority loaded:**  Sample volume for first test: 95 μL Sample volume for each additional test from same sample cup: 45 μLRoutinely loaded: Sample volume for first test: 150 μL Sample volume for each additional test from same sample cup: 45 μL**Stability when separated from cells/gel:** **20 to 25°C** Not specified. Remove to storage at least once per shift.**2 to 8°C** 6 days**-20°C** 14 days**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** **Alinity c** 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 kit in 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.

**Architect i1000SR:**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:• 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.• Load the reagent kit on the ARCHITECT iSystem.• Verify that all necessary reagents are present.• Ensure that septums are present on all reagent bottles.• 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. |
|  | **Alinity c and Architect i1000:**

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Alinity i Free T4 Reagent Kit  | 07P7020 | **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**Opened**: 2 to 8°C Until expiration dateStore 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 the potential to compromise reagent performance. |
| Alinity i Free T4 Calibrators | 07P7001 | **Store at:** 2 to 8°C**Unopened:** Until expiration date**Opened expiration:** Until expiration date. Store tightly capped with new replacement cap.Return to refrigerated storage after use. 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.For additional information on printing assay parameters, refer to the Alinity ci-series Operations Manual, Section 5.  |
| Abbott Architect Free T4 Reagent Kit | 07K6529 | **Store at:** 2 to 8°C**Unopened:** Until printed expiration date**Onboard expiration:** 30 daysReagents 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. |
| Abbott Architect Free T4 Calibrators | 07K6502 | **Store at:** 2 to 8°C**Unopened:** Until expiration date**Opened expiration:** Until 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 agentsNo special reagent disposal indicated.Safety data sheets (MSDS/SDS) available on [Children’s Intranet](https://starnet.childrenshc.org/emergency-and-safety/) |
| **Calibration** | **Alinity ci and Architect i1000SR:**

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| Assay Range: | 0.42 – 5.00 ng/dL |
| Reference Material: | Abbott Alinity i Free T4 CalibratorsAbbott Architect Free T4 Calibrators |
| Suggested Calibration Levels: | CAL A: 0.0CAL B: 0.5CAL C: 1.0CAL D: 2.0CAL E: 3.5CAL F: 6.0 |
| Calibration Scheme: | 6 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 with every calibration and at least every 6 months. |

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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** | An Abbott study was performed on the ARCHITECT i System. The Free T4 assay is designed to have a mean potential interference from hemoglobin, bilirubin, triglycerides, and protein of < 10% at the levels indicated below and applies to both analyzers:Hemoglobin ≤ 500 mg/dLBilirubin ≤ 20 mg/dLTriglycerides ≤ 3000 mg/dLProtein ≤ 12 g/dLFT4 is usually measured by automated analog immunoassays. In most instances, this will result in accurate results. However, abnormal types or quantities of binding proteins found in some patients and most often related to other illnesses or drug treatments may interfere in the accurate measurement of FT4 by analog immunoassays. These problems can be overcome by measuring FT4 by equilibrium dialysis, free from interfering proteins.  |
| **Reference Intervals** | **Male and Female:** in ng/dL0 to 14 days: 0.7 - 3.21 15 to 29 Days: 0.7 - 2.5330 Days to < 1 Year: 0.7 - 1.71 to < 19 Years: 0.7 - 1.37Adult: 0.70 - 1.48  |
| **Critical Values** | None specified |
| **Limitations** | Results should be used in conjunction with other data; e.g., symptoms, results of other thyroid tests, clinical impressions, etc.If the Free T4 results are inconsistent with clinical evidence, additional testing is suggested to confirm the result. It is recommended that neonates be tested by another method if results do not coincide with other data; e.g., symptoms, results of other thyroid tests, clinical impressions, etc. A separate order for children less than 1 year of age is orderable to send to Mayo Medical Laboratories. FT4 is usually measured by automated analog immunoassays. In most instances, this will result in accurate results. However, abnormal types or quantities of binding proteins found in some patients and most often related to other illnesses or drug treatments may interfere in the accurate measurement of FT4 by analog immunoassays. These problems can be overcome by measuring FT4 by equilibrium dialysis, free from interfering proteins. |
| **Dilutions** | Do not dilute. |
| **Result Reporting** | **Alinity c and Architect i1000SR:*** Results between 0.42 and 5.0 ng/dL without error messages are released
* Results below 0.42 without error messages are reported as < 0.42 ng/dL
* Results > 5.0 are reported as > 5.0 ng/dL

**Test Code LFT4:**Results between 0.8 to 5.0 ng/dL without error messages are releasedResults greater than 5.0 ng/dL: report as > 5.0 ng/dL after repeat analysisResults <0.8 will order a new test to be sent to Mayo Laboratory for Free T4 by Equilibrium DialysisChildren less than 1 year should be tested by Free T4 at Mayo Medical Laboratories, MML test code FRT4. |
| **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 Free T4 Reagent Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised February 2018
2. Abbott Alinity i Free T4 Calibrator Kit Package Insert, Abbott Diagnostics Division, Abbott Park, IL USA. Revised December 2017
3. Abbott Architect Free T4 Reagent Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised April 2017
4. Abbott Architect Free T4 Calibrator Kit Package Insert, Abbott Diagnostics Division, Abbott Park, IL USA. Revised June 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 29, 2020 | New Procedure for Abbott analyzers |
| 1 | Erin Bartos | October 28, 2020 | Added Alinity ci analyzer, added i1000 analyzer. References, reference intervals, AMR, calibrators, reagents, sample, etc. for new testing/new analyzer. Children less than 1 year should be tested by MML |