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T-Uptake

cobas®

T-uptake

REF	Σ	SYSTEM
11731394 122	200	Elecsys 2010 MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

Intended use

Immunoassay for the in vitro quantitative determination of thyroxine-binding capacity (TBC or T4-uptake) 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

The thyroid hormone thyroxine (T4) is physiologically part of the regulating circuit of the thyroid gland and has an effect on general metabolism. The determination of the T4 concentration is of importance in laboratory diagnostics for differentiating between euthyroid, hyperthyroid, and hypothyroid conditions.[1,2,3] As the major fraction of the total thyroxine is bound to transport proteins (TBG, prealbumin, and albumin), the determination of total thyroxine only provides correct information when the thyroxine-binding capacity in serum is normal. The free thyroid hormones are in equilibrium with the hormones bound to the carrier proteins. A change in the TBG concentration can lead to elevated or lowered total T4 concentrations being measured although the free T4 concentration is in the euthyroid range.

The performance of a T-uptake or TBC assay provides a measure of the available thyroxine-binding sites.[4,5,6]

Determination of the free thyroxine Index (fT4I) from the quotient of total T4 and TBI (thyroxine-binding index = result of the T-uptake determination) takes into account changes in the thyroid hormone carrier proteins and the thyroxine level.

The Elecsys T-Uptake assay is an immunological method for determining the T-uptake (TBC), in which exogenous T4 is added to saturate the TBG.

Test principle

Modified competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 15 µL of sample, exogenous T4, and biotinylated T4-polyhapten. The T4 occupies the free binding sites in the serum sample.
- 2nd incubation: After addition of a T4-specific antibody labeled with a ruthenium complex[a)], the polyhapten and the antibody derivative react to form a complex, the concentration of which is inversely proportional to the concentration of the excess, exogenous T4. This immunological complex becomes bound to the added streptavidin-coated microparticles 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/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration.

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

Reagents - working solutions

The reagent rackpack is labeled as T UP.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL:

Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Poly-T4~biotin (gray cap), 1 bottle, 18 mL:

Biotinylated T4 polyhapten 70 ng/mL; thyroxine 60 ng/mL; phosphate buffer 100 mmol/L, pH 7.0; preservative.

R2 Anti-T4-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 18 mL:

Polyclonal anti-T4-antibody (sheep) labeled with ruthenium complex 120 ng/mL; phosphate buffer 100 mmol/L, pH 7.0; preservative.

Precautions and warnings

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

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

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

All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

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

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on Elecsys 2010 and **cobas e** 411	8 weeks
on MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602	5 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Undiluted serum collected using standard sampling tubes or tubes containing separating gel.

Li-, Na-, NH_4^+-heparin, K_3-EDTA, sodium citrate and sodium fluoride/potassium oxalate plasma (undiluted).

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 8 days at 2-8 °C, 3 months at -20 °C. 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 manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect 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 performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 11731505122, T-Uptake CalSet, 4 x 1 mL
- REF 11731416190, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2

- REF 11731416160, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2 (for USA)
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS 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
- 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 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, 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
- REF 11298500160, Elecsys SysClean, 5 x 100 mL system cleaning solution (for USA)

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

Calibration

Traceability: This method has been standardized using a clinically defined human serum panel with a mean TBI of 1.0. The measurements obtained are indexes. The normal value was determined in a study on a normal group of sera and arbitrarily set at 1.

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The calibration is adapted to the analyzer using the relevant 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 defined limits

Quality control

For quality control, use PreciControl Universal.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours 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.

Calculation

The analyzer automatically calculates the T-uptake (TBC) concentration of each sample as TBI values.

In order to obtain further information on the thyroid function (also taking into account the T4 value), the free thyroxine index should be determined.

fT4I = T4 : TBI

Limitations - interference

The assay is unaffected by icterus (bilirubin < 701 µmol/L or < 41 mg/dL), hemolysis (Hb < 1.2 mmol/L or < 2 g/dL), lipemia (Intralipid < 2000 mg/dL) and biotin (< 164 nmol/L or < 40 ng/mL).

Criterion: Recovery within ± 10 % of initial value.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 339 IU/mL and samples from dialysis patients.

In vitro tests were performed on 26 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.[7]

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.[8]

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

TBI: 0.200-1.90

Values below the lower detection limit are reported as < 0.200 TBI. Values above the measuring range are reported as > 1.90 TBI.

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: TBI: 0.200

The lower 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

Samples for T-uptake determinations cannot be diluted, as T4 in the blood is present in free and protein-bound forms which are in equilibrium. A change in the concentration of the binding proteins alters this equilibrium and consequently also the binding capacity being measured.

Expected values

Elecsys T-Uptake results (TBI) calculated in 974 serum samples from euthyroid subjects in Japan, Belgium, and Germany:

TBI: Median 1.0 (range: 2.5th-97.5th percentile): 0.8-1.3

FT4 index:

Elecsys T4/TBI results calculated in 825 serum samples from euthyroid subjects in Germany and Japan (range: 2.5th-97.5 th percentile):

62-164 nmol/L or 4.8-12.7 µg/dL

The following values were determined for the 99 % percentile range of results in a total of 275 serum and plasma samples from healthy subjects in the USA:

57-147 nmol/L or 4.4-11.4 µg/dL

Values < 0.8 are indicative of hyperthyroidism or low TBG concentrations while values > 1.3 are indicative of hypothyroidism or elevated TBG concentrations.

For detailed information about reference intervals in children, adolescents and pregnant women, refer to the brochure "Reference Intervals for Children and Adults", English: REF 04640292, German: REF 04625889.

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.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

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

Elecsys 2010 and **cobas e** 411 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean TBI	SD TBI	CV %	SD TBI	CV %
Human serum 1	1.80	0.012	0.7	0.016	0.9
Human serum 2	0.95	0.020	2.2	0.035	3.3
Human serum 3	0.55	0.028	5.1	0.064	11.7
PreciControl Universal 1	1.09	0.026	2.4	0.029	2.7
PreciControl Universal 2	0.94	0.031	3.3	0.034	3.7

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers						
	Repeatability			**Intermediate precision**		
Sample	Mean TBI	SD TBI	CV %	Mean TBI	SD TBI	CV %
Human serum 1	0.71	0.01	2.0	0.68	0.03	4.9
Human serum 2	0.87	0.02	2.6	0.92	0.04	3.9
Human serum 3	1.40	0.01	0.8	1.41	0.02	1.5
PreciControl Universal 1	1.22	0.01	0.8	1.23	0.03	2.4
PreciControl Universal 2	0.86	0.02	2.0	0.83	0.04	4.8

Method comparison

A comparison of the Elecsys T-Uptake assay (y) with the Enzymun-Test TBK method (x) using clinical samples gave the following correlations:

Number of samples measured: 145

Passing/Bablok[9]	Linear regression
y = 1.03x - 0.15	y = 0.98x - 0.09
τ = 0.717	r = 0.915

The sample TBI values were between 0.8 and 1.4.

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 %.

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing

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