

Immunoassay

REF










CME0201 / CME0202 / CME0203 / CME0204 / CME0205


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

T4 CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of T4 (thyroxine) in human serum.

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| Key to Graphical Symbols Used | | | |
|---|---|---|-----------------------------------|
|  | batch code |  | use by |
|  | manufacturer |  | contains sufficient for <n> tests |
|  | <i>in vitro</i> diagnostic medical device |  | temperature limitation |
|  | catalogue number |  | consult instructions for use |
|  | authorized representative in the European Community | | |

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Contact your local dealers for all product related questions in your local language

Introduction

T4 (thyroxine or 3, 5, 3', 5'-tetraiodo-L-thyronine) is the major hormone produced by the thyroid gland. It has a molecular weight of 777 daltons and is synthesized by iodination of tyrosine residues on thyroglobulin. Proteolytic cleavage of follicular thyroglobulin releases T4 into the bloodstream. Greater than 99% of T4 is reversibly bound to three plasma proteins in the blood, TBG (thyroxine binding globulin) binds 70%, TBPA (thyroxine binding pre-albumin) binds 20%, and albumin binds 10%. Approximately 0.03% of T4 is in the free, unbound state in blood at any time.^{1,2}

Diseases effecting thyroid function may present a wide array of confusing symptoms. Measurement of total T4 by immunoassay is the most reliable and convenient screening test available to determine the presence of thyroid disorders in patients. Increased levels of T4 have been found in hyper-thyroidism due to Grave's disease and Plummer's disease and in acute and subacute thyroiditis. Low levels of T4 have been associated with congenital hypothyroidism, myxedema, chronic thyroiditis (Hashimoto's disease), and with some genetic abnormalities.^{3,4,5}

Measurement Principle

This assay is based upon the one-step competitive method. The sample, anti-T4 coated microparticles and enzyme labeled T4 are combined. During the incubation, T4 labeled on Enzyme Conjugate and T4 present in the sample compete for binding to antibodies coated on microparticles. After washing, a complex is generated between the solid phase and enzyme-linked antigens by immunological reactions. Chemiluminescent Substrate is added and the complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLU's. The RLU is inversely proportional to the amount of T4 in the samples.

Materials provided


1. Calibrators

6 vials each containing 1.0 mL of calibrator A through F. The matrix is human serum containing PBS (phosphate buffered saline). Contains a selection of preservatives.

Calibrators provided ready to use.

2. Reagent pack

Reagent pack provided ready to use.

|  | 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-T4 coated microparticles in PBS buffer containing BSA. Contains a selection of preservatives.

● Enzyme Conjugate

Horseradish-peroxidase labeled T4 derivant in Tris-NaCl buffer containing BSA (bovine serum albumin) and ANS. 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. Chemiluminescent Substrate
5. System Wash for washing the pipetting needles
6. Wash Buffer used in the washing procedure
7. Distilled water or deionized water

Metrological Traceability of Calibrators

The measurand or analyte in this T4 calibrators is traceable to the material purchased from NICBPB (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 °C. Do not freeze. Avoid strong light.
2. Refrigerate the reagent pack at 2-10 °C for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10°C 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 °C in an upright position.

4. Seal and return the remaining calibrators at 2-8 °C 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 °C. 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 °C for no more than 8 hours, for longer use samples should be capped and stored at 2-8 °C up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 °C. 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 T4 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 T4 value exceeding 30 µg/dL may be diluted manually. Thyroid hormone free human serum 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 T4 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 µg/dL.

Conversion formula: µg/dL × 12.87 = nmol/L

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. Serum T4 concentration is dependent upon a multiplicity of factors: hypothalamus gland function and its regulation, TBG concentration, and the binding of T4 to TBG. Thus, total T4 concentration alone is not sufficient to assess clinical status.
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 0.5 – 30 µg/dL. If T4 concentrations above the measuring range to be expected, it is recommended to dilute samples with Thyroid hormone free human serum, the recommended dilution is 1:4 of this test, under this condition, allowing samples to be up to approximately 150 µg/dL.

Biological Reference Interval

A normal range of 5.0 µg/dL to 13 µg/dL (central 95% interval) was obtained by testing serum samples from 795 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 (µg/dL) | Within-run | Total |
|--------|----|-----------------|------------|-------|
| | | | %CV | %CV |
| 1 | 80 | 4.12 | 3.34 | 5.74 |
| 2 | 80 | 10.26 | 2.62 | 4.23 |
| 3 | 80 | 17.10 | 4.47 | 7.51 |

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

2. Analytical Sensitivity

Limit of Blank: 0.5µg/dL.

Limit of Detection: 1µg/dL.

Limit of Quantitation: 2µg/dL with a coefficient of variation of ≤20%.

3. Analytical Specificity

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

| Substance | Concentration (ng/mL) | Measured Value (µg/dL) |
|-----------|--------------------------|---------------------------|
| T3 | 500 | ≤1.5 |
| rT3 | 50 | ≤1.5 |

Interference: No interference with 40 mg/dL of bilirubin, 500 mg/dL of hemoglobin, 1000 mg/dL of triglycerides.

4. Measurement Accuracy by Correlation

A comparison study was performed where samples were tested using this assay and a microparticle based T4 test which was already available on the market. Data were analyzed and are summarized in the following table.

| Correlation Method | Number of Samples | Intercept | Slope | Correlation Coefficient |
|--------------------|-------------------|-----------|--------|-------------------------|
| Linear Regression | 198 | 0.3533 | 0.9313 | 0.9565 |

Literature References

1. Young DS. Effects of drugs on clinical laboratory tests. *Ann. Clin. Biochem.* 1997;34 (Pt 6):579-581.
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5. Demers LM, Spencer CA. Laboratory medicine practice guidelines: laboratory support for the diagnosis and monitoring of thyroid disease. *Clin. Endocrinol. (Oxf).* 2003;58(2):138-140.