ms_06445896190V2.0

Tg II

Thyroglobulin

cobas®

REF	Σ	SYSTEM
		Elecsys 2010
		MODULAR ANALYTICS E170
06445896 190	100	**cobas e** 411
		cobas e 601
		cobas e 602

English

Please note

Thyroglobulin (Tg) determinations can be affected by the presence of Tg autoantibodies (anti-Tg) in some patient samples. These autoantibodies may interfere with the assay used to measure Tg, causing false high or false low Tg values.[1,2]

The measured Tg value of a patient's sample can also vary depending on the assay used. The laboratory finding must therefore always contain a statement on the Tg assay method used. Tg values determined on patient samples by different assays 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 patient monitoring, the Tg values obtained upon changing to the new procedure must be confirmed by parallel measurements with both methods.[2,3]

Intended use

Immunoassay for the in vitro quantitative determination of thyroglobulin in human serum and plasma. Determination of Tg is used as an aid in monitoring after 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 approximately 660 kDa.[4] 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 (thyroid peroxidase) 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.[5]

During synthesis of Tg 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 be found in the blood of healthy individuals not suffering from thyroid diseases.

Elevated Tg concentrations have been reported in different thyroid conditions like Hashimoto's disease, Graves' disease, thyroid adenoma, and thyroid carcinoma. The determination of Tg can also be helpful to distinguish between subacute thyroiditis and factitious thyrotoxicosis. In cases of congenital hypothyroidism the determination of Tg can be used to differentiate between the complete absence of the thyroid gland and thyroid hypoplasia or other pathological conditions.[5,6,7]

The main application of Tg testing is the post-operative follow-up of patients with differentiated thyroid carcinoma (DTC). As the thyroid gland is the only known source of Tg, the serum Tg level will drop to a very low or undetectable concentration after total or near-total thyroidectomy and successful radioiodine ablation of the residual thyroid tissue. In patients who have undergone a partial thyroidectomy Tg levels will still be measurable depending on how much tissue is remaining after surgery. Detectable levels of serum Tg after total thyroidectomy are indicative of persistent or recurrent DTC. In consequence significantly increasing Tg levels are interpreted as a sign of recurrence of the disease.[8,9,10,11,12,13]

Using very sensitive Tg assays an increased number of 'thyroglobulin-positive' patients may be observed, even if patients show no clinical evidence of disease.[13] These patients cannot be defined as disease-free and should be monitored according to current guidelines. Different cut-off values are published to distinguish between monitoring patients and patients with recurrence of disease who need further diagnosis and treatment. Alternatively, institutional cut-off levels can be established to tailor the follow-up strategies to the local patient population and the thyroglobulin test used.[8,9,10,13]

All Tg results should be interpreted in conjunction with the total clinical presentation of the patient, including symptoms, clinical history, data from additional tests (i.e. neck ultrasound, whole body scan) and other appropriate information.

Tg determinations can be affected by the presence of Tg autoantibodies causing false high or false low Tg values. Therefore anti-Tg determinations are recommended for all Tg samples to rule out this interference.[1,2]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: Tg from 35 µL of sample, a biotinylated monoclonal Tg-specific antibody and monoclonal Tg-specific antibodies 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 II.

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, 9 mL:
Biotinylated monoclonal anti-Tg antibody (mouse) 1 mg/L; Bis-Tris buffer 50 mmol/L, pH 6.3; preservative.

R2 Anti-Tg-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 mL:
Monoclonal anti-Tg antibodies (mouse) labeled with ruthenium complex 3.1 mg/L; Bis-Tris buffer 50 mmol/L, pH 6.3; 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	84 days (12 weeks)
on the analyzers	28 days (4 weeks)

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K_2- and K_3-EDTA plasma.

Stable for 48 hours at 15-25 °C, 72 hours at 2-8 °C, 1 month at -20 °C.[14] 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 06445900190, Tg II CalSet, for 4 x 1 mL
- REF 11731416190, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2 or REF 06445918190, PreciControl Thyro Sensitive, for 4 x 2 mL
- REF 03609987190, Diluent MultiAssay, 2 x 16 mL sample diluent
- Anti-Tg assay, to verify the presence of antibodies to Tg in patient samples (e.g. Anti-Tg assay, REF 04738578191)
- REF 06513107190, Tg II 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 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 against CRM (Certified Reference Material) 457, of the BCR (Community Bureau of Reference) of the European Union.[15]

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 Thyro Sensitive.

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 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 II Confirmatory Test) or preferably verified by the determination of anti-Tg (e.g. Elecsys Anti-Tg assay).[1,2]

Limitations - interference

The assay is unaffected by icterus (bilirubin < 1128 µmol/L or < 66 mg/dL), hemolysis (Hb < 0.373 mmol/L or < 0.6 g/dL), lipemia (Intralipid < 22.8 mmol/L or < 2000 mg/dL) and biotin (< 123 nmol/L or < 30 ng/mL), IgG ≤ 2 g/dL, IgA ≤ 1.6 g/dL and IgM ≤ 0.5 g/dL.

Criterion: Recovery within ± 10 % of initial value for samples ≤ 2 ng/mL or ± 25 % of initial value for samples > 2 ng/mL.

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 600 IU/mL.

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

In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.

The following special thyroid drugs were tested with concentrations shown in the table below. No interference with the assay was found. Criterion: Recovery within ± 10 % of initial value.

Drug	Concentration (µg/mL)
Iodid	0.2
Carbimazol	30
Thiamazol	80
Propylthiouracil	300
Perchlorat	2000
Propranolol	240
Amiodaron	200
Prednisolon	100
Hydrocortison	200
Fluocortolon	100
Octreotid	0.3
L-T3	0.5
D-T3	0.5
L-T4	5
D-T4	5

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 II Confirmatory Test) or preferably verified by the determination of anti-Tg (e.g. Elecsys Anti-Tg assay).[1,2]

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

Make sure that in the Special Wash List (Screen → Utility → Special Wash → Immune) the Elecsys Tg II assay is combined with Anti-Tg.

From test	Step	To test	Step 0	Step 1	Step 2
Anti-Tg	1	Tg II	-	x	-

The described additions to the Special Wash List have to be entered manually. Please refer to the operator's manual.

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

0.04-500 ng/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.04 ng/mL. Values above the measuring range are reported as > 500 ng/mL (or up to 5000 ng/mL for 10-fold diluted samples).

Lower limits of measurement

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)

Limit of Blank = 0.02 ng/mL

Limit of Detection = 0.04 ng/mL

Limit of Quantitation = 0.1 ng/mL with a total allowable error of ≤ 30 %

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable error of ≤ 30 %.

When reporting results below LoQ a higher uncertainty needs to be taken into consideration.

Dilution

Samples with Tg concentrations above the measuring range can be diluted with Diluent MultiAssay. The recommended dilution is 1:10 (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.

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

3.5-77 ng/mL

These values correspond to the 2.5th and 97.5th percentiles of results obtained from a total of 478 healthy Caucasian subjects (254 males, 224 females).

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 protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	0.180	0.010	5.5	0.017	9.2
Human serum 2	1.11	0.024	2.2	0.034	3.0
Human serum 3	1.59	0.019	1.2	0.042	2.6
Human serum 4	89.3	2.71	3.0	3.71	4.2
Human serum 5	247	6.14	2.5	7.83	3.2
Human serum 6	470	9.14	1.9	17.9	3.8
PC U[b)]1	20.8	0.421	2.0	1.08	5.2
PC U2	67.0	0.900	1.3	3.39	5.1

b) PC U = PreciControl Universal

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	0.289	0.014	4.8	0.017	5.9
Human serum 2	1.10	0.028	2.5	0.050	4.5
Human serum 3	1.56	0.031	2.0	0.062	4.0
Human serum 4	87.5	2.90	3.3	4.09	4.7
Human serum 5	242	5.85	2.4	10.4	4.3
Human serum 6	456	10.4	2.3	19.8	4.3
PC U1	19.5	0.419	2.2	0.896	4.6
PC U2	61.1	1.20	2.0	2.52	4.1

Method comparison

A comparison of the Elecsys Tg II assay (y) with a commercial assay (x) using clinical samples gave the following correlations:

Number of samples measured: 94

Passing/Bablok[16]	Linear regression
$y = 0.936x + 0.105$	$y = 0.917x + 0.877$
$\tau = 0.892$	$r = 0.981$

The sample concentrations were between approximately 0.2 and 300 ng/mL.

Analytical specificity

The following cross-reactivities were found, tested with thyroglobulin concentrations of approximately 5 and 50 ng/mL:

Cross-reactant	Concentration tested	Cross-reactivity %
TSH	1000 mIU/L	1.94
TBG	200000 ng/mL	0.008

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 Spencer CA, LoPresti JS. Technology Insigth: measuring thyroglobulin and thyroglobulin autoantibody in patients with differentiated thyroid cancers. Nat Clin Pract Endocrinol Metab 2008;4(4):223-233.

3 Clark P, Franklyn J. Can we interpret serum thyroglobulin results? Ann Clin Biochem 2012;49:313–322.

4 Malthiéry Y, Lissitzky S. Primary structure of human thyroglobulin deduced from sequence of its 8448-base complementary DNA. Eur J Biochem 1987;165:491-498.

5 Kronenberg HM, Melmed S, Polonsky KS, et al. Williams Textbook of Endocrinology. Saunders Elsevier, Philadelphia, 12th edition, 2011.

6 Torréns JI, Burch HB. Serum thyroglobulin measurement. Utility in clinical practice. Endocrinol Metab Clin North Am 2001;30(2):429-467.

7 Pacini F, Pinchera A. Serum and tissue thyroglobulin measurement: Clinical applications in thyroid disease. Biochemie 1999;81:463-467.

8 Pacini F, Schlumberger M, Dralle H, et al. European consensus for the management of patients with differentiated thyroid carcinoma of the follicular epithelium. Eur J Endocrinol 2006;154:787–803.

9 Cooper DS, Doherty GM, Haugen BR, et al. Revised American Thyroid Association Management Guidelines for Patients with Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid 2009;19(11):1-48.

10 Pitoia F, Ward L, Wohllk N. et al. Recommendations of the Latin American Thyroid Society on diagnosis and management of differentiated thyroid cancer. Arq Bras Endocrinol Metab 2009;53(7):884-897.

11 Mazzaferri EL, Robbins RJ, Spencer CA, et al. A Consensus Report of the Role of Serum Thyroglobulin as a Monitoring Method for Low-Risk Patients with Papillary Thyroid Carcinoma. J Clin Endocrinol Metab 2003;88:1433-1441.

12 Zucchelli G, Iervasi A, Ferdeghini M, et al. Serum thyroglobulin measurement in the follow-up of patients treated for differentiated thyroid cancer. Q J Nucl Med Mol Imaging 2009;53:482-489.

13 Elisei R, Pinchera A. Advances in the follow-up of differentiated or medullary thyroid cancer. A Nat Rev Endocrinol 2012;8:466-475.

14 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.

15 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.

16 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

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com