Appendix (2)

11810 456001V18

T3

Triiodothyronine

200 tests **11731360** 122

• Indicates analyzers on which the kit can be used

cobas e 601	**cobas e** 411	MODULAR ANALY TICS E170	Elecsys 2010	Elecsys 1010
•	•	•	•	•

English

Intended use

Immunoassay for the in vitro quantitative deter mination of total triiodothyronine 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

Triiodothyronine(T3)is thehormoneprincipally responsiblefor thedevelopment of the ef fects of the thyroid hormones on the various target organs.
T3 (3,5,3'-triiodothyronine) is mainly for med extrathyroidally, particularly in the liver, by enzymatic 5'-deiodination of T4. Accordingly, the T3 concentration in serum is more a reflection of the functional state of the peripheral tissue than the secretor y perfor mance of the thyroid g land.
A reduction in the conver sion of T4 to T3 results in a decrease in the T3 concentration. It occurs under the influence of medicaments such as propanolol, g lucocorticoids or amiodarone and in severe non-thyroidal illness (NTI), and is referred to as "low T3 syndrome". As with T4, over 99 % of T3 is bound to transport proteins. However, the af finity of T3 to them is around 10-fold lower. [1,2,3,4]

The deter mination of T3 is utilized in the diagnosis of T3-hyperthyroidism, the detection of early stag es of hyperthyroidism and for indicating a diagnosis of thyrotoxicosis factitia. [5,6,7]
The Elecsys T3 assay employs a competitive test principle with polyclonal antibodies specifically directed against T3. Endogenous T3, released by the action of 8-anilino-1-naphthalene sulfonic acid (ANS), competes with the added biotinylated T3-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: 30 µL of sample and a T3-specific antibody labeled with a ruthenium complex; bound T3 is released from the binding proteins in the sample by ANS.
- 2nd incubation: Af ter addition of streptavidin-coated microparticles and biotinylated T3, 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-T3-Ab~$Ru(bpy)^{2+}_3$ (gray cap), 1 bottle, 16 mL: Polyclonal anti-T3-antibody (sheep) labeled with ruthenium complex 75 ng/mL; ANS 0.8 mg/mL; phosphate buffer 100 mmol/L, pH 7.4; preservative.

R2 T3~biotin (black cap), 1 bottle, 16 mL: Biotinylated T3 3 ng/mL; ANS 0.8 mg/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 T3 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 MODULAR ANALY TICS E170 and **cobas e** 601
8 weeks	on Elecsys 2010 and **cobas e** 411
8 weeks (stored alter nately in the refrigerator and on the analyzer - ambient temperature 20-25 °C; up to 20 hours opened in total)	on Elecsys 1010

Specimen collection and pr eparation

Only the specimens listed below were tested and found acceptable. Serum collected using standard sampling tubes or tubes containing separating g el.
Li-, Na-, NH^+_4-heparin, K_3-EDTA , sodium citrate, and sodium fluoride/potassium oxalate plasma.
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. Stable for 7 days at 2-8 °C, 1 month at -20 °C. [4] 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)

- Cat. N o. 117315 48122, T3 CalSet, for 4 x 1 mL
- Cat. N o. 11731416122, PreciControl Univer sal, for 2 x 3 mL each of PreciControl Univer sal 1 and 2 or
  Cat. N o. 11731416190, PreciControl Univer sal, for 2 x 3 mL each of PreciControl Univer sal 1 and 2
- Cat. N o. 11731416160, PreciControl Univer sal, for 2 x 3 mL each of PreciControl Univer sal 1 and 2 (for USA)
- General laborator y equipment
- Elecsys 1010/2010, MODULAR ANALY TICS E170 or **cobas e** analyzer

Accessories for Elecsys 1010/2010 and **cobas e** 411 analyzers:

- Cat. N o. 11662988122, ProCell, 6 x 380 mL system buffer

Triiodothyronine

- Cat. N o. 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- Cat. N o. 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- Cat. N o. 11933159001, Adapter for SysClean
- Cat.N o. 11706829001, Elecsys 1010 AssayCup,12 x 32 reactionvesselsor Cat. N o. 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- Cat. N o. 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALY TICS E170 and **cobas e** 601 analyzers:

- Cat. N o. 0 4880340190, ProCell M, 2 x 2 L system buffer
- Cat. N o. 0 4880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- Cat. N o. 12135027190, CleanCell M, 1 x 2 L measuring cell cleaning solution (for USA)
- Cat. N o. 03023141001, PC/CC-Cups, 12 cups to prewar m ProCell M and CleanCell M before use
- Cat. N o. 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- Cat. N o. 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 8 4 reaction vessels or pipette tips, waste bags
- Cat. N o. 03023150001, WasteLiner, waste bags
- Cat. N o. 03027651001, SysClean Adapter M

Accessories for all analyzers:

- Cat. N o. 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution

Only available in the USA:

- Cat. N o. 117 76690160, Elecsys T3 CalCheck, 3 concentration ranges

Assay

For optimum perfor mance 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 parameter s via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

MODULAR ANALY TICS E170, Elecsys 2010 and **cobas e** analyzers: Bring the cooled reagents to approx. 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid the for mation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.

Elecsys 1010 analyzer: Bring the cooled reagents to approx. 20-25 °C and place on the sample/reagent disk of the analyzer (ambient temperature 20-25 °C). Avoid the for mation of foam.

Open bottle caps **manually** before use and **close manually** af ter use. Store at 2-8 °C af ter use.

Calibration

Traceability: This method has been standardized against reference standards by weighing T3 into analyte-free human serum matrix.

Ever y Elecsys T3 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 T3 CalSet.

Calibration freq uency: Calibration must be perfor med 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:

MODULAR ANALY TICS E170, Elecsys 2010 and **cobas e** analyzers:

- af ter 1 month (28 days) when using the same reagent lot
- af ter 7 days (when using the same reagent kit on the analyzer)

Elecsys 1010 analyzer:

- with ever y reagent kit
- af ter 7 days (ambient temperature 20-25 °C)
- af ter 3 days (ambient temperature 25-32 °C)

For all analyzers:

- as required: e.g. quality control findings outside the specified limits

Quality control

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

Controls for the various concentration ranges should be run as single deter minations at least once ever y 24 hours when the test is in use, once per reagent kit, and af ter ever y calibration. The control inter vals and limits should be adapted to each laborator y's individual requirements. Values obtained should fall within the defined limits.

Each laborator y should establish corrective measures to be taken if values fall outside the limits.
Follow the applicable gover nment regulations and local guidelines for quality control.

Calculation

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

Conver sion factor s: nmol/L x 0.651 = ng/mL
nmol/L x 65.09998 = ng/dL
ng/mL x 1.536 = nmol/L

Limitations - inter fer ence

The assay is unaffected by icterus (bilirubin < 599 µmol/L or < 35 mg/dL), hemolysis (Hb < 1.2 mmol/L or < 2.0 g/dL), lipemia (Intralipid < 1800 mg/dL), and biotin < 40.9 nmol/L or < 10 ng/mL.
Criterion: Recover y 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 af ter the last biotin administration.

N o inter ference was observed from rheumatoid factor s up to a concentration of 1500 IU/mL and samples from dialysis patients.
In vitro tests were perfor med on 26 commonly used pharmaceuticals. N o inter ference with the assay was found.
Therapy with amiodarone can lead to depressed T3 values.
Phenytoin, phenylbutazone, and salicylates cause release of T3 from the binding proteins, thus leading to a reduction in the total T3 hormone level at normal fT3 levels.[8]
Autoantibodies to thyroid hormones can inter fere 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.[9]
Pathological concentrations of binding proteins (TBG, albumin) can lead to total T3 values outside the normal range being found despite a euthyroid metabolic state (e.g. in NTI[b] -patients, pregnancy, use of oral contraceptives). In such cases a fT3 or fT4 deter mination is indicated.
In rare cases, inter ference due to extremely high titer s of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These ef fects are minimized by suitable test design.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical histor y, clinical examination and other findings.

b) NTI = non thyroidal illness

Measuring range

0.300-10.00 nmol/L or 0.195-6.51 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.300 nmol/L or < 0.195 ng/mL. Values above the measuring range are reported as > 10.00 nmol/L or > 6.51 ng/mL.

Dilution

N ot necessary due to the broad measuring range.

Expected values

1.3-3.1 nmol/L or 0.8-2.0 ng/mL: euthyroid
The values correspond to the 2.5th and 97.5th percentiles of findings from a total of 514 healthy test subjects.

Status: MCE Elecsys 2010, status 1996, verified 1st quarter 1998

For detailed infor mation about reference inter vals in children, adolescents and pregnant women, refer to the brochure "Reference Inter vals for Children and Adults", Cat. N o. English: 0 46 40292, German: 0 4625889.

This booklet also contains results of a detailed study about influencing factor s on thyroid parameter s 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 Biochemistr y - NACB).

Each laborator y should investigate the transferability of the expected values to its own patient population and if necessary deter mine its own reference ranges.

T3

Triiodothyronine

cobas®

Specific performance data

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

Pr ecision

Reproducibility was deter mined using Elecsys reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the N CCLS (National Committee for Clinical Laborator y Standards): 6 times daily for 10 days (n = 60); within-run precision on MODULAR ANALY TICS E170 analyzer, n = 21. The following results were obtained:

Elecsys 1010/2010 and **cobas e** 411 analyzers

Total pr ecision			Within-run pr ecision					
CV	SD		CV	SD		Mean		Sample
%	ng/mL	nmol/L	%	ng/mL	nmol/L	ng/mL	nmol/L	
5.4	0.05	0.07	3.6	0.03	0.04	0.79	1.22	HS[c] 1
4.7	0.09	0.14	4.2	0.08	0.12	1.87	2.87	HS 2
5.4	0.18	0.27	5.3	0.18	0.27	3.31	5.09	HS 3
4.8	0.07	0.10	4.1	0.06	0.09	1.38	2.12	PC U[d] 1
4.1	0.17	0.26	3.5	0.14	0.22	4.11	6.31	PC U2

c) HS = human serum
d) PC U = PreciControl Univer sal

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

Total pr ecision					Within-run pr ecision					
CV	SD		Mean		CV	SD		Mean		Sample
%	ng/mL	nmol/L	ng/mL	nmol/L	%	ng/mL	nmol/L	ng/mL	nmol/L	
4.5	0.04	0.06	0.80	1.24	3.1	0.02	0.04	0.77	1.19	HS 1
3.4	0.05	0.08	1.49	2.28	2.2	0.03	0.05	1.41	2.16	HS 2
3.7	0.17	0.26	4.61	7.08	1.5	0.07	0.11	4.45	6.83	HS 3
3.4	0.05	0.08	1.58	2.42	1.3	0.02	0.03	1.54	2.36	PC U1
3.4	0.13	0.20	3.78	5.81	1.3	0.05	0.07	3.79	5.83	PC U2

Analytical sensitivity (lower detection limit)

0.300 nmol/L or 0.195 ng/mL
The lower detection limit represents the lowest analyte level that can be distinguished from zero.

Method comparison

A comparison of the Elecsys T3 assay (y) with the Enzymun-Test T3 method (x) using clinical samples gave the following correlations (nmol/L): Number of samples measured: 300

Linear regression	Passing/Bablok[10]
y = 1.18x - 0.35	y = 1.26x - 0.56
r = 0.957	τ = 0.75 4

The sample concentrations were between approx. 0.5 and 9 nmol/L (0.3 and 5.9 ng/mL).

Analytical specificity

For the antibody derivative used, the following cross-reactivities were found: D-T3 100 %; L-T4 < 0.16 %; D-T4 < 0.16 %; L-rT3 < 0.04 %; L-T2 < 1.0 %; 3,3',5-triiodothyroacetic acid 106 %; 3,3',5,5'-tetraiodothyroacetic acid < 0.01 %.

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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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