| **Total Thyroxine (T4)** |
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| **Purpose** | This procedure provides instructions for performing TOTAL THYROXINE (T4) ON ABBOTT INSTRUMENTATION. The Alinity i Total T4 (TT4) assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of thyroxine (Total T4) in human serum and plasma on the Alinity i analyzer. |
| **Policy Statements** | This procedure applies to all personnel responsible for operating Alinity ci at Children’s Minnesota Laboratory in Minneapolis. |
| **Principle** | This assay is a two-step immunoassay for the quantitative determination of thyroxine (Total T4) in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology.Sample and anti-T4 coated paramagnetic microparticles are combined and incubated. Bound T4 is removed from the binding sites on thyroxine binding globulin, prealbumin and albumin. The 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 Total 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) is an iodine-containing hormone which has a molecular weight of approximately 777 daltons and is secreted by the thyroid gland. T4 and its associate thyroid hormone T3 are responsible for regulating diverse biochemical processes throughout the body which are essential for normal metabolic and neural activity.Although T3 has greater biologic potency2, T4 is normally present in human serum in approximately 50-fold excess of circulating T3 and accounts for more than 90% of the circulating protein-bound iodine. T4 is 99.9% bound to serum thyroxine binding proteins (TBP). The hormone is transported bound primarily to thyroxine binding globulin (TBG) and secondarily by thyroxine binding prealbumin (TBPA) and albumin. Less than 0.05% of the total circulating T4 is unbound and therefore biologically active. Clinically, T4 measurements have long been recognized as an aid in the assessment and diagnosis of thyroid status. Elevated T4 values are characteristically seen in patients with overt hyperthyroidism, while T4 levels are generally depressed in patients with overt hypothyroidism. Normal T4 levels accompanied by high T3 values are seen in patients with T3- thyrotoxicosis. T4 levels are altered by physiological or pathological changes in TBP capacity. Thyroxine binding globulin (TBG) capacity has a pronounced effect on the concentration of thyroid hormones. Consequently, T4 levels may be elevated with increased concentrations of TBG, such as in pregnancy, administration of oral contraceptives or estrogen, infectious and chronic active hepatitis, biliary cirrhosis or congenital increase in TBG levels. Conversely, when TBG levels are decreased, such as in nephrotic syndrome, androgen therapy, glucocorticoid therapy, major systemic illness or congenital decrease of TBG, T4 may be reduced. Drugs which compete for protein binding sites, such as phenylbutazone, diphenylhydantoin or salicylates, can result in a depressed T4 measurement. Serum T4 levels in neonates and infants are higher than values in the normal adult, due to the increased concentration of TBG in neonate serum.While in many cases T4 values give good indications of thyroid status, T4 values should be normalized for individual variations in thyroxine binding protein (TBP) capacity. The Free Thyroxine Index (FTI) is conventionally used to achieve this measurement.To ensure maximum diagnostic accuracy, the final definition of thyroid status should be determined in conjunction with other thyroid function tests such as TSH, Free T4, Total T3, FTI and clinical evaluation by the physician. |
| **Analyzer** | **Minneapolis: Abbott Alinity i (Sunquest method code: MACI)****Backup:** Hold samples until instrument is back in service. If urgent or directed by provider, send to Mayo Medical Laboratories (T4) |
| **Sunquest Test Codes** | **T4** Thyroxine Total |
| **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/plasmaMaximum number of replicates sampled from the same sample cup: 10Priority loaded: Sample volume for first test: 74 μL Sample volume for each additional test from same sample cup: 24 μLLoaded routinely: Sample volume for first test: 150 μL Sample volume for each additional test from same sample cup: 34 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** 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.
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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Alinity i Total 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 daysStore 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 Total T4 Calibrator Kit | 07P7001 | **Store at:** 2 to 8°C**Unopened:** Until printed 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. |

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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 Special reagent disposal not 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: | 3.0 – 24.0 µg/dL |
| Reference Material: | Alinity i Total T4 Calibrator Kit |
| Suggested Calibration Levels: | CAL A: 0.0CAL B: 3.0CAL C: 6.0CAL D: 12.0CAL E: 18.0CAL F: 24.0 |
| 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 at least every six 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** | A study was performed based on guidance from CLSI document EP7-A on the ARCHITECT i System. Potential interference was assessed with the ARCHITECT Total T4 assay and the mean interference was determined to be < 10% for hemoglobin, bilirubin, triglycerides, and protein at the levels indicated below:Hemoglobin ≤ 500 mg/dLBilirubin ≤ 20 mg/dLTriglycerides ≤ 3000 mg/dLProtein ≤ 12 g/dL |
| **Reference Intervals** | **Male and Female**: 0 to <1 year: 5.87 - 13.7 1 to <9 years: 6.16-10.3 9 to <12years: 5.48 to 9.31 12 to <14 years: Female: 5.08 - 8.34 Male: 5.01 - 8.28 14 to <19 years: Female: 5.46 - 13.0 Male: 4.68 - 8.62 **Adult**: 4.87 - 11.72 |
| **Critical Values** | None specified |
| **Limitations** | For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other thyroid tests, clinical impressions, etc.If the Total 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. When serial specimens are being evaluated, the same type of specimen should be used throughout the study. |
| **Dilutions** | Samples should not be diluted for the Alinity i Total T4 assay. |
| **Result Reporting** | * Results between 3.0 and 24.0 without error messages are released
* Results below 3.0 without error messages are reported as < 3.0 µg/dL.
* Results > 24.0 are reported as > 24.0 µg/dL.
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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 Total T4 Reagent Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised February 2018
2. Abbott Alinity i Total T4 Calibrator Kit Package Insert, Abbott Diagnostics Division, Abbott Park, IL USA. Revised February 2018
3. [CALIPER Reference Interval Studies](https://caliper.research.sickkids.ca/#/login;next=search;queryParams=%7B%7D), accessed 10/27/2020.
4. 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** |
| 1 | Erin Bartos | October 28, 2020 | New Procedure for Abbott Alinity analyzer |
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