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Immunoassay

REF **CME0801 / CME0802 / CME0803 / CME0804 / CME0805**

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

TG CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of TG (Thyroglobulin) 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

TG (thyroglobulin) is a dimeric protein with a molecular weight of approximately 660 kDa; it is used by the thyroid gland to produce the T4 (thyroxine) and T3 (triiodothyronine), each TG molecule forms approximately 10 thyroid hormone molecules[1]. Elevated TG concentrations have been reported in different thyroid conditions such as thyroid adenoma and thyroid carcinoma[2]. The determination of TG can also be helpful to distinguish between sub-acute thyroiditis and factitious thyrotoxicosis[3]. TG testing is mainly for the post-operative follow-up of patients with differentiated thyroid carcinoma (DTC)[6]. As the TG is entirely produced by thyroid gland, the serum TG level will drop to a very low or undetectable concentration after total or near-total thyroidectomy and successful radioiodine ablation of the residual thyroid tissue[4,5]. Detectable levels of serum TG after total thyroidectomy are indicative of persistent or recurrent DTC. In consequence significantly increasing TG levels are interpreted as a sign of recurrence of the disease[6].

Measurement Principle

This assay is based upon the one-step sandwich method. The sample, anti-TG coated microparticles and enzyme labeled anti-TG are combined. During the incubation, TG present in the sample is allowed to react simultaneously with the two antibodies, resulting in the TG being sandwiched between the solid phase and enzyme-linked antibodies. After washing, a complex is generated between the solid phase, the TG within the sample and enzyme-linked antibodies 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 TG in the samples.

Materials provided

1. Calibrators

6 vials each containing 1.0 mL of calibrator A through F. The matrix is HEPES-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	11.0 mL*1	11.0 mL*2	11.0 mL*5	5.5mL*2

- Microparticles Solution

Anti-TG coated microparticles in PBS buffer containing BSA. Contains a selection of preservatives.

- Enzyme Conjugate

Horseradish-peroxidase labeled anti-TG in 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 TG calibrators is traceable to the material purchased from CRM (Certified Reference Material) 457, of the BCR Community Bureau of Reference) of the European Union.

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: 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; for longer use, store opened calibrators in aliquots and freeze at -20 ℃. Avoid multiple freeze-thaw cycles.

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, 50 μL of samples 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 operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers sample into the reaction vessel
  - Adds Microparticles Solution and Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of TG 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, calibration is required every 28 days.
- 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 a TG value exceeding 500 ng/mL may be diluted via the program of the analyzer. Diluent Universal is used to dilute the samples. The software takes the dilution into account when reporting the result.

Measurement Results

The sample test results are determined automatically by the system software. The amount of TG in the samples is determined from the measured light production by means of the stored calibration data. Sample test results can be reviewed using the appropriate screen. Refer to the Assay Analyzer's operation manual on reviewing sample results.
The default unit for this assay is ng /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. 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.
4. Performance of this test has not been established with neonatal samples.
5. 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.
6. This test measures concentrations within the range of 0.1-500ng/mL. If TG concentrations above the measuring range to be expected, it is recommended to dilute samples with Diluent Universal, the recommended dilution is 1:9 of this test, under this condition, allowing samples to be up to approximately 5000ng /mL.
7. The presence of TG autoantibodies (anti-TG) in human serum can interfere with the TG assay.

Biological Reference Interval

A normal range of 1.0 ng/mL to 39.0 ng/mL (central 95% interval) was obtained by testing serum samples from 273 individuals defined as normal by clinician. 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 (ng/mL)	Within-run	Total
			%CV	%CV
1	80	5.65	2.83	3.34
2	80	89.63	2.07	4.00
3	80	206.86	4.43	5.10

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

2. Analytical Sensitivity

Limit of Blank: 0.1ng/mL.
Limit of Detection: 0.2ng/mL.
Limit of Quantitation: 0.3 ng/mL with a coefficient of variation of ≤20%.

3. Analytical Specificity

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

Substance	Concentration	Measured Value
T4	100 μg/dL	≤0.1 ng/mL
T3	30 ng/mL	≤0.1 ng/mL

Interference: No interference with 18 mg/dL of bilirubin, 200 mg/dL of hemoglobin, 2000 mg/dL of triglycerides.

4. Relative Agreement

A comparison study was performed where samples were tested using this assay and a microparticle based TG test which was already available on the market. Data for relative agreement are summarized in the following table. The agreement is 99.52% (209/210).

		This Assay		
		Positive	Negative	Total
Reference Test	Positive	67	1	68
	Negative	0	142	142
	Total	67	143	210

5. High Dose Hook Effect

A sample spiked with TG up to 50000 ng/mL was determined, the concentration result obtained was ≥500 ng/mL.

Literature References

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