Tg

Thyroglobulin (Tg)

cobas®

REF	Σ	SYSTEM
05118921 190	100	Elecsys 2010 MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

Please note

Thyroglobulin determinations can be affected by the presence of anti-thyroglobulin antibodies (anti-Tg) in patient samples.[1] The measured Tg value of a patient's sample can vary depending on the testing procedure used. The laboratory finding must therefore always contain a statement on the Tg assay method used. Tg values determined on patient samples by different testing procedures cannot be directly compared with one another and could be the cause of erroneous medical interpretations. If there is a change in the Tg assay procedure used while monitoring therapy, then the Tg values obtained upon changing over to the new procedure must be confirmed by parallel measurements with both methods.

Intended use

Immunoassay for the in vitro quantitative determination of thyroglobulin in human serum and plasma.

Determination of Tg is used in the confirmation of a diagnosis of thyroid disease and for monitoring progress after total thyroid ablation.

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

Summary

Thyroglobulin (Tg) is a glycoprotein with a molecular weight of approx. 660 kD. It basically consists of two protein chains of 300 kD and 330 kD, linked together by disulfide bridges.[2]

Tg is synthesized in large quantities by the thyrocytes and released into the lumina of the thyroid follicles. Production of Tg is stimulated by TSH, intrathyroidal iodine deficiency and the presence of thyroid-stimulating immunoglobulins.

Tg plays a decisive role in the synthesis of the peripheral thyroid hormones T3 and T4. It contains about 130 tyrosine residues, some of which can be iodinated to monoiodo- and diiodothyrosine (MIT and DIT) in the presence of TPO (thyroperoxidase) and iodide.[3] The subsequent coupling of MIT and DIT to form T3 and T4 also takes place on the Tg-matrix with the involvement of TPO.

During Tg-synthesis by the thyrocytes and the transport of Tg to the follicles, small quantities of the protein can pass into the bloodstream. Accordingly, low concentrations of Tg can also be found in the blood of persons not suffering from thyroid diseases. Low levels of circulating Tg therefore indicate the presence of thyroid tissue. Following successful total thyroidectomy, Tg is no longer detectable.[4]

In cases of congenital hypothyroidism the determination of Tg can be used to distinguish between the complete absence of the thyroid gland and thyroid hypoplasia or other pathological conditions.

On the other hand, injury to the follicle wall can result in larger quantities of Tg passing into the blood. Tg is therefore regarded in particular as a marker for the morphological integrity of the thyroid gland.[5,6]

The determination of Tg can also be useful in distinguishing between subacute thyroiditis and factitious thyrotoxicosis.[7] In the latter case, low Tg values are to be expected due to the suppression of TSH.

The presence of antibodies to Tg can falsify the result of a Tg determination.

Anti-Tg determinations in all Tg samples are recommended to rule out this interference.[8]

The Elecsys Tg assay employs monoclonal antibodies directed specifically against human thyroglobulin.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: Tg from 20 µL of sample, a biotinylated monoclonal Tg-specific antibody, and a monoclonal Tg-specific antibody labeled with a ruthenium complex[a)] react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the 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/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 and a master curve provided via the reagent barcode.

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

Reagents - working solutions

The reagent rackpack is labeled as TG.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-Tg-Ab~biotin (gray cap), 1 bottle, 10 mL: Biotinylated monoclonal anti-Tg antibody (mouse) 0.7 mg/L; phosphate buffer 90 mmol/L, pH 6.1; preservative.

R2 Anti-Tg-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 10 mL: Monoclonal anti-Tg antibody (mouse) labeled with ruthenium complex 1.0 mg/L; phosphate buffer 90 mmol/L, pH 6.1; 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.

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Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on the analyzers	8 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes.

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

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 24 hours at 15-25 °C, 3 days at 2-8 °C, 1 month 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 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 11820940122, Tg CalSet, for 4 x 1 mL
- REF 11731416190, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2 or
  REF 11776452122, PreciControl Tumor Marker, for 2 x 3 mL each of PreciControl Tumor Marker 1 and 2
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or
  REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- Anti-Tg assay, to verify the presence of antibodies to Tg in patient samples (e.g. Anti-Tg assay, REF 04738578)
- REF 05107555, Tg Confirmatory Test
- Distilled or deionized water
- 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 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- 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 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.

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approx. 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 against CRM (Certified Reference Material) 457, of the BCR (Community Bureau of Reference) of the European Union.[10]

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve 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 or PreciControl Tumor Marker.

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.

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Calculation

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

Interpretation of the results

When interpreting the test results the possibility of anti-Tg antibodies in the sample should be taken into account. Results should be either confirmed with the confirmatory test (e.g. Elecsys Tg Confirmatory Test) or preferably verified by the determination of anti-Tg (e.g. Elecsys Anti-Tg assay).[8]

Limitations - interference

The assay is unaffected by icterus (bilirubin < 616 µmol/L or < 36 mg/dL), hemolysis (Hb < 1.2 mmol/L or < 1.9 g/dL), lipemia (triglycerides < 22.8 mmol/L or < 2000 mg/dL) and biotin (< 327 nmol/L or < 80 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 2500 IU/mL.

There is no high-dose hook effect at Tg concentrations up to 120000 ng/mL.

In vitro tests were performed on 21 commonly used pharmaceuticals. No interference with the assay was found.

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.

Tg determinations can be affected by the presence of anti-thyroglobulin antibodies (anti-Tg) or by non-specific effects in patient sera. Results should be either confirmed with the Tg recovery test (e.g. Elecsys Tg Confirmatory Test) or preferably verified by the determination of anti-Tg (e.g. Elecsys Anti-Tg assay).[1,8]

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

0.100-1000 ng/mL or µg/L (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.100 ng/mL (µg/L). Values above the measuring range are reported as > 1000 ng/mL (µg/L) (or up to 5000 ng/mL (µg/L) for 5-fold diluted samples).

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: < 0.1 ng/mL (µg/L)

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 with Tg concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:5 (either automatically by the MODULAR ANALYTICS E170, Elecsys 2010 or **cobas e** analyzers or manually). The concentration of the diluted sample must be > 50 ng/mL (µg/L).

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** software automatically takes the dilution into account when calculating the sample concentration.

Expected values

Studies performed by clinical centers in Austria, Spain, and the USA on samples from 130 healthy subjects showed the following values: 1.4-78 ng/mL or µg/L (5^{th}-95^{th} percentile).

Status: MCE, Elecsys Tg assay with Elecsys 1010/2010 analyzers, study No. B99P001, 9/2000; values recalculated after restandardization: 10/2001.

Tg should no longer be measurable following complete ablation of thyroid tissue by thyroidectomy and radioiodine therapy. More extensive diagnostic measures are indicated if in such patients there is a rise in the Tg concentration.[11]

For detailed information about reference intervals in children, adolescents and pregnant women, refer to the brochure "Reference Intervals for Children and Adults", REF English: 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 accordance with 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 ng/mL (µg/L)	SD ng/mL (µg/L)	CV %	SD ng/mL (µg/L)	CV %
Human serum 1	4.11	0.08	1.8	0.12	3.0
Human serum 2	26.9	0.36	1.4	0.61	2.3
Human serum 3	184	2.00	1.1	3.40	1.8
PC U[b)]1	8.75	0.15	1.7	0.24	2.7
PC U2	14.8	0.22	1.5	0.36	2.5

b) PC U = PreciControl Universal

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers						
	Repeatability			**Intermediate precision**		
Sample	Mean ng/mL (µg/L)	SD ng/mL (µg/L)	CV %	Mean ng/mL (µg/L)	SD ng/mL (µg/L)	CV %
Human serum 1	3.38	0.13	3.8	3.42	0.12	3.6
Human serum 2	27.9	0.31	1.1	28.2	0.62	2.2
Human serum 3	84.6	0.48	0.6	82.8	1.78	2.2
PC U1	17.7	0.20	1.1	17.5	0.41	2.3
PC U2	75.6	0.55	0.7	73.9	2.34	3.2

Method comparison

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

Number of samples measured: 98

Passing/Bablok[12]	Linear regression
y = 0.96x - 0.56	y = 1.02x - 3.20
τ = 0.946	r = 0.995

The sample concentrations were between approx. 2 and 680 ng/mL (µg/L).
b) A comparison of the Elecsys Tg assay (y) with a commercially available Tg test (x) using clinical samples gave the following correlations:
Number of samples measured: 64

Passing/Bablok[12]	Linear regression
y = 1.27x + 5.4	y = 1.03x + 24.9
τ = 0.791	r = 0.916

The sample concentrations were between approx. 1.1 and 290 ng/mL (µg/L).

Analytical specificity

For the monoclonal antibodies used, no cross-reactivities were found with the following substances:
60 µg/dL T4, 60 ng/mL T3, 54 µg/mL thyroxine binding globulin (TBG), 10 g/dL human albumin, 5.4 g/L immunoglobulin A (IgA), 4.1 g/dL immunoglobulin G (IgG).

Functional sensitivity

< 1.0 ng/mL (µg/L)
The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of 20 %.

References

1 Erali M, Bigelow RB, Meikle AW. ELISA for thyroglobulin in serum: recovery studies to evaluate autoantibody interference and reliability of thyroglobulin values. Clin Chem 1996;42(5):766-770.
2 Malthiery Y, Lissitzky S. Primary structure of human thyroglobulin deduced from sequence of its 8448-base complementary DNA. Eur J Biochem 1987;165:491-498.
3 Saboori AM, Rose NR, Butscher WG, et al. Modification of a Nonincinerative Method for Determination of Iodine in Iodoproteins. Anal Biochem 1993;214:335-338.
4 Rüter A, Smeds S, Lennquist S. Value of Serum Thyroglobulin Measurement in Patients Operated on for Well Differentiated Thyroid Carcinoma. Eur J Surg 1998;164:665-671.
5 Druetta L, Croizet K, Bornet H, et al. Analyses of the molecular forms of serum thyroglobulin from patients with Graves' disease, subacute thyroiditis or differentiated thyroid cancer by velocity sedimentation on sucrose gradient and Western blot. Eur J Endocrino 1998;139:498-507.
6 Pacini F, Pinchera A. Serum and tissue thyroglobulin measurement: Clinical applications in thyroid disease. Biochemie 1999;81:463-467.
7 Refetoff S, Lever EG. The value of serum thyroglobulin measurement in clinical practice. JAMA 1983;250:2352-2357.
8 Spencer C. International Thyroid Testing Guidelines. National Academy of Clinical Biochemistry, August 2001;Section 3E,11-14.
9 Guder WG, Narayanan S, Wisser H, et al. List of Analytes; Preanalytical Variables. Brochure in: Samples: From the Patient to the Laboratory. GIT-Verlag, Darmstadt 1996:20/21. ISBN 3-928865-22-6.
10 Feldt-Rasmussen U, Profilis C, Colinet E, et al. Purification and assessment of stability and homogeneity of human thyroglobulin reference material (CRM 457). Exp Clin Endocrinol 1994;102:87-91.
11 Follow-up and management of differentiated thyroid carcinoma: a European perspective in clinical practice. Eur J Endocrinol 2004;151:539-548.
12 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

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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