| **Thyroglobulin Antibody (Anti- Tg)** |
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| **Purpose** | This procedure provides instructions for performing THYROGLOBULIN ANTIBODY (also known as Anti-Tg) ON ABBOTT INSTRUMENTATION. The Alinity i Anti-Tg assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of the IgG class of thyroglobulin autoantibodies (anti-Tg) in human serum and plasma on the Alinity i analyzer.The Alinity i Anti-Tg assay is to be used as an aid in the diagnosis of autoimmune thyroid disease. |
| **Policy Statements** | This procedure applies to all personnel responsible for operating the Abbott Alinity i at Children’s Minnesota Laboratory in Minneapolis. |
| **Principle** | This assay is a two-step immunoassay for the quantitative determination of the IgG class of thyroglobulin autoantibodies (anti-Tg) in human serum and plasma using chemiluminescentmicroparticle immunoassay (CMIA) technology. Sample, Tg coated paramagnetic microparticles, and assay diluent are combined and incubated. The anti-Tg present in the sample binds to the Tg coated microparticles. The mixture is washed. Anti-human IgG 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 anti-Tg 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** | Autoimmune thyroiditis was first described by Hashimoto in 1912 and autoimmune thyroid disease with associated goitre is termed Hashimoto’s thyroiditis. The presence of anti-Tg in patients with this disease was first demonstrated in 1956 by Roitt, et al using a precipitin reaction. Unlike autoantibodies to thyroid peroxidase (anti-TPO), autoantibodies to thyroglobulin do not appear to be pathogenic and may simply be indicators of disease. They have been found to be polyclonal in nature and are also heterogeneous with respect to heavy chain subclass. Thyroglobulin is a glycoprotein of 670 000 daltons, which is comprised of two identical subunits and represents the major protein found in the thyroid. This protein provides 40 tyrosine residues, of the 140 in the molecule, used for iodination during the biosynthesis of thyroxine (T4) and triiodothyronine (T3) and, therefore, is responsible for the accumulation of iodine by the thyroid gland.Although anti-Tg are found in conjunction with anti-TPO in the majority of cases of Hashimoto’s thyroiditis, Primary Myxedema and Graves’ disease, up to 1% of cases of hypothyroidism are associated with anti-Tg alone.13 Anti-Tg are associated with cases of mild hypothyroidism or hyperthyroidism, and are frequently found in patients with other autoimmune diseases such as Rheumatoid Arthritis, Pernicious Anemia and Type I Diabetes. Anti-Tg are detected in 30-60% of cases of thyroid carcinoma patients. In such patients, measurement of Tg antigen must take into account the likelihood of the presence of significant levels of anti‑Tg, since measurement and detection of Tg antigen may be influenced by the presence of anti-Tg. Furthermore, low levels of anti-Tg are also found in up to 20% of asymptomatic individuals, particularly the elderly and more often in women than men, although the clinical significance of these autoantibodies is unclear. |
| **Analyzer** | **Minneapolis: Abbott Alinity i (Sunquest method code: MACI)****Backup:** Hold samples until instrument is back in service. If directed by provider, send to Mayo Medical Laboratories for reduced turn-around time. (Code ATA) |
| **Sunquest Test Codes** | **ATAB** |
| **Specimen** | Sample: Serum or Serum Separator Tube (SST)**Preferred:** SST**Alternative:** Lithium heparin, sodium heparin, EDTA, plasma separator with lithium heparin**Minimum sample volume:** 1.0 mL blood, 0.25 mL serum/plasmaPriority loaded: Sample volume for first test: 75 μL Sample volume for each additional test from same sample cup: 25 μLRoutinely loaded: Sample volume for first test: 150 μL Sample volume for each additional test from same sample cup: 25 μL**Stability when separated from cells/gel:** **20 to 25°C** 8 hours**2 to 8°C** 72 Hours (remove from clot/gel if testing is delayed more than 8 hours.)**-20°C** 30 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 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.
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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Alinity i Anti Tg Reagent Kit | 09P3420 | **Store at:** 2 to 8°C**Unopened:** Until manufacturer’s printed expiration date**On-board**: System temperature for 30 days |
| Alinity i Anti Tg Calibrators | 09P3401 | **Store at:** -10°C or colder until expiration date**Unopened:** Until manufacturer’s printed expiration date**Opened expiration:** 2-8°C 30 days after thaw, not to exceed expiration date on bottle.  |

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| **Risk and Safety** | **CAUTION:** This product contains human-sourced and/or potentially infectious components. 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 these reagents 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.The human-sourced material used in the microparticles is nonreactive for HBsAg, HIV-1 RNA, anti-HCV, and anti-HIV-1/HIV-2.No special disposal 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: | 3.00 to 1000.00 IU/mL |
| Reference Material: | Abbott Alinity i Anti-Tg Calibrator Kit |
| Suggested Calibration Levels: | CAL A: 0.0CAL B: 5.0CAL C: 62.5CAL D: 125.0CAL E: 500.0CAL F: 1000.0 |
| Calibration Scheme: | The Alinity i Anti-Tg assay utilizes a 4 Parameter Logistic Curve fitdata reduction method (4PLC, Y-weighted) to generate a calibrationand results. |
| Calibration Frequency: | As required by quality control results, after major instrument maintenance or replacement of critical parts, as directed by technical support or field service, at least every 6 months. |
| AMR | AMR is verified with every calibration. |

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| **Quality Control** | **QC Material:** Bio-Rad Liquichek Specialty Immunoassay Control Levels 1, 2, & 3**Frequency:** Three levels each day of use**Stability:** 30 days when stored at 2-8° C and exposure to room temperature is minimized.**Preparation**: Allow the product to thaw for approximately one hour at room temperature, or until completely thawed. Gently swirl until homogenous with no visible signs of precipitate. Minimize exposure to room temperature; store tightly capped and refrigerated between uses. **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 based on guidance from NCCLS EP7-A was performed for the ARCHITECT Anti-Tg assay. Specimens with anti-Tg levels between 53.41 and 320.25 IU/mL were supplemented with the following potentially interfering compounds. The average amount of interference observed during the study ranged from -3.8% to +1.7%.Bilirubin ≤ 20 mg/dLHemoglobin ≤ 1000 mg/dLTotal Protein (Low) 4 g/dLTotal Protein (High) 10 g/dLTriglycerides ≤ 2000 mg/dLSee the assay Instructions for Use for other conditions and known autoimmune diseases which did not cross-react or interfere in studies completed by Abbott. |
| **Reference Intervals** | 0.0-12.5 IU/mL |
| **Critical Values** | None specified. |
| **Limitations** | Antibody measurement represents one parameter in a multicriteria diagnostic process. When making a diagnosis of thyroid disease, a combination of test methods should be used in conjunction with clinical symptoms.About 20% of asymptomatic specimens may present with anti-Tg autoantibodies reflecting the prevalence in apparently healthy populations. The prevalence of anti-Tg may also depend on age, gender, and geographic region of the selected population.Some specimens may not dilute linearly because of the heterogeneity of the autoantibodies with respect to physiochemical properties.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 that employ mouse monoclonal antibodies. Assay results that are not consistent with other clinical observations may require additional information for diagnosisHeterophilic 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: | 1:10 |
| Maximum Manual Dilution: | 1:20Add 10 μL of the sample to 190 μL of Alinity i Anti-Tg Calibrator A. |
| Diluent: | Alinity i Anti Tg Calibrator A |
| 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 automated 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 3 IU/mL, do not report the result. Rerun and/or investigate for other possible causes of error. |

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| **Result Reporting** | * Results between 3 and 1000 without error messages are released
* Results below 3 without error messages are reported as < 3 IU/mL.
* Results > 1000 should be diluted using the onboard automated 1:10 dilution. Release results without error messages following this dilution.
* Results > 10000 following automated dilution are diluted manually 1:20. Release results without error messages following this dilution.
* Results > 20000 following manual dilution are reported as >20000 IU/mL
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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 Anti-Tg Reagent Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised February 2018
2. Abbott Alinity i Anti-Tg Calibrator Kit Package Insert, Abbott Diagnostics Division, Abbott Park, IL USA. Revised March 2018
3. CALIPER Reference Interval Studies, accessed 10/27/2020.
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Michelle Anton |  | New Procedure for Abbott analyzers |
| 1 | Erin Bartos | October 28, 2020 | Added AMR, reference interval, references, corrected instrument, QC materials, interferences, calibrators, etc for new instrument. |