ARCHITECT®

SYSTEM

E

Anti-TPO

REF 2K47

840242/R2

Anti-TPO

Customer Service
United States: 1-877-4ABBOTT
International: Call your Abbott Representative

This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

CE ABBOTT LABORATORIES Abbott Park, IL 60064 USA

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Key to symbols used

REF	List Number	LOT	Lot Number
IVD	For *In Vitro* Diagnostic Use		Expiration Date
2°C 8°C	Store at 2-8°C	REACTION VESSELS	Reaction Vessels
		SAMPLE CUPS	Sample Cups
	Consult instructions for use	SEPTUM	Septum
SN	Serial Number	REPLACEMENT CAPS	Replacement Caps
EC REP	Authorized Representative	REAGENT LOT	Reagent Lot
CE ABBOTT LABORATORIES Abbott Park, IL 60064 USA	Legal Manufacturer	ASSAY CD-ROM	Assay CD-ROM
		CONTROL NO.	Control Number

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

ABBOTT
Diagnostics Division

NAME

ARCHITECT® Anti-TPO

INTENDED USE

ARCHITECT Anti-TPO is a Chemiluminescent Microparticle Immunoassay (CMIA) for the quantitative determination of the IgG class of thyroid peroxidase autoantibodies (anti-TPO) in human serum and plasma on the ARCHITECT *i* System. The ARCHITECT Anti-TPO assay is intended for use as an aid in the diagnosis of thyroid disease.

SUMMARY AND EXPLANATION OF TEST

It was first demonstrated by Trotter *et al.* in 1957[1] and subsequently by Roitt and Doniach in 1958[2] 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).[3]

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 T_3 and T_4.[4] 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.[5,6] 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.[7,8] Unlike autoantibodies to thyroglobulin (anti-Tg), autoantibodies to TPO fix complement,[9] are potentially deleterious and may have a pathogenic role in (destructive) autoimmune thyroid disease.[10,11] 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.[12] 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.[13-17]

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.[18] 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.[19-21] They are also detectable at low levels in up to 20% of asymptomatic individuals,[22] particularly the elderly[23] and more often in women than in men, although the clinical significance of these autoantibodies is unclear.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

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 and wash, 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 *i** system optics. For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

**i* = immunoassay

REAGENTS

Reagent Kit, 100 Tests

NOTE: Some kit sizes are not available in all countries; please contact your local distributor.

ARCHITECT Anti-TPO Reagent Kit (2K47)

- **MICROPARTICLES** 1 or 4 Bottles (6.6 mL) Thyroid peroxidase (recombinant) coated microparticles in MES buffer with protein (bovine) stabilizer. Preservative: antimicrobial agents.
- **CONJUGATE** 1 or 4 Bottles (5.9 mL) Anti-human IgG (mouse, monoclonal) acridinium labeled conjugate in MES buffer with protein (bovine) stabilizer. Preservative: antimicrobial agents.
- **ASSAY DILUENT** 1 or 4 Bottles (10.0 mL) Assay Diluent in MES buffer. Preservative: antimicrobial agents.

Other Reagents

ARCHITECT *i* Pre-Trigger Solution

- **PRE-TRIGGER SOLUTION** Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

ARCHITECT *i* Trigger Solution

- **TRIGGER SOLUTION** Trigger Solution containing 0.35N sodium hydroxide.

ARCHITECT *i* Wash Buffer

- **WASH BUFFER** Wash Buffer containing phosphate buffered saline solution. Preservative: antimicrobial agent.

WARNINGS AND PRECAUTIONS

IVD For *In Vitro* Diagnostic Use.

Safety Precautions

- ⚠ **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.[24] Biosafety Level 2[25] or other appropriate biosafety practices [26,27] should be used for materials that contain or are suspected of containing infectious agents.
- Microparticles contain Potassium Ferricyanide and are classified per applicable European Community (EC) Directives as: Harmful (Xn). The following are the appropriate Risk (R) and Safety (S) phrases.

R32	Contact with acids liberates very toxic gas.
S35	This material and its container must be disposed of in a safe way.
S36	Wear suitable protective clothing.
S46	If swallowed, seek medical advice immediately and show this container or label.

- The ARCHITECT *i* Trigger Solution contains sodium hydroxide (NaOH) and is classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.

R41	Risk of serious damage to eyes.
S25	Avoid contact with eyes.
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S35	This material and its container must be disposed of in a safe way.
S36/39	Wear suitable protective clothing and eye/face protection.
S46	If swallowed, seek medical advice immediately and show this container or label.

- For product not classified as dangerous per European Directive 1999/45/EC - Safety data sheet available for professional user on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a kit or between reagent kits.**
- Prior to loading the ARCHITECT Anti-TPO Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septa MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septa are not used according to the instructions in this package insert.**
- **To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.**
- Prior to placing the septum on an uncapped reagent bottle, squeeze the septum in half to confirm that the slits are open. If the slits appear sealed, continue to gently squeeze the septum to open the slits.
- Once a septum has been placed on an open reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts which have no effect on assay efficacy.
- For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Storage Instructions

- The ARCHITECT Anti-TPO Reagent Kit must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
- When stored and handled as directed, reagents are stable until the expiration date.
- The ARCHITECT Anti-TPO Reagent Kit may be stored on board the ARCHITECT *i* System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.
- Reagents may be stored on or off the ARCHITECT *i* System. If reagents are removed from the system, store them at 2-8°C (with septa and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.** After reagents are removed from the system, one must initiate a scan to update the onboard stability timer.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid and must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

- The ARCHITECT Anti-TPO assay file must be installed on the ARCHITECT *i* System from the ARCHITECT *i* Assay CD-ROM Addition B prior to performing the assay. For detailed information on assay file installation and on viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.
- For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.
- For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.
- The default result unit for the ARCHITECT Anti-TPO assay is IU/mL.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

- The following specimen collection tubes may be used in the ARCHITECT Anti-TPO assay.

	Glass	Plastic
Serum	• No additive (uncoated)	• Serum separator tubes
Plasma	• Lithium heparin • Plasma separator tubes with lithium heparin • EDTA	• Lithium heparin • Plasma separator tubes with lithium heparin • Sodium heparin • EDTA

Other anticoagulants have not been validated for use with the ARCHITECT Anti-TPO assay. Follow the manufacturer's processing instructions for serum or plasma collection tubes.

- When serial specimens are being evaluated, the same type of specimen should be used throughout the study.
- The ARCHITECT *i* System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify the correct specimen types are used in the ARCHITECT Anti-TPO assay.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated specimens
  - cadaver specimens or body fluids other than human serum or plasma
  - obvious microbial contamination
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- Inspect all samples for bubbles. Remove bubbles with an applicator stick prior to analysis. Use a new applicator stick for each sample to prevent cross contamination.
- Serum and plasma specimens should be free of fibrin, red blood cells or other particulate matter.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.

Preparation for Analysis

- Patient specimens with a cloudy or turbid appearance must be centrifuged prior to testing. Following centrifugation, avoid the lipid layer (if present) when pipetting the specimen into a sample cup or secondary tube.
- Specimens must be mixed THOROUGHLY after thawing by LOW speed vortexing or by gently inverting, and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results. Multiple freeze-thaw cycles of specimens should be avoided.
- All samples (patient specimens, controls, and calibrators) should be tested within 3 hours of being placed on board the ARCHITECT System. Refer to the ARCHITECT System Operations Manual, Section 5, for a more detailed discussion of onboard sample storage constraints.

Storage

- 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. Specimens removed from the separator gel, cells or clot may be stored up to 72 hours at 2-8°C.
- Specimens can be stored up to 30 days frozen at -10°C or colder.

Shipping

- Before shipping specimens, it is recommended that specimens be removed from the serum or plasma separator, red blood cells or clot. When shipped, specimens must be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens must be shipped frozen (dry ice). Do not exceed the storage time limitations identified in this section of the package insert.

PROCEDURE

Materials Provided:

- 2K47 ARCHITECT Anti-TPO Reagent Kit

Materials Required but not Provided:

- ARCHITECT *i* System
- 3K53 ARCHITECT *i* **ASSAY CD-ROM** WW (excluding US) Addition B
- 2K47-01 ARCHITECT Anti-TPO Calibrators
- 2K47-10 ARCHITECT Anti-TPO Controls
- ARCHITECT *i* **PRE-TRIGGER SOLUTION**
- ARCHITECT *i* **TRIGGER SOLUTION**
- ARCHITECT *i* **WASH BUFFER**
- ARCHITECT *i* **REACTION VESSELS**
- ARCHITECT *i* **SAMPLE CUPS**
- ARCHITECT *i* **SEPTUM**
- ARCHITECT *i* **REPLACEMENT CAPS**
- Pipettes or pipette tips (optional)

For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

Assay Procedure

- Before loading the ARCHITECT Anti-TPO Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment.
  - Invert the microparticle bottle 30 times.
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles remain adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott Laboratories representative.**
  - Once the microparticles have been resuspended, remove and discard the cap. Wearing clean gloves, remove a septum from the bag. Squeeze the septum in half to confirm that the slits are open. Carefully snap the septum onto the top of the bottle.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.
  - For information on ordering patient specimens and controls, refer to the ARCHITECT System Operations Manual, Section 5.
- Load the ARCHITECT Anti-TPO Reagent Kit on the ARCHITECT *i* System.
  - Verify that all necessary assay reagents are present. Ensure that septa are present on all reagent bottles.

- The minimum sample volume is calculated by the system and is printed on the Orderlist report. No more than 10 replicates may be sampled from the same sample cup. Verify adequate sample cup volume is present prior to running the test.
  - Priority: 75 µL for the first ARCHITECT Anti-TPO test plus 25 µL for each additional ARCHITECT Anti-TPO test from the same sample cup.
  - ≤ 3 hours on board: 150 µL for the first ARCHITECT Anti-TPO test plus 25 µL for each additional ARCHITECT Anti-TPO test from the same sample cup.
  - To minimize the effects of evaporation, all samples (patient specimens, calibrators and controls) must be tested within 3 hours of being placed on board the ARCHITECT *i* System.
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- Prepare Calibrators and Controls.
  - ARCHITECT Anti-TPO Calibrators and Controls should be prepared according to their respective package inserts.
  - To obtain the recommended volume requirements for the ARCHITECT Anti-TPO Calibrators and Controls, hold the bottles **vertically** and dispense 5 drops of each calibrator or control into each respective sample cup.
- Load samples.
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN. The system performs the following functions:
  - Moves the sample to the aspiration point.
  - Loads a reaction vessel (RV) into the process path.
  - Aspirates and transfers sample into the RV.
  - Advances the RV one position and transfers assay diluent and microparticles into the RV.
  - Mixes, incubates, and washes the reaction mixture.
  - Adds conjugate to the RV.
  - Mixes, incubates, and washes the reaction mixture.
  - Adds pre-trigger and trigger solutions.
  - Measures chemiluminescent emission to determine the quantity of anti-TPO in the sample.
  - Aspirates contents of RV to liquid waste and unloads RV to solid waste.
  - Calculates the result.
- For optimal performance, it is important to follow the routine maintenance procedures defined in the ARCHITECT System Operations Manual, Section 9. If your laboratory requires more frequent maintenance, follow those procedures.

Specimen Dilution Procedures

Specimens with an anti-TPO value exceeding 1000.00 IU/mL are flagged with the code ">1000.00" and may be diluted with the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

- If using the 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 2000.00 IU/mL are flagged with the code ">2000.00" when run using the Automated Dilution Protocol. These specimens may be diluted by following the Manual Dilution Procedure.

Manual Dilution Procedure

- Manual dilutions should be performed as follows:
  - The suggested dilution for an anti-TPO test is 1:20.
  - Prior to diluting the specimen, dispense approximately 10 drops of ARCHITECT Anti-TPO Calibrator A into a clean test tube for use in the next step.
  - Transfer 190 µL of ARCHITECT Anti-TPO Calibrator A from the test tube prepared in the prior step into another clean test tube and add 10 µL of the patient specimen.
  - The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution. This will be the reported result. The dilution should be performed so that the diluted result (before the dilution factor is applied) reads greater than 5.61 IU/mL.
  - For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- To perform an ARCHITECT Anti-TPO calibration, test Calibrators A, B, C, D, E, and F in duplicate. A single sample of each ARCHITECT Anti-TPO Control level must be tested to evaluate the assay calibration. Ensure that assay control values are within the concentration ranges specified in the control package insert. Calibrators should be priority loaded.
- Calibration Range: 0.00 - 1000.00 IU/mL.
- Once an ARCHITECT Anti-TPO calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - A reagent kit with a new lot number is used.
  - Controls are out of range.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

QUALITY CONTROL PROCEDURES

The recommended control requirement for the ARCHITECT Anti-TPO assay is a single sample of each control level be tested once every 24 hours each day of use. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific procedures.

The ARCHITECT Anti-TPO Control values must be within the acceptable ranges specified in the control package insert. If a control is out of its specified range, the associated test results are invalid and must be retested. Recalibration may be indicated.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B. The ARCHITECT Anti-TPO assay belongs to method group 1. Use ARCHITECT Anti-TPO Calibrators in place of MasterCheck® as described in the ARCHITECT System Operations Manual, Appendix B.

RESULTS

Calculation

The ARCHITECT Anti-TPO assay uses a 4 Parameter Logistic Curve Fit (4PLC, Y-weighted) data reduction method to generate a calibration curve.

Flags

- Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the ARCHITECT System Operations Manual, Section 5.

LIMITATIONS OF THE PROCEDURE

- 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.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).[28] Such specimens may show either falsely elevated or depressed values when tested with assay kits that employ mouse monoclonal antibodies.[28,29] 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.[30] The presence of heterophilic antibodies in a patient specimen may cause anomalous values to be observed.[30] Additional information may be required for diagnosis.

EXPECTED VALUES

In a study, human serum specimens were collected from a population of 236 apparently healthy individuals. All specimens delivered TSH values within the normal reference range. Of this study population, 9 specimens delivered positive results on a commercially available anti-TPO assay device and were excluded from further normal range analysis. The 97.5 percentile concentration of the remaining population was 5.61 IU/mL. In this study population, the normal range is < 5.61 IU/mL. A total of 97.8% (222/227) of the population gave values within this normal range.* This normal range is suggested as a guideline and each laboratory should establish a normal range appropriate to their patient populations, giving due consideration to age, gender, geographical location and their clinical practice.

* Representative data; results in individual laboratories may vary from these data.

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

The ARCHITECT Anti-TPO assay is designed to have an assay precision of ≤ 10% total CV for samples ≥ 5.61 IU/mL.

A study was performed for the ARCHITECT Anti-TPO assay with guidance from the National Committee for Clinical Laboratory Standards (NCCLS) Protocol EP5-A.[31] ARCHITECT Anti-TPO Positive Control and three human panels were assayed using three lots of reagents in replicates of two at two separate times per day for 20 days on three instruments. Each reagent lot used a single calibration curve throughout the study. Data from this study are summarized in the following table.*

Sample	Instrument	Reagent Lot	n	Mean Conc. (IU/mL)	Within Run SD	Within Run %CV	Total SD	Total %CV
Positive Control	1	A	80	74.85	2.11	2.8	2.16	2.9
		B	80	74.20	1.89	2.6	2.04	2.7
		C	80	74.63	2.01	2.7	2.17	2.9
	2	A	80	77.42	2.11	2.7	3.25	4.2
		B	80	75.32	1.92	2.5	2.54	3.4
		C	80	74.59	1.73	2.3	2.57	3.4
	3	A	80	75.41	2.13	2.8	2.52	3.3
		B	80	75.48	1.90	2.5	2.13	2.8
		C	80	76.66	2.42	3.2	2.87	3.7
Panel 1	1	A	80	1.57	0.08	4.8	0.10	6.5
		B	80	1.46	0.06	3.8	0.09	5.8
		C	80	1.64	0.09	5.6	0.10	6.1
	2	A	80	1.60	0.09	5.3	0.12	7.6
		B	80	1.53	0.06	3.9	0.11	7.2
		C	80	1.52	0.10	6.7	0.12	7.7
	3	A	80	1.47	0.08	5.3	0.11	7.8
		B	80	1.47	0.07	4.7	0.13	8.5
		C	80	1.52	0.14	9.5	0.15	9.8
Panel 2	1	A	80	20.98	0.65	3.1	0.76	3.6
		B	80	21.14	0.61	2.9	0.66	3.1
		C	80	21.51	0.71	3.3	0.75	3.5
	2	A	80	21.27	0.61	2.9	0.98	4.6
		B	80	21.62	0.66	3.0	0.90	4.2
		C	80	20.82	0.67	3.2	0.85	4.1
	3	A	80	21.00	0.73	3.5	0.86	4.1
		B	80	21.77	0.60	2.7	0.84	3.8
		C	80	21.24	0.70	3.3	0.89	4.2
Panel 3	1	A	80	214.78	5.14	2.4	6.48	3.0
		B	80	221.79	4.73	2.1	5.82	2.6
		C	80	216.71	5.36	2.5	6.36	2.9
	2	A	80	219.32	4.41	2.0	8.61	3.9
		B	80	224.54	4.04	1.8	13.37	6.0
		C	80	218.73	5.76	2.6	13.18	6.0
	3	A	80	212.91	6.11	2.9	6.84	3.2
		B	80	225.46	5.15	2.3	5.67	2.5
		C	80	228.17	5.80	2.5	7.09	3.1

*Representative data; results in individual laboratories may vary from these data.

Functional Sensitivity

In a study, human panels ranging in concentration from 0.16-1.20 IU/mL were tested in replicates of 2 over 10 days on one instrument using two reagent lots and three calibrations for a total of 40 replicates per panel. The total %CVs (combining variance components for replicate, run, day and reagent lot) were calculated and plotted against the mean concentration. A reciprocal curve was fitted through the data and the functional sensitivity value was calculated as the concentration corresponding to the 20% CV level of the fitted curve. The lowest ARCHITECT Anti-TPO assay value exhibiting a 20% CV is 0.50 IU/mL.*

* Representative data; results in individual laboratories may vary from these data.

Analytical Sensitivity

The ARCHITECT Anti-TPO assay is designed to have an analytical sensitivity of ≤ 1.0 IU/mL. The analytical sensitivity of the ARCHITECT Anti-TPO assay, defined as the concentration at two standard deviations above the ARCHITECT Anti-TPO Calibrator A (0.0 IU/mL) was calculated to be 0.16 IU/mL* at the 95% level of confidence (based upon one study with n=48 runs, 10 replicates of Calibrator A and 4 replicates of Calibrator B per run).

* Representative data; results in individual laboratories may vary from these data.

Linearity

The ARCHITECT Anti-TPO assay is linear between 3.0 and 1000.0 IU/mL based on a study performed with guidance from NCCLS protocol EP6-A.[32]

Autodilution Verification

The ARCHITECT Anti-TPO automated dilution protocol is designed to recover within 15% of manually diluted specimens. In a study, the automated dilution protocol (1:2) was compared to a manual 1:2 dilution procedure using 9 human specimens with anti-TPO levels that were greater than Calibrator E (250 IU/mL). The manual dilution was performed with ARCHITECT Anti-TPO Calibrator A. The observed percent recovery results are summarized in the following table.*

Sample ID	Automated Dilution (IU/mL)	Manual Dilution (IU/mL)	% Recovery**
1	861.25	859.27	100.2
2	684.49	703.64	97.3
3	844.36	847.62	99.6
4	724.55	757.09	95.7
5	709.46	688.49	103.1
6	1105.65	1106.18	100.0
7	948.43	931.80	101.8
8	840.77	851.72	98.7
9	966.48	998.45	96.8

* Representative data; results in individual laboratories may vary from these data.

$$**\%\ \text{Recovery} = \frac{\text{Automated Dilution (IU/mL)}}{\text{Manual Dilution (IU/mL)}} \times 100$$

Interference

Interference from elevated levels of bilirubin, hemoglobin, triglycerides, and total protein in the ARCHITECT Anti-TPO assay is designed to be ≤ 15% at the levels indicated.

A study based on guidance from the NCCLS Protocol EP7-A[33] was performed for the ARCHITECT Anti-TPO assay. 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%.*

Potentially Interfering Substance	Potentially Interfering Substance Concentration
Bilirubin	20 mg/dL
Hemoglobin	1000 mg/dL
Total Protein (Low)	4 g/dL
Total Protein (High)	10 g/dL
Triglycerides	1000 mg/dL

* Representative data; results in individual laboratories may vary from these data.

Evaluation of Autoimmune Disease Specimens and High Titer IgG Samples

Potential interference from autoimmune disease specimens and high titer IgG samples in the ARCHITECT Anti-TPO assay is designed to be ≤ 15%. In a study, the ARCHITECT Anti-TPO assay was evaluated by testing specimens with known autoimmune diseases and elevated IgG. Specimens were evaluated with anti-TPO levels spiked between 131.44 and 568.78 IU/mL. Mean absolute % interference is summarized in the following table.*

Clinical Condition	Mean Absolute % Interference
Anti-Nuclear Antibody (ANA)	1.6
Rheumatoid Arthritis (RA)	1.6
Systemic Lupus Erythematosus (SLE)	1.1
Insulin Dependent Diabetes Mellitus (IDDM)	1.0
Crohn's Disease	2.4
Multiple Sclerosis	1.7
Ulcerative Colitis	1.5
Hyperglobulinemia (high IgG)	0.9

* Representative data; results in individual laboratories may vary from these data.

Evaluation of Other Potential Interferents

Potential interference from HAMA and rheumatoid factor (RF) in the ARCHITECT Anti-TPO assay is designed to be ≤ 15%. In a study, the ARCHITECT Anti-TPO assay was evaluated by testing specimens with HAMA and RF to further assess the clinical specificity. Specimens positive for HAMA and specimens positive for RF were evaluated for % interference with anti-TPO levels spiked between 163.0 and 184.3 IU/mL. Mean absolute % interference is summarized in the following table.*

Other Potential Interferents	Number of Specimens	Mean Absolute % Interference
HAMA Positive	10	2.1
RF Positive	10	1.6

* Representative data; results in individual laboratories may vary from these data.

Clinical Sensitivity

In two studies, clinical sensitivity was evaluated by testing 139 clinically defined Hashimoto's thyroiditis specimens and 125 Graves' disease specimens. The clinical diagnosis was based on the criteria of the respective laboratory. The presence of autoantibodies against thyroglobulin and/or TPO was not necessarily a diagnostic criterion of these Graves' disease and Hashimoto's thyroiditis specimens. Data from these studies are summarized in the following table.*

	Hashimoto's Thyroiditis		Graves' Disease	
	n	% Positive	n	% Positive
Study 1	89	64.0	75	92.0
Study 2	50	74.0	50	100.0

* Representative data; results in individual laboratories may vary from these data.

Concordance

The performance of the ARCHITECT Anti-TPO was compared to a commercially available immunoassay for the determination of anti-TPO. A total of 500 specimens were evaluated in a study, encompassing a population of apparently healthy individuals and patients with autoimmune thyroid disease (Graves' disease and Hashimoto's thyroiditis). Specimens were tested in replicates of one using the ARCHITECT Anti-TPO assay with three reagent lots on three instruments and compared with a commercially available immunoassay (Comparison Assay). Data from this study are summarized in the following table.*

	Comparison Assay	
ARCHITECT Anti-TPO	Negative	Positive
Negative	242	32
Positive	5	221
Concordance = 92.6 %		

* Representative data; results in individual laboratories may vary from these data.

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