| **Thyroperoxidase Antibody (Anti-TPO)** |
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| **Purpose** | This procedure provides instructions for performing THYROPEROXIDASE ANTIBODY (also known as Anti-TPO) ON ABBOTT INSTRUMENTATION. The Alinity i Anti-TPO assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of the IgG class of thyroid peroxidase autoantibodies (anti-TPO) in human serum and plasma on the Alinity i analyzer.The Alinity i Anti-TPO 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 Alinity ci at Children’s Minnesota Laboratory. |
| **Principle** | This assay is a two-step immunoassay for the quantitative determination of anti-TPO in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology. Sample, TPO coated paramagnetic microparticles, and assay diluent are combined and incubated. The anti-TPO present in the sample binds to the TPO 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-TPO 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** | It was first demonstrated by Trotter et al. in 19571 and subsequently by Roitt and Doniach in 19582 that many patients with Hashimoto’s thyroiditis had detectable autoantibodies in their blood directed at a thyroid antigen distinct from thyroglobulin. This antigen was termed thyroid microsomal and it has since been demonstrated that most if not all anti-thyroid microsomal autoantibodies recognize thyroid peroxidase (TPO).TPO is a membrane-bound glycoprotein enzyme with an approximate mass of 107kD. The *in vivo* function is the iodination of tyrosine in the synthesis of T3 and T4. Autoimmune reactivity to TPO is believed to be polyclonal and heterogeneous in nature with a minimum of six antigenic determinants being recognized, comprising both conformational and linear epitopes. In addition, the proportion of each immunoglobulin class (G or M) or subclass (G1 – G4) as well as their affinity varies widely from patient to patient. Unlike autoantibodies to thyroglobulin (anti-Tg), autoantibodies to TPO fix complement, are potentially deleterious and may have a pathogenic role in (destructive) autoimmune thyroid disease. Anti-TPO antibodies are found often in conjunction with anti-Tg in the majority of cases of Hashimoto’s thyroiditis, Primary Myxedema, and Graves’ disease. The relationship of autoimmune thyroid disease to pregnancy has been the subject of considerable interest with the recognition of the postpartum thyroid disease syndromes.Anti-TPO antibodies are demonstrable in most cases of postpartum thyroiditis and it has been found that the presence of autoantibody in early pregnancy was associated with a high risk of asymptomatic postpartum hypothyroidism. It is common to find anti-TPO antibodies in the absence of autoantibodies to thyroglobulin, particularly in patients with small goitres and up to 64% of cases of autoimmune hypothyroidism have been reported to be associated with anti-TPO antibodies alone. In addition, anti-TPO antibodies are frequently found in patients with other autoimmune diseases such as Rheumatoid Arthritis, Addison’s Disease and Type I Diabetes. They are also detectable at low levels in up to 20% of asymptomatic individuals, particularly the elderly and more often in women than in men, although the clinical significance of these autoantibodies is unclear. |
| **Analyzer** | **Minneapolis: Abbott Alinity i (Sunquest method code: MACI)**Back up: Hold samples until instrument is back in service. If directed by provider, send to Mayo Medical Laboratories. |
| **Sunquest Test Codes** | **ATPO** |
| **Specimen** | Sample: Serum or Plasma (with or without gel barrier)**Preferred:** SST**Alternative:** Lithium Heparin, Sodium Heparin, EDTA**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: 60 μL Sample volume for each additional test from same sample cup: 10 μLRoutinely loaded: Sample volume for first test: 150 μL Sample volume for each additional test from same sample cup: 10 μLSample volumes do not include dead volume of **Stability when separated from cells/gel:** **20 to 25°C** 8 hours**2 to 8°C** 72 hours**-20°C** 30 daysAvoid multiple freeze/thaw cycles.**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-TPO Reagent Kit | 09P3521 | **Store at:** 2 to 8°C**Unopened:** Until manufacturer’s printed expiration date**On-board**: 30 days |
| Alinity i Anti-TPO Calibrators | 09P3501 | **Store at:** -10°C or colder (unopened) 2 to 8°C (opened)**Unopened:** Until manufacturer’s printed expiration date**Opened expiration:** 30 days after thaw, not to exceed the expiration date printed on the bottle.  |

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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:** *Assay Diluent***WARNING**Causes serious eye irritation.**The following warnings and precautions apply to:** *Microparticles* **WARNING** Contains potassium ferricyanide.Suspected of damaging fertility or the unborn child.**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.The human-sourced material used in Calibrators B through F is reactive for anti-TPO and nonreactive for HBsAg, anti-HIV-1/HIV- 2, and anti-HCV.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 – 1000 IU/mL |
| Reference Material: | Abbott Alinity i Anti-TPO Calibrator Kit |
| Suggested Calibration Levels: | CAL A: 0.0CAL B: 5.0CAL C: 20.0CAL D: 62.5CAL E: 250.0CAL F: 1000.0 |
| Calibration Scheme: | 6 Levels |
| 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 was performed based on guidance from NCCLS Protocol EP7-A.37 Specimens with anti-TPO levels between 45.07 and 361.64 IU/mL were supplemented with the following potentially interfering compounds. The average amount of interference observed during the study ranged from -3.6% to +3.7%.Bilirubin ≤ 20 mg/dLHemoglobin ≤ 1000 mg/dLTotal Protein (Low) 4 g/dLTotal Protein (High) 10 g/dLTriglycerides ≤ 1000 mg/dL |
| **Reference Intervals** | 0.0-8.9 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- TPO autoantibodies reflecting the prevalence in apparently healthy populations. The prevalence of anti-TPO 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 diagnosis.Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. The presence of heterophilic antibodies in a patient specimen may cause anomalous values to be observed. Additional information may be required for diagnosis.See package insert for known autoimmune disease that do not interfere or cross-react with this assay.No high-dose hook effect was observed when samples containing up to approximately 17000 IU/mL of Anti-TPO antibody were assayed. |
| **Dilutions** |

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| Max Auto Dilution: | 1:2 |
| Maximum Manual Dilution: | 1:20 Add 10 μL of the sample to 190 μL of the Alinity i Anti-TPO Calibrator A |
| Diluent: | Alinity i Anti-TPO 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 and 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 3, 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 IU/mL without error messages are released
* Results below 3 without error messages are reported as < 3 IU/mL.
* Results > 1000 IU/mL should be diluted using the onboard automated 1:2 dilution. Release results without error messages following this dilution.
* Results > 2000 IU/mL following automated dilution may be manually diluted 1:20 using Alinity i Anti-TPO Calibrator A. Release results without error messages following this dilution.
* Results >20000 following the 1:20 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-TPO Reagent Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised February 2018
2. Abbott Alinity i Anti-TPO 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** |
|  | Elauteria Earnhardt | May 18, 2020 | New Procedure for Abbott analyzers |
| 1 | Erin Bartos | October 28, 2020 | Corrected analyzer, added dilutions, AMR, calibrator information, reference interval, QC material, references, ETC for Alinity i |