Appendix (3)

11965263001V15

T4

Thyroxine

cobas®

REF **200 tests** **12017 709** 122

• Indicates analyzers on which the kit can be used

cobas e 602	**cobas e** 601	**cobas e** 411	MODULAR ANALY TICS E170	Elecsys 2010
•	•	•	•	•

English

Intended use

Immunoassay for the in vitro quantitative deter mination of thyroxine in human serum and plasma.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary [1,2,3]

The hormone thyroxine (T4) is the main product secreted by the thyroid g land and is an integral component of the hypothalamus-anterior pituitar y-thyroid regulating system. It has the function of anabolically influencing metabolism. Thyroxine is for med in a coupling reaction from two DIT molecules (3,5-diiodotyrosine) in the thyroid g land. It is stored bound to thyrog lobulin in the lumina of the thyroid follicles and is secreted as required under the influence of TSH.[1,2]

The major part (> 99 %) of total thyroxine (T4) in serum is present in protein-bound for m. As the concentrations of the transport proteins in serum are subject to exog enous and endogenous ef fects, the status of the binding proteins must also be taken into account in the assessment of the thyroid hormone concentration in serum. If this is ignored, changes in the binding proteins (e.g. due to estrog en-containing preparations, during pregnancy or in the presence of a nephrotic syndrome etc.) can lead to erroneous assessments of the thyroid metabolic state.[3,4,5,6,7]

The deter mination of T4 can be utilized for the following indications: the detection of hyperthyroidism, the detection of primary and secondary hypothyroidism, and the monitoring of TSH-suppression therapy.[8]

The Elecsys T4 assay employs a competitive test principle with an antibody specifically directed against T4. Endogenous T4, released by the action of 8-anilino-1-naphthalene sulfonic acid (ANS), competes with the added biotinylated T4-derivative for the binding sites on the antibodies labeled with the ruthenium complex[a].

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)^{2+}_3$)

Test principle

Competition principle. Total duration of assay : 18 minutes.

- 1st incubation: 15 µL of sample and a T4-specific antibody labeled with a ruthenium complex; bound T4 is released from binding proteins in the sample by ANS.
- 2nd incubation: Af ter addition of streptavidin-coated microparticles and biotinylated T4, the still-free binding sites of the labeled antibody become occupied, with for mation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a volt ag e to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are deter mined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

R eagents - working solutions

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-T4-Ab~Ru(bpy) $^{2+}_3$ (gray cap), 1 bottle, 18 mL: Polyclonal anti-T4-antibody (sheep) labeled with ruthenium complex 100 ng/mL; ANS 1 mg/mL; phosphate buffer 100 mmol/L, pH 7.4; preservative.

R2 T4~biotin (black cap), 1 bottle, 18 mL: Biotinylated T4 20 ng/mL; phosphate buffer 100 mmol/L, pH 7.4; preservative.

Pr ecautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laborator y reagents. Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

Avoid the for mation of foam with all reagents and sample types (specimens, calibrator s, and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All infor mation required for correct operation is read in via the respective reagent barcodes.

S torage and stability

Store at 2-8 °C.

Store the Elecsys T4 reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use. Stability:

up to the stated expiration date	unopened at 2-8 °C
12 weeks	af ter opening at 2-8 °C
8 weeks	on the analyzers

Specimen collection and pr eparation

Only the specimens listed below were tested.

Serum collected using standard sampling tubes or tubes containing separating g el.

Li-, Na-heparin, K3-EDTA , and sodium citrate plasma.

When sodium citrate is used, the results must be corrected by + 10 %. Criterion: Recover y within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95. When sodium fluoride/potassium oxalate are used, the values found are by approx. 26 % lower than those for serum.

Stable for 7 days at 2-8 °C, 30 days at -20 °C.[9] Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturer s were tested. Sample collection systems from various manufacturer s may contain dif fering materials which could af fect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before perfor ming the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.

Ensure the patients' samples, calibrator s, and controls are at ambient temperature (20-25 °C) before measurement.

Because of possible evaporation ef fects, samples, calibrator s, and controls on the analyzers should be measured within 2 hours.

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

- REF 12017 717122, T4 CalSet, 4 x 1 mL
- REF 11731416122, PreciControl Univer sal, for 2 x 3 mL each of PreciControl Univer sal 1 and 2 or REF 11731416190, PreciControl Univer sal, for 2 x 3 mL each of PreciControl Univer sal 1 and 2
- General laborator y equipment
- Elecsys 2010, MODULAR ANALY TICS E170 or **cobas e** analyzer

Accessories for Elecsys 2010 and **cobas e** 411 analyzers:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean

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- REF 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 0 4880340190, ProCell M, 2 x 2 L system buffer
- REF 0 4880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically before use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

Bring the cooled reagents to approx. 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: The Elecsys T4 test has been checked by ID-GC/MS (isotope dilution gas chromatography mass spectrometry) on various control materials.[10] Every Elecsys T4 reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys T4 CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the specified limits

Quality control

For quality control, use Elecsys PreciControl Universal 1 and 2. Other suitable control material can be used in addition.

Controls for the various concentration ranges should be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every 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 limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in nmol/L, µg/dL or ng/L).

Conversion factors: nmol/L x 0.07 7688 = µg/dL
µg/dL x 12.872 = nmol/L
nmol/L x 0.77688 = µg/L

Limitations - interference

The assay is unaffected by icterus (bilirubin < 633 µmol/L or < 37 mg/dL), hemolysis (Hb < 1.4 mmol/L or < 2.3 g/dL), lipemia (triglycerides < 28.5 mmol/L or < 2500 mg/dL), and biotin < 409 nmol/L or < 100 ng/mL.

Criterion: Recovery within ± 10 % of initial value.

In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 2400 IU/mL and samples from dialysis patients.

In vitro tests were performed on 15 commonly used pharmaceuticals. No interference with the assay was found.

The test cannot be used in patients receiving treatment with lipid-lowering agents containing D-T4. If the thyroid function is to be checked in such patients, the therapy should first be discontinued for 4-6 weeks to allow the physiological state to become re-established.[11]

Autoantibodies to thyroid hormones can interfere with the assay. Binding protein anomalies seen with FDH (familial dysalbuminemic hyperthyroxinemia), for example, may cause values which, while characteristic of the condition, deviate from the expected results.[12]

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

5.40-320.0 nmol/L or 0.420-24.86 µg/dL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 5.40 nmol/L or < 0.420 µg/dL. Values above the measuring range are reported as > 320.0 nmol/L or > 24.86 µg/dL.

Lower limits of measurement

Lower detection limit

Lower detection limit: 5.40 nmol/L (0.420 µg/dL)

The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Dilution

Not necessary due to the broad measuring range.

Expected values

Measurements with the Elecsys T4 assay on 2526 serum samples from euthyroid test subjects in Germany and Japan yielded the following values (2.5th-97.5th percentile):

66-181 nmol/L or 5.1-14.1 µg/dL

FT4 Index (T4/TBI) calculated from 825 serum samples from euthyroid test subjects measured with the Elecsys T4 assay and the Elecsys T-Uptake assay (2.5th-97.5th percentile):

62-164 nmol/L or 4.8-12.7 µg/dL

Following values were determined for the 99 % percentile range from 275 serum and plasma samples from healthy test subjects in USA:

59-154 nmol/L or 4.6-12.0 µg/dL FT4 Index:

57-147 nmol/L or 4.4-11.4 µg/dL

For detailed information about reference intervals in children, adolescents and pregnant women, refer to the brochure "Reference Intervals for Children and Adults", REF English: 0 46 40292, German: 0 4625889.

This booklet also contains results of a detailed study about influencing factors on thyroid parameters in a well characterized reference group of adults. Different inclusion and exclusion criteria were applied (e.g. sonographic results (thyroid volume and density) as well as criteria according to the guidelines of the National Academy of Clinical Biochemistry - NACB).

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

T4

Thyroxine

cobas®

Specific performance data

Representative perfor mance data on the analyzers are given below. Results obtained in individual laboratories may dif fer.

Pr ecision

Precision was determined using Elecsys reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laborator y Standards Institute): 6 times daily for 10 days (n = 60); repeatability on the MODULAR ANALY TICS E170 analyzer, n = 21. The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers

Intermediate pr ecision			R epeatability[b]					
CV	SD		CV	SD		Mean		Sample
%	µg/dL	nmol/L	%	µg/dL	nmol/L	µg/dL	nmol/L	
6.9	0.18	2.31	4.7	0.12	1.56	2.59	33.4	HS[c] 1
3.7	0.35	4.56	2.7	0.26	3.38	9.59	123	HS 2
3.0	0.54	6.98	2.5	0.46	5.97	18.4	237	HS 3
3.3	0.29	3.78	2.3	0.20	2.54	8.79	113	PC U[d]1
2.7	0.28	4.90	2.0	0.28	3.58	14.0	181	PC U2

b) Repeatability = within-run precision
c) HS = human serum
d) PC U = PreciControl Univer sal

MODULAR ANALY TICS E170, **cobas e** 601 and **cobas e** 602 analyzers

Intermediate pr ecision					R epeatability					
CV	SD		Mean		CV	SD		Mean		Sample
%	µg/dL	nmol/L	µg/dL	nmol/L	%	µg/dL	nmol/L	µg/dL	nmol/L	
3.7	0.19	2.40	5.09	65.6	1.3	0.09	1.13	6.55	8 4.3	HS 1
3.4	0.21	2.67	6.15	79.1	1.8	0.09	1.14	4.90	63.1	HS 2
4.2	0.75	9.67	18.0	231	1.7	0.32	4.07	18.9	243	HS 3
3.8	0.27	3.51	7.22	92.9	1.3	0.09	1.15	7.01	90.3	PC U1
3.3	0.49	6.29	14.7	190	1.7	0.24	3.07	14.2	182	PC U2

Method comparison

A comparison of the Elecsys T4 assay (y) with the Enzymun-Test T4 method (x) using clinical samples gave the following correlations (nmol/L): Number of samples measured: 71

Linear regression	Passing/Bablok[13]
y = 0.75x + 9.88	y = 0.77x + 7.77
r = 0.975	τ = 0.841

The sample concentrations were between 8 and 250 nmol/L (0.6 and 19 µg/dL).

Analytical specificity

For the antibody derivative used, the following cross-reactivities were found: L-T4 and D-T4 100 %; L-T3 1.53 %; D-T3 1.38 %; 3-iodo-L-tyrosine 0.002 %; 3,5-diiodo-L-tyrosine 0.01 %; 3,3',5,5'-tetraiodothyroacetic acid 38.5 %.

Refer ences

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For further infor mation, please refer to the appropriate operator 's manual for the analyzer concerned, the respective application sheets, the product infor mation, and the packag e inserts of all necessary components.

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