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Immunoassay

REF **CME0501 / CME0502 / CME0503 / CME0504 / CME0505**

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

FT4 CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of FT4 (free thyroxine) 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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Introduction

FT4 (free thyroxine) is an indicator of thyroxine activity in the body. It can also be measured as total thyroxine, which also depends on the thyroxine that is bound to TBG (thyroxine-binding globulin).[1] Under normal thyroid condition, as the concentrations of the carrier proteins alter, the total T4 level changes but the FT4 concentration remains constant. Thus, measurement of FT4 concentrations correlates better with clinical status than total T4 levels. FT4 immunoassays depend upon serum protein-bound T4 dissociation to stabilize the FT4 concentration during assay perturbations, interassay differences in perturbations combined with variation in serum protein-bound T4 concentrations could produce discordant FT4 measurements.[2]

For example, the increase in total T4 associated with pregnancy, oral contraceptives and estrogen therapy occasionally result in total T4 levels over the limits of normal while the FT4 concentration remains within the normal reference range. Masking of abnormal thyroid function can also occur in both hyper and hypothyroid conditions by alterations in the TBG concentration.

The total T4 can be elevated or lowered by TBG changes such that the normal reference levels result is observed. Again, the FT4 concentration typically uncovers the patient's actual clinical status. Neither FT4 immunoassay accurately reflects established free T4 changes during pregnancy. TT4 and the FT4 retained an appropriate inverse relationship with TSH throughout pregnancy and appear to provide a more reliable free T4 estimate.[3]

Measurement Principle

This assay is based upon the one-step competitive method. The sample, T4 derivant coated microparticles and enzyme labeled anti-T4 are combined. During the incubation, T4 derivant coated on microparticles and FT4 present in the sample compete for binding to the enzyme labeled antibodies. After washing, a complex is generated between the solid phase 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 inversely proportional to the amount of FT4 in the samples.

Materials provided

1. Calibrators

6 vials each containing 1.0 mL of calibrator A through. The matrix is human serum. 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

- Microparticles Solution

T4 derivant coated microparticles in PBS (phosphate buffered saline) buffer containing BSA. Contains a selection of preservatives.

- Enzyme Conjugate

Horseradish-peroxidase labeled anti-T4 in Tris-NaCl buffer containing BSA (bovine serum albumin). 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 FT4 calibrators is traceable to the manufacturer's working calibrators. Traceability process is based on EN ISO 17511. The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

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. Once the reagent pack is open, it can be stored at 2-8 ℃ for 1 month.
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. Do not dilute samples as T4 exists in both free and combined form in blood, and in an equilibrated status. Binding protein concentration variation will break the equilibrated status.
12. 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 FT4 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 substrate solution 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.

Measurement Results

The sample test results are determined automatically by the system software. The amount of FT4 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 pmol/L.
Conversion formula: pmol/L $\times$ 0.0777 = ng/dL

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.
4. Serum FT4 concentration is dependent upon a multiplicity of factors: hypothalamus gland function and its regulation, TBG concentration, and the binding of T4 to TBG. Thus, FT4 concentration alone is not sufficient to assess clinical status.
5. In NTI (severe non-thyroidal illness), the assessment of thyroid status becomes very difficult. TSH measurements are recommended to identify thyroid dysfunction.
6. Serum FT4 values may be elevated under conditions such as pregnancy or administration of oral contraceptives.
7. The interpretation of FT4 is complicated by a variety of drugs that can affect the binding of T4 to the thyroid hormone carrier proteins or interfere with its metabolism to T3.
8. In rare conditions associated with extreme variations in albumin binding capacity for T4-such as FDH (familial dysalbuminemic hyperthyroxinemia)-direct assessment of FT4 may be misleading.
9. Autoantibodies to thyroid hormones and hormone binding inhibitors may interfere with the assay.
10. 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.[4,5]
11. Samples cannot be diluted for the FT4 determinates.
12. This test measures concentrations within the range of 2.5-100pmol/L.

Biological Reference Interval

A normal range of 10 pmol/L to 22 pmol/L (central 95% interval) was obtained by testing serum samples from 247 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 (pmol/L)	Within-run	Total
			%CV	%CV
1	80	9.68	3.14	7.67
2	80	15.44	2.31	6.66
3	80	46.87	4.10	7.81

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

2. Analytical Sensitivity

Limit of Blank: 2.5pmol/L.
Limit of Detection: 4pmol/L.
Limit of Quantitation: 4.5pmol/L 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)	Cross reactivity (%)
T3	500	≤0.003
rT3	500	≤0.01

Interference: No interference with 20 mg/dL of bilirubin, 1000 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 FT4 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	0.9449	0.9748	0.9582

Literature References

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2. Nelson JC. Underestimates of serum free thyroxine (T4) concentrations by free T4 immunoassays. *Journal of Clinical Endocrinology & Metabolism*. 1994;79(1):76-79.
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4. Kinders RJ, Hass GM. Interference in immunoassays by human anti-mouse antibodies. *Eur. J. Cancer*. 1990;26(5):647-648.
5. Spencer C, Takeuchi M, Kazarosyan M, et al. Interlaboratory/intermethod differences in functional sensitivity of immunometric assays of thyrotropin (TSH) and impact on reliability of measurement of subnormal concentrations of TSH. *Clin Chem*. 1995;41(3):367-374.