| **Thyroperoxidase Antibody (Anti-TPO)** | |
| --- | --- |
| **Purpose** | This procedure provides instructions for performing THYROPEROXIDASE ANTIBODY  on the Abbott Architect i1000SR. |
| **Policy Statements** | This procedure applies to all personnel responsible for performing testing on the Abbott Architect i1000SR. |
| **Principle** | The ARCHITECT Anti-TPO assay is a two-step immunoassay for the quantitative determination of anti-TPO in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex. In the first step, sample, assay diluent and TPO coated paramagnetic microparticles are combined and incubated. Anti-TPO present in the sample binds to the TPO coated microparticles. After washing, anti-human IgG acridinium-labeled conjugate is added in the second step. Following another incubation cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of anti-TPO in the sample and the RLUs detected by the ARCHITECT System optics. |
| **Clinical Significance** | It was first demonstrated by Trotter *et al.* in 1957 and subsequently by Roitt and Doniach in 1958 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. |
| **Instrument** | **PRIMARY METHOD: Abbott Architect i1000SR**  Backup Method**:** Mayo Medical Laboratories |
| **Sunquest Test Code** | ATPO |
| **Specimen** | **Preferred Sample type:** Serum/SST  Also acceptable: Lithium Heparin, Sodium Heparin, or EDTA  **Sample Draw Volume:** 1.2 mL whole blood  **Minimum processed sample volume:** 150 µL of serum or plasma  **Stability:** 8 hours at room temperature, 72 hours at 2-8°C, 30 days at -20°C or colder. Avoid more than 5 freeze/thaw cycles. If testing will be delayed for more than 8 hours, remove serum or  plasma from the serum or plasma separator, red blood cells or clot.  **Transport:** Ship refrigerated 2-8°C to Minneapolis lab.  **Rejection criteria:** Unlabeled/mislabeled specimens, incorrect sample type  **Preparation:**   1. Serum specimens should be centrifuged following complete clot formation, according to Specimen Processing procedures prior to analysis. Plasma specimens can be centrifuged immediately 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. Lipemic samples should be ultrafuged. 4. Specimens should be free of particulate matter. 5. Transfer serum or plasma to a properly labeled sendout tube. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time. |
| **Reagents** | |  |  |  |  | | --- | --- | --- | --- | | ***Product Description*** | ***Product Code*** | ***Stability*** | | | Anti-TPO Reagent | 02K47-27 | **Store at:** 2 – 8 °C  **Unopened/Opened:** Manufacturer expiration date.  **On-board:** 30 Days | | Anti-TPO Calibrator | 02K47-01 | **Store at:**  -20°C (Allow to thaw for 45-60 minutes)  **Unopened**: Manufacturer expiration date.  **Opened**: Store at 2-8°C, stable for 30 days after thaw. | | Pre-Trigger Solution | 06E23-65 | Refer to Supply Status on Analyzer | | Trigger Solution | 06C55-60 | Refer to Supply Status on Analyzer | | Wash Buffer | 06C54-58 | Refer to Supply Status on Analyzer | | Reaction Vessels | 07C15 (-02 or -03) | N/A | |
| **Risk and Safety:** | Contains potassium ferricyanide. Wear protective gloves, protective clothing and use eye protection. If exposed or concerned, get medical attention. If reagent bottle is used up (no tests remaining,) the bottles may be discarded in regular trash, otherwise, recap and dispose of in the appropriate Hazardous Waste Container. |
| Calibration/ Verification/AMR | |  |  | | --- | --- | | Analytical Measuring Range: | 3.0-1000.0 IU/mL | | Reference Material: | Anti-TPO Calibrator 02K47-01 | | Suggested Calibration Levels | A – 0.0 IU/mL  B – 5.0 IU/mL  C – 20.0 IU/mL  D – 62.5 IU/mL  E – 250.0 IU/mL  F – 1000.0 IU/mL | | Verification Scheme: | n=6 | | Verification Frequency: | * For each new lot of reagent * After major maintenance or service, if indicated by quality control results * As indicated in laboratory quality control procedures | | AMR | Verification of AMR is accomplished with each calibration.   * Cal Verification and AMR verification are performed at least once every six (6) months. | |  | | | |
| **Quality Control** | Bio-Rad Liquichek Specialty Immunoassay Control, Level 1, 2 and 3.  **Frequency:** Three levels each day of use.  **Stability:** 30 Days at 2-8°C.  **Preparation**: Allow controls to thaw at room temperature.  **Sunquest Control names:** Level 1 = C-LQSI1, Level 2 = C-LQSI2, Level 3= C-LQSI3  **Acceptable ranges:**   * Ranges are current in Sunquest and the instrument. Refer to the Quality Control in Chemistry procedure for QC exception codes. * If a control value is outside the confidence interval, the determination must be repeated. If the repeat determination confirms the deviation, a new reference curve should be established. * Do not release patient results until the cause of deviation has been identified and corrected * When a new lot of assayed control is received, validate the manufacturer’s insert range by running the new lot in parallel with the current lot, and confirming that the results obtained are within the stated range * When a new lot of unassayed control is received, verify new ranges by running the new lot in parallel with the current lot 30 times, and calculate a new range using the method mean ± 3 SD. Ranges are current in Sunquest and the instrument. Refer to the Quality Control Procedure for QC exception codes. |
| **Interferences** | **•** 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.  **•** Heterophilic 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. |
| **Reference Range** | 0.0-8.9 IU/mL |
| **Critical Values** | None specified |
| **Limitations** | * Antibody measurement represents one parameter in a multi-criteria 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. * Assay results that are not consistent with other clinical observations may require additional information for diagnosis. * The instrument reporting system contains error messages to warn the operator of specific malfunctions. Refer to Operator’s Manual for troubleshooting specific error messages. |
| **Dilutions** | Specimens with an Anti-TPO value exceeding 1000.00 IU/mL are flagged “>1000.00” and should be diluted using the Automated Dilution Protocol and/or the Manual Dilution Procedure.  **Automated Dilution Protocol**  The system performs a 1:2 dilution of the specimen and automatically calculates the concentration of the specimen before dilution and reports the result. Specimens with an anti‑TPO value exceeding 2000 IU/mL are flagged with the code “> 2000.00” when run using the Automated Dilution Protocol. These specimens should be diluted by the Manual Dilution Procedure.  **Manual Dilution Procedure**  Recommended and maximum dilution is 1:20   1. To maintain integrity of the Anti-TPO Calibrator A, place 450 uL in a clean cup. 2. To make the 1:20 dilution, add exactly 20.0 uL patient serum/plasma to 380.0 uL ARCHITECT Anti-TPO Calibrator A and mix well, avoiding bubbles. 3. The operator must enter the dilution factor in the Patient order screen. The system will use this dilution factor to automatically calculate the dilution result. For detailed information on ordering dilutions, refer to the [ARCHITECT System Operations Manual](https://starnet.childrenshc.org/References/labsop/chem/operator/abbott-architect-operations-manual.pdf), Section 5. 4. Record manual dilution on the dilution log   If at any point the sample is insufficient to complete dilution testing, append the “-UNQ” (Unable to Quantitate Further) code to the appropriate “>” test result in Sunquest. |
| **Result Reporting** | * Results between 3-1000 IU/mL will autofile. * Results <3 IU/mL will be reported as <3.0 IU/mL rather than the numerical value. * Results >9.0 IU/mL should have above reference range flag in Sunquest. * Results >1000 IU/mL will trigger an autodilution on the Architect. * Results >2000 IU/mL will be manually diluted using the Manual Dilution Procedure. * Results >20000 IU/mL should be reported as >20,000 IU/mL rather than the numerical value. |
| **Specimen Storage** | Promptly stopper tested specimen and store upright in specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 14 days in specimen storage freezer. |
| **References** | 1. Abbott Architect Anti-TPO Reagent Package insert Abbott Laboratories, Abbott Park, IL, 60064. Revised Date February 2015. 2. Abbott Architect Anti-TPO Calibrator Package insert, Abbott Laboratories, Abbott Park, IL 60064. Revised April 2015. 3. Abbott Architect Anti-TPO Safety Data Sheet, Abbott Diagnostics, Abbott Park, IL 60064. Revised July 30, 2015. 4. Bio-Rad Liquichek Specialty Immunoassay Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618 September 2017. 5. [CALIPER Reference Range Studies](https://app3.ccb.sickkids.ca/caliper/caliperlogin), Accessed 4/20/2018 |
| **Historical Record** | |  |  |  |  | | --- | --- | --- | --- | | **Version** | **Written/Revised By** | **Effective Date** | **Summary of Revisions** | | 1 | Kelsi Brown/ Erin Bartos | May 15, 2018 | New Procedure | |  |  |  |  | |  |  |  |  | |