Snibe Diagnostic

CE REF 130203011M: 100 tests
130603011M: 50 tests

MAGLUMI® Anti-TPO (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of Anti-TPO in human serum using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer (including Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 1000 Plus, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X8, MAGLUMI X3 and MAGLUMI X6) and Biolumi series Integrated System (including Biolumi CX8).

SUMMARY AND EXPLANATION OF THE TEST

Thyroid peroxidase, called thyroperoxidase (TPO) or iodide peroxidase is a membrane-bound glycoprotein enzyme with an approximately mass of 107 kD, which is expressed mainly in the thyroid where it secreted into colloid. TPO could oxidize iodide ions to form iodine atoms for addition onto tyrosine residues on thyroglobulin for the generation of thyroxine (T4) or triiodothyronine (T3), the thyroid hormones[1].

The autoimmune thyroid diseases (AITD) include Graves' disease, lymphocytic thyroiditis (Hashimoto's thyroiditis and primary myxedema), and post-partum thyroid dysfunction and are all diseases in which the immune system is primarily responsible for the disease process (although not necessarily the initiator). The thyroid autoantibodies to thyroid peroxidase (TPOAb) and thyroglobulin (TgAb) are a secondary response to thyroid injury. Both are polyclonal IgG antibodies and the amounts present correlate with lymphocytic infiltration of the thyroid, have complement fixing cytotoxic activity and TPO autoantibodies correlate with thyroidal damage. The finding of TPOAb in serum indicates thyroid autoimmunity and may hence be associated with present or future occurrence of other organ-specific autoimmune diseases[2-4].High anti-TPO titers are found in up to 90% of patients with chronic Hashimoto's thyroiditis. In Graves' disease, 70% of the patients have an elevated titer[5]. As AITD occurs commonly with other autoimmune diseases, such as insulin-dependent diabetes mellitus, which places them at increased risk of developing AITD, the presence of TPOAb is helpful in selecting which patients require monitoring of their thyroid function[2].

Assays for TPOAb are more sensitive for the diagnosis of autoimmune thyroiditis than TgAb, but with quantitative sensitive assay one or both antibodies are found in almost 100% of patients. TPOAb is of higher affinity and is usually present in higher concentrations than TgAb. High concentrations of thyroid antibodies confirm the diagnosis of primary autoimmune disease in patients in whom the clinical picture is unclear. Generally the higher the TPOAb concentration, the more severe the disease process, as TPOAb has been implicated as a cytotoxic agent in the destructive thyroiditic process[2].

PRINCIPLE OF THE TEST

The Anti-TPO assay is a sandwich chemiluminescence immunoassay.

The sample (or calibrator/control, if applicable), buffer and magnetic microbeads coated with TPO antigen are mixed thoroughly and incubated. After precipitation in a magnetic field, decant the supernatant, and perform a wash cycle. Then add ABEI labeled with purified TPO antigen, and incubate to form sandwich complexes. After precipitation in a magnetic field, decant the supernatant, and then perform another wash cycle. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of anti-TPO present in the sample (or calibrator/control, if applicable).

KIT COMPONENTS

Material Provided

Components	Contents	100 tests (REF: 130203011M)	50 tests (REF: 130603011M)
Magnetic Microbeads	Magnetic microbeads coated with TPO antigen, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	Containing BSA and TPO antibody, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator High	Containing BSA and TPO antibody, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Buffer	Containing BSA, NaN_3 (<0.1%).	12.5 mL	7.0 mL
ABEI Label	Purified TPO antigen labeled with ABEI, containing BSA, NaN_3 (<0.1%).	12.5 mL	7.0 mL
Diluent	0.9% NaCl.	25.0 mL	15.0 mL
Internal Quality Control	Containing BSA and TPO antibody, NaN_3 (<0.1%).	2.0 mL	2.0 mL
All reagents are provided ready-to-use.			

Accessories Required But Not Provided

MAGLUMI and Biolumi Series:

Reaction Module	REF: 630003
Starter 1+2	REF: 130299004M, 130299027M
Wash Concentrate	REF: 130299005M
Light Check	REF: 130299006M
Reaction Cup	REF: 130105000101

Please order accessories from Shenzhen New Industries Biomedical Engineering Co., Ltd. (SNIBE) or our authorized representatives.

CALIBRATION

Traceability: This method has been standardized against the NIBSC Research Standard 66/387.

Test of assay specific calibrators allows the RLU values to adjust the assigned master curve. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve(10 calibrations) provided via the reagent Radio Frequency Identification (RFID) CHIP.

Recalibration is recommended if any of the following conditions occurs:

- After each change of lots (Reagent or Starter 1+2).

- Every week and/or each time a new reagent kit is used (recommended).
- After instrument service is required.
- If controls lie outside the expected range.

QUALITY CONTROL

Follow government regulations or accreditation requirements for quality control frequency.
Internal quality control is only applicable with MAGLUMI and Biolumi systems. For instructions for use and target value refer to ***Anti-TPO (CLIA) Quality Control Information***. User needs to judge results with their own standards and knowledge.
For detailed information about entering quality control values, refer to the corresponding Analyzer Operating Instructions.
To monitor system performance and chart trends, commercially available quality control materials are required. Treat all quality control samples the same as patient samples. A satisfactory level of performance is achieved when analyte values obtained are within the acceptable Control Range for the system or within your range, as determined by an appropriate internal laboratory quality control scheme. If the quality control results do not fall within the Expected Values or within the laboratory's established values, do not report results. Take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instructions for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact your local technical supporters or distributors for assistance.

SPECIMEN COLLECTION AND PREPARATION

- Use standard sampling tubes or tubes containing separating gel. Collect blood aseptically following the universal precautions for venipuncture.
- 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 clotting, the presence of fibrin may cause erroneous results. Samples must be free of fibrin and other particulate matter.
- Do not use hemolyzed or grossly lipemic specimens as well as specimens containing particulate substance or exhibiting obvious microbial contamination. Inspect all specimens for bubbles, and remove bubbles before analysis for optimal results.
- Avoid repeating freeze-thaw cycles. The serum sample can be only frozen and thawed one time. Specimens must be mixed thoroughly after thawing.
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or a secondary tube. Care should be taken to transfer only the clarified specimen without the lipemic material.
- All samples (patient specimens and controls) should be tested within 3 hours when placed on board the MAGLUMI and Biolumi Systems. Refer to the SNIBE service for more details of onboard sample storage constraints.
- Specimens removed from the separator, cells or clot may be stored up to 24 hours at 2-8°C.
- Before shipping specimens, it is recommended that specimens be removed from the serum separator, red blood cells or clot. When shipped, specimens should be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens should be shipped frozen.
- The sample volume required for a single determination of Anti-TPO is 10 µL.

WARNING AND PRECAUTIONS FOR USERS

- IVD
- For *In Vitro* Diagnostic Use.
- Follow the package insert carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

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 29 CFR 1910.1030 Occupational exposure to bloodborne pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- All samples, biological reagents and materials used in the assay should be considered potentially able to transmit infectious agents. They should therefore be disposed in accordance with the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.
- This product contains Sodium Azide. Dispose of contents and containers must be in accordance with all local, regional and national regulations.
- Refer to safety data sheets which are available on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not interchange reagent components from different reagents or lots.
- Prior to loading the reagent kit on the system for the first time, the reagent kit requires mixing to re-suspend magnetic microbeads that have settled during shipment.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and samples.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts which have no effect on assay efficacy.
- For detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

STORAGE AND STABILITY

- Sealed: Stored at 2-8°C until the expiration date.
- Opened at 2-8°C: Minimum stability is 4 weeks.
- On-board: Minimum stability is 4 weeks.
- To ensure the best kit performance, it is recommended to place opened kits in the refrigerator after the end of the intraday test work. It is still possible to keep on using the kit beyond the opened or on-board period if the controls are found within the expected ranges.
- Keep upright for storage to facilitate later proper resuspension of magnetic microbeads.
- Keep away from sunlight.

TEST PROCEDURE

Preparation of the Reagent

- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are totally resuspended homogenous prior to use.
- To ensure proper test performance, strictly adhere to the corresponding Analyzer Operating Instructions. Each test parameter is identified via a RFID CHIP on the Reagent kit. For further information please refer to the corresponding Analyzer Operating Instructions.

DILUTION

Samples with concentrations above the measuring range can be diluted.
After manual dilution, multiply the result by the dilution factor. After dilution by the analyzers, the analyzer software automatically takes the dilution into account when calculating the sample concentration.
The automatic sample dilution is available after dilution settings are done in the MAGLUMI and Biolumi series Fully-auto chemiluminescence immunoassay analyzer user software. Please refer to the corresponding Analyzer Operating Instructions.

LIMITATIONS

- A skillful operation and strict adherence to the instructions are necessary to obtain reliable results. Procedural directions must be followed exactly and careful operation must be used to obtain valid results. Any modification of the procedure is likely to alter the results.
- Specimens from patients taking medicines and/or medical treatment may produce misleading results. For additional information, refer to one of the published summaries.
- For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or received immunotherapy may contain human anti-mouse antibodies (HAMA), which may result in falsely elevated or decreased values. Moreover, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples as well. Additional clinical or diagnostic information may be required to determine patient status.
- Some specimens may not dilute linearly because of the heterogeneity of the autoantibodies with respect to physiochemical properties.

RESULTS

Calculation of Results

The analyzer automatically calculates the Anti-TPO concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in IU/mL. For further information please refer to the corresponding Analyzer Operating Instructions.

Interpretation of Results

The expected range for the Anti-TPO assay was obtained by testing 230 apparently healthy individuals in China, and gave the following expected value:
<30 IU/mL (95th percentile).
Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory establish its own expected ranges.

PERFORMANCE CHARACTERISTICS

Precision

Precision for the Anti-TPO assay was determined as described in the CLSI EP5-A2, 2 controls and 3 human serum pools containing different concentration of analyte were assayed in duplicate at two independent runs per day for 20 testing days. The results are summarized in the following table:

Sample	Mean(IU/mL) (N=80)	Within-Run		Between-Run		Total	
		SD(IU/mL)	%CV	SD(IU/mL)	%CV	SD(IU/mL)	%CV
Serum Pool 1	18.551	0.974	5.25	0.704	3.80	1.202	6.48
Serum Pool 2	34.925	1.635	4.68	0.964	2.76	1.898	5.43
Serum Pool 3	299.743	8.246	2.75	0.844	0.28	8.289	2.77
Control 1	45.170	1.700	3.76	1.336	2.96	2.162	4.79
Control 2	110.661	3.831	3.46	1.406	1.27	4.080	3.69

Limit of Blank (LoB)

The LoB for the Anti-TPO assay is 0.38 IU/mL.

Limit of Detection (LoD)

The LoD for the Anti-TPO assay is 2.0 IU/mL.

Measuring Range

0.38-1000 IU/mL (defined by the limit of blank and the maximum of the master curve). Values below the limit of blank are reported as <0.38 IU/mL. Values above the measuring range are reported as >1000 IU/mL.

Dilution Recovery

Two human serum samples (50 IU/mL and 600 IU/mL) of anti-TPO were serially diluted at the radio of 1:2, 1:4 and 1:8 with sample dilution and assayed for recovery. The result is summarized in the following table:

Sample	Dilution	Expected Concentration (IU/mL)	Determined Concentration (IU/mL)	%Recovery
S1	stock	50	-	-
	1:2	25	24.692	98.77
	1:4	12.5	11.950	95.60
	1:8	6.25	6.004	96.07
S2	stock	600	-	-
	1:2	300	304.100	101.37
	1:4	150	147.000	98.00
	1:8	75	68.950	91.93

Method Comparison

A total of 150 clinical samples in the range of 5.190 to 944.850 IU/mL were tested using the Anti-TPO assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as: y=0.956x+3.466, r^2=0.9584.

Specificity
There is no significant interference with thyroglobulin (<300 ng/mL). The specificity of the anti-TPO assay system was assessed by testing specimens with known autoimmune diseases and elevated IgG. Clinical anti-TPO negative samples, which contain potential cross-reactants including ANA, RA and Hyperglobulinemia (high IgG) were used to evaluate the cross-reactivity of the Anti-TPO assay. Of all the potential cross-reactants, none were found to cause false positive in the Anti-TPO assay. The results were summarized in the following table:

Condition	Number of negative samples	Number of the Anti-TPO positive results
Anti-Nuclear Antibody (ANA)	15	0
Rheumatoid Arthritis (RA)	15	0
Hyperglobulinemia (high IgG)	10	0

Drugs up to the following concentrations did not interfere with the assay:
- Phenylbutazone 15.0 mg/dL
- Sodium salicylate 50.0 mg/dL
- Aspirin 50.0 mg/dL
- Ibuprofen 50.0 mg/dL
- Acetaminophen 20.0 mg/dL
- Phenytoin 5.0 mg/dL
- Amiodarone 20.0 mg/dL
- Propylthiouracil 30.0 mg/dL

Endogenous Interference
Substances up to the following concentrations did not interfere with the assay:
- Bilirubin 66 mg/dL
- Triglyceride 1500 mg/dL
- Hemoglobin 2100 mg/dL
- Total Protein 12 g/dL

REFERENCES

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3. Feldt-Rasmussen, U. Analytical and clinical performance goals for testing autoantibodies to thyroperoxidase, thyroglobulin, and thyrotropin receptor. Clin.Chem. 42, 160–163 (1996).
4. Utiger RD. The pathogenesis of autoimmune thyroid disease. N Eng Med 1991;325:278-279.
5. Pfannenstiel P, Hotze LA, Saller B. Schilddrüsen-Krankheiten. Diagnose und Therapie. 3rd completely revised edition; Berliner Med. Verl. Anst., Berlin 1999.

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SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
2°C 8°C	Temperature limit (Store at 2-8 °C)		Use-by date
Σ	Contains sufficient for		Keep away from sunlight
	This way up	EC REP	Authorized representative in the European Community
IVD	*In vitro* diagnostic medical device	CONTENTS	Kit components
REF	Catalogue number	LOT	Batch code