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Immunoassay

REF **CME0701 / CME0702 / CME0703 / CME0704 / CME0705**

50 tests*1 / 100 tests*1 / 100 tests*2 / 100 tests*5 / 50 tests*2

Anti-TPO CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of anti-TPO (antibody to thyroid peroxidase) in human serum.

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		

EC REP

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CE IVD

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Introduction

TPO is an enzyme expressed mainly in the thyroid that liberates iodine for addition onto tyrosine residues on thyroglobulin for the production of T4 (thyroxine) or T3 (triiodothyronine), thyroid hormones.[1] In humans, thyroperoxidase is encoded by the TPO gene.[2] Antibodies to thyroid peroxidase have been shown to be characteristically present from patients with Hashimoto thyroiditis (95%), idiopathic myedema (90%) and Graves' Disease (80%).[3,4] In fact 72% of patients positive for anti-TPO exhibit some degree of thyroid dysfunction. This has led to the clinical measurement becoming a valuable tool in the diagnosis of thyroid dysfunction. Measurements of antibodies to TPO have been done, in the past, by PHA (Passive Hemaglutination).[5,6] However, PHA tests do not have the sensitivity of enzyme immunoassay and are limited by subjective interpretation. This product, with the enhanced sensitivity of chemiluminescence immunoassay, permits the detection of subclinical levels of antibodies to TPO. In addition, the results are quantitated by a luminometer, which eliminates subjective interpretation. The assay of serum anti-TPO by chemiluminescence immunoassy is useful in the differential diagnosis of autoimmune thyroid disease.[7]

Measurement Principle

This assay is based upon the two-step indirect method. In the first step, the diluted sample and the TPO coated microparticles are combined. During the incubation, the anti-TPO present in the sample binds to the antigen coated on the microparticles. After the washing, in the second step, Enzyme Conjugate is added to the reaction mixture. During the incubation, the anti-TPO present in the reaction mixture reacts with enzyme labeled anti-human IgG. Then a complex is generated between the solid phase and enzyme-linked anti-human IgG by immunological reactions. Chemiluminescent Substrate is added and the complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is proportional to the amount of anti-TPO in the samples.

Materials provided

1. Calibrators

6 vials each containing 1.0 mL of calibrator A through F. The matrix is Tris-NaCl buffer containing BSA (bovine serum albumin). Contains a selection of preservatives.
Calibrators provided ready to use.

2. Reagent pack

Reagent pack provided ready to use.

Σ	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3mL*2	2.3mL*5	1.2mL*2
Enzyme Conjugate	5.5mL*1	11.0mL*1	11.0mL*2	11.0mL*5	5.5mL*2
Sample Diluent	5.5mL*1	11.0mL*1	11.0mL*2	11.0mL*5	5.5mL*2

- Microparticles Solution

TPO coated microparticles in PBS (phosphate buffered saline) buffer with BSA. Contains a selection of preservatives.

- Enzyme Conjugate

Horseradish-peroxidase labeled anti-human IgG in Tris-NaCl buffer with bovine serum. Contains a selection of preservatives.

- Sample Diluent

Tris-NaCl buffer containing BSA. Contains a selection of preservatives.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzer which is AutoLumo A2000 Plus, AutoLumo A2000 Plus B or AutoLumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample cup(s) or tube(s) for sample containing
4. Diluent Universal
5. Chemiluminescent Substrate
6. System Wash for washing the pipetting needles
7. Wash Buffer used in the washing procedure
8. Distilled water or deionized water

Metrological Traceability of Calibrators

The measurand or analyte in this anti-TPO calibrators is traceable to the material purchased from NICPBP (National Institute for the Control of Pharmaceutical and Biological Products), China.

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. CAUTION: the calibrators contain material of human origin, which has been tested and found non-reactive for HBsAg, HIV-1 and HIV-2, HCV and syphilis. This assay contains materials of animal origin. Bovine components originate from countries where BSE has not been reported.
6. Do not smoke, drink, eat or use cosmetics in the working area.
7. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
8. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
9. Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
10. Do not use reagents beyond the labeled expiry date.
11. Do not mix or use components from kits with different batch codes.
12. When storing the calibrators, be certain the vials are securely sealed.
13. Ensure the microparticles are re-suspended before loading it on the analyzer.
14. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
15. Do not substitute any reagent in this kit from other manufacturers or other lots.
16. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.

Storage

1. Store the kit at 2-8 ℃. Do not freeze. Avoid strong light.
2. Refrigerate the reagent pack at 2-10 ℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10℃ for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10 ℃ in an upright position.
4. Seal and return the remaining calibrators at 2-8 ℃ immediately after the experiment, under which conditions the stability will be retained for 2 months.

Sample

1. Collect serum samples in accordance with correct medical practices.
2. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples.
3. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
4. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
5. Insufficient processing of sample or disruption of the sample during transportation may cause depressed results.
6. Avoid grossly hemolytic, lipemic or turbid samples.
7. Cap and store the samples at 18-25 ℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8 ℃ up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 ℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
8. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
9. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
10. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
11. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Measurement Procedure

1. Check the consumable materials

- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit

- Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests

- Place the sample cups or tubes on the sample rack, 30 μL of sample for each test. But consider the sample container and 150 μl of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically conduct test operation. The analyzer perform the following functions:
  - Moves the sample to the set point.
  - Loads a reaction vessel into the process path.
  - Aspirates and transfers Diluent Universal and sample into the reaction vessel.
  - Aspirates and transfers diluted sample into the reaction vessel.
  - Adds Sample Diluent and Microparticles Solution to the reaction vessel.
  - Mixes, incubates and washes the reaction mixture.
  - Adds Enzyme Conjugate.
  - Mixes, incubates and washes the reaction mixture.
  - Adds Chemiluminescent Substrate.
  - Measures chemiluminescent emission to determine the quantity of anti-TPO in the sample.
  - Discards the used reaction vessel.
  - Calculates the result.
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve

- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample cups or tubes and place them on the sample rack. Conduct duplicate detection on the system.
- Load the sample rack and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements.
  - A reagent kit and Chemiluminescent Substrate with new batch code is used.
  - Beyond the expiration date of a calibration curve.
  - Important parts of the analyzer are replaced or repaired.
- Refer to the Assay Analyzer's operation manual.

5. Dilute the sample

Samples with an anti-TPO value exceeding 400 IU/mL may be diluted via manually. Diluent Universal is used to dilute the samples. After dilution, multiply the result by the dilution factor.

Measurement Results

The sample test results are determined automatically by the system software. The amount of anti-TPO in the samples in the samples is determined from the measured light production by means of the stored calibration data. Refer to the Assay Analyzer's operation manual on reviewing the stored data.

The default unit for this assay is IU/mL.

Control Procedure

Controls for the various concentration ranges should be run individually when the test is in use, once per reagent kit, and following each calibration.
The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.
Follow the applicable government regulations and local guidelines for quality control.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop HAMA (human Anti-mouse antibodies). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies. Additional information may be required for diagnosis.
4. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulin, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
5. Performance of this test has not been established with neonatal samples.
6. This test measures concentrations within the range of 2 IU/mL - 400 IU/mL. If anti-TPO concentrations above the measuring range to be expected, it is recommended to dilute samples with Diluent Universal, the recommended dilution is 1:4 of this test, under this condition, allowing samples to be up to approximately 2000 IU/mL.

Biological Reference Interval

The following reference interval was obtained using percentile method by testing serum samples from 188 individuals defined as normal by a clinician. The 95 percentile concentration of the population was less than 30 IU/mL, and the 99 percentile concentration was less than 40 IU/mL. On the basis of this study population, the expected normal range is <30 IU/mL. Samples with concentration values ranging between 30 and 40 IU/mL should be considered equivocal.
It is recommended that each laboratory establish its own normal range which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

Performance Characteristics

1. Measurement Precision

3 samples were assayed in duplicate, twice per day across 20 testing days. Data from this study are summarized in the following table.

Sample	n	Mean (IU/mL)	Within-run	Total
			%CV	%CV
1	80	30.64	2.49	3.33
2	80	82.05	3.44	4.07
3	80	169.97	3.22	3.63

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

2. Analytical Sensitivity

Limit of Blank: 2 IU/mL.
Limit of Detection: 4 IU/mL.
Limit of Quantitation: 8 IU/mL with a coefficient of variation of $\leq$20%.

3. Analytical Specificity

Cross reaction: No cross reaction with following substances at listed concentration levels.

Substance	Concentration IU/mL	Measured Value IU/mL
Anti-TG	10000	≤20

Interference: No interference with 20 mg/dL of bilirubin, 500 mg/dL of hemoglobin, 1000 mg/dL of triglycerides.

4. Relative Agreement

A comparison study was performed where samples were tested using this assay and a microparticle based anti-TPO test which was already CE marked. Data for relative agreement are summarized in the following table. The agreement is 96.63% (316/327).

		This Assay		
		Positive	Negative	Total
Reference Test	Positive	110	8	118
	Negative	3	206	209
	Total	113	214	327

5. High Dose Hook Effect

A sample spiked with anti-TPO up to 1000 IU/mL was determined, the concentration result obtained was $\geq$400 IU/mL.

Literature References

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