| **Triiodothyronine (TT3), Total** |
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| **Purpose** | This procedure provides instructions for performing TRIIODOTHYRONINE (TT3) on ABBOTT INSTRUMENTATION. The Alinity i Total T3 (TT3) assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of total triiodothyronine (Total T3) 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 total triiodothyronine (Total T3) in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology.Sample and anti-T3 coated paramagnetic microparticles are combined and incubated. The T3 present in the sample binds to the anti-T3 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** | 3,5,3’ Triiodothyronine (T3) is a thyroid hormone with a molecular weight of 651 daltons and a half-life in serum of 1.5 days. T3 circulates in the blood as an equilibrium mixture of free and protein bound hormone. T3 is bound to thyroxine binding globulin (TBG), prealbumin, and albumin. The actual distribution of T3 among these binding proteins is controversial as estimates range from 38-80% for TBG, 9-27% for prealbumin, and 11-35% for albumin. The binding of these proteins is such that only 0.2-0.4% of the total T3 is present in solution as unbound or free T3. This free fraction represents the physiologically active thyroid hormone.It has become apparent in recent years that T3 plays an important role in the maintenance of the euthyroid state. Serum T3 measurements can be a valuable component of a thyroid screening panel in diagnosing certain disorders of thyroid function as well as conditions caused by iodine deficiency. Clinically, measurements of serum T3 concentration are especially valuable in diagnosing hyperthyroidism and in following the course of therapy for this disorder. Under conditions of strong thyroid stimulation, the T3 measurement provides a good estimation of thyroid reserve. Recognition of a thyroid dysfunction called T3-thyrotoxicosis, associated with an increased serum T3 level but normal thyroxine (T4), free T4, and in vitro Uptake results have further highlighted the importance of serum T3 measurements. Dietary iodine deficiency results in inadequate production of thyroid hormones despite the presence of normal thyroid tissue. In these cases, the serum T4 concentration is often low while the Thyroid Stimulating Hormone (TSH) concentration is elevated. Elevated TSH associated with low T4 is normally indicative of hypothyroidism. However, in iodine deficiency, these results together with normal or slightly elevated serum T3 are indicative of euthyroid status in most individuals.T3 levels are also affected by conditions which affect TBG concentration. Slightly elevated T3 levels may occur in pregnancy or during estrogen therapy, while depressed levels may occur during severe illness, malnutrition, in renal failure and during therapy with anti-thyroid drugs, propranolol and propylthiouracil and salicylates. In patients with severe or chronic illnesses, many abnormalities of thyroid hormone balance occur. T4 production and the extent of serum thyroid hormone binding may be independently abnormal, resulting in a low, normal or high free T4 estimate. Serum T3 concentrations are often low; TSH levels may be normal or slightly elevated. Total T3 measurements may be valuable when hyperthyroidism is suspected and the free T4 estimate is normal. The Alinity i Total T3 assay is to be used as an aid in the assessment of thyroid status. |
| **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 (T3) |
| **Sunquest Test Codes** | **TT3** Total Triiodothyronine (Total T3) |
| **Specimen** | Sample: Plasma or Serum (with or without gel barrier)**Preferred:** Lithium Heparin**Alternative:** SST, Sodium Heparin, EDTA **Minimum sample volume:** 0.6 mL blood, 0.2 mL serum/plasmaWhen serial specimens are being evaluated, the same type of specimen should be used throughout the study. Performance has not been established for the use of neonatal specimens. Children under 1 year of age should be tested with Mayo Medical Laboratories test code T3.Maximum number of replicates sampled from the same sample cup: 10Priority loaded: Sample volume for first test: 70 μL Sample volume for each additional test from same sample cup: 20 μLLoaded routinely: Sample volume for first test: 150 μL Sample volume for each additional test from same sample cup: 20 μL**Stability when separated from cells/gel:** **20 to 25°C** Not specified. Remove to storage at least once every 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 2 hours 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 T3 Reagent Kit  | 07P9420 | **Store at:** 2 to 8°C**Unopened:** Until printed 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 2 hours before use. **On-board**: 30 daysIf removed from the system, 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 the potential to compromise reagent performance. |
| Alinity i Total T3 Calibrators | 07P9401 | **Store at:** 2 to 8°C**Unopened:** Until printed expiration date**Opened expiration:** Until printed 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 Operation Manual, Section 5. |
| Alinity i Total T3 Manual Diluent | 07P9440 | **Store at:** 2 to 8°C**Unopened/Opened:** Until printed expiration date Store tightly capped. Store in an upright position. Return to refrigerated storage after use. |

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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**The following warnings and precautions apply to:** *Conjugate* **WARNING** Contains methylisothiazolones.  May cause an allergic skin reaction.**The following warnings and precautions apply to:** *Microparticles* Contains sodium azide. Contact with acids liberates very toxic gas.**The following warnings and precautions apply to:** *Cal A- Cal F***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.Special 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: | 40 – 600 ng/dL |
| Reference Material: | Abbott Alinity i Total T3 Calibrator kit |
| Suggested Calibration Levels: | CAL A: 20CAL B: 50CAL C: 75CAL D: 150CAL E: 350CAL F: 600 |
| 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 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** | Abbott performed a study on the ARCHITECT i System. Potentially Interfering Endogenous Substances:The testing demonstrated ≤ 10% mean interference at the levels indicated below.Hemoglobin ≤ 500 mg/dLBilirubin ≤ 20 mg/dLTriglycerides ≤ 3000 mg/dLProtein ≤ 12 g/dL |
| **Reference Intervals** | **Female: (ng/dL)** 4 Days to < 1 Year: 84.6 - 234 1 to < 12 Years: 113 - 189 12 to < 15 Years: 97.7 - 176 15 to < 17 Years: 92.5 - 142 17 to < 19 Years: 89.8 - 168 **Male:** (**ng/dL**)4 Days to < 1 Year: 84. 6 - 2341 to < 12 Years: 113 - 18912 to < 15 Years: 97.7 - 176 15 to < 17 Years: 93.8 - 15617 to < 19 Years: 89.8 – 168**Adult**: 35 to 193 ng/dL |
| **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 T3 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 test is orderable for children under 1 year of age is available for Mayo Medical Laboratories test code T3.  |
| **Dilutions** |

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| Max Auto Dilution: | None |
| Maximum Manual Dilution: | 1:2Add a minimum of 75 μL of the sample to 75 μL of Alinity i Total T3 Manual Diluent. |
| Diluent: | Abbott Alinity i Total T3 Manual Diluent |
| Manual Dilution: | Follow Abbott [Alinity Operator’s Manual](https://starnet.childrenshc.org/References/labsop/chem/operator/alinity-ci-series-operations-manual.pdf) 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 40 ng/dL, do not report the result. Rerun and/or investigate for other possible causes of error. |

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| **Result Reporting** | * Results between 40 and 600 ng/dL without error messages are released
* Results below 40 without error messages are reported as < 40 ng/dL.
* Results > 600 should be diluted using the onboard manual 1:2 dilution. Release results without error messages following this dilution.
* Results > 1200 following automated dilution are reported as > 1200 ng/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 T3 Reagent Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised April 2020
2. Abbott Alinity i Total T3 Calibrator Kit Package Insert, Abbott Diagnostics Division, Abbott Park, IL USA. Revised February 2018
3. Abbott Alinity i Total T3 Manual Diluent Insert, Abbott Diagnostics Division, Abbott Park, IL USA. Revised February 2018
4. [CALIPER Reference Interval Studies](https://caliper.research.sickkids.ca/#/login;next=search;queryParams=%7B%7D), accessed 10/27/2020.
5. 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 i analyzer, children under 1 year of age have separate test code |
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