Elecsys Anti-Tg

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REF		Σ	SYSTEM
06368697190	06368697500	100	**cobas e** 411 **cobas e** 601 **cobas e** 602

English

System information

For **cobas e** 411 analyzer: test number 710
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 133

Please note

The measured anti-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 anti-Tg assay method used. Anti-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 anti-Tg assay procedure used while monitoring therapy, then the anti-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 antibodies to thyroglobulin in human serum and plasma. The anti-Tg determination is used as an aid in the detection of autoimmune thyroid diseases.

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 produced in the thyroid gland and is a main component in the lumen of the thyroid follicle. In synergy with the enzyme thyroid-specific peroxidase (TPO), Tg has an essential function in the iodination of L-tyrosine and in the formation of the thyroid hormones T4 and T3.[1] Both Tg and TPO are potentially autoantigenic.[2,3]

Elevated serum concentrations of antibodies against Tg (Tg-autoantibodies) are found in subjects with autoimmunity-based thyroiditis.[2,3] High concentrations of anti-Tg together with anti-TPO are present in most patients with chronic lymphocytic-infiltrative thyroiditis (Hashimoto's disease).[3] The frequency of thyroglobulin antibodies is approximately 50-80 % in subjects with autoimmune-thyroiditis, including Hashimoto's disease, and approximately 30-50 % in individuals with Graves' disease.[3,4,5,6] The anti-Tg assay can also provide useful information for monitoring the course of Hashimoto's thyroiditis and for differential diagnosis.[3,7] This includes cases of suspected autoimmune thyroiditis of unknown origin with negative anti-TPO test results,[8,9] and to distinguish Hashimoto's thyroiditis from nontoxic nodular goiter or from other forms of thyroiditis.[4]

Anti-Tg has also been reported as a useful surrogate diagnostic marker for differentiated thyroid cancer when serum Tg is negative,[10] and for ruling out interference by Tg autoantibodies when measuring serum Tg using a Tg test.[11,12]

Although the sensitivity of the procedure can be increased by simultaneously determining additional thyroid antibodies (anti-TPO, anti-TSHR), a negative result does not definitively rule out the presence of an autoimmune disease. The antibody titer does not correlate with the clinical activity of the disease. Titers that are elevated initially can become negative if the disease persists for a longer period of time or if remission occurs. If antibodies reappear after remission, relapse is likely.

The Elecsys Anti-Tg assay uses human antigen and monoclonal human anti-Tg antibodies.[13]

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 10 µL of sample are incubated with biotinylated Tg and the antibodies of the sample bind the antigen.
- 2nd incubation: After addition of anti-Tg antibodies labeled with ruthenium complex[a] and streptavidin-coated microparticles, the immunocomplex produced 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 or e-barcode.

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

Reagents - working solutions

The reagent rackpack is labeled as A-TG.

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

R1 Tg~biotin (gray cap), 1 bottle, 10 mL:
Biotinylated Tg (human) 0.200 mg/L; TRIS buffer 100 mmol/L, pH 7.0; preservative.

R2 Anti-Tg-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 10 mL:
Monoclonal anti-Tg antibodies (human) labeled with ruthenium complex 0.620 mg/L; TRIS 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.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

2-methyl-2H-isothiazol-3-one hydrochloride

EUH 208 May produce an allergic reaction.

Product safety labeling follows EU GHS guidance.

All human material should be considered potentially infectious.

All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV.

The initial thyroid glandular tissue extract containing the human thyroglobulin has shown to be free from HBsAg and antibodies to HCV and HIV.

The testing methods used assays approved by the FDA or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[14,15]

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.

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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	6 weeks
on the analyzers	6 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.

K_2- and K_3-EDTA plasma.

Criterion: Recovery within 85-115 % of serum value or slope 0.85-1.15 + intercept within < ± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Do not use Li-heparin or sodium citrate plasma.

Stable for 3 days at 2-8 °C, 1 month at -20 °C. Freeze only once.[16]

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 06368603190, Anti-Tg CalSet, for 4 x 1.5 mL
- REF 05042666191, PreciControl ThyroAB, for 4 x 2.0 mL
- General laboratory equipment
- **cobas e** analyzer

Additional materials for the **cobas e** 411 analyzer:

- 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, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Additional materials for **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, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Additional materials for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution
- REF 11298500160, ISE Cleaning Solution/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 against the NIBSC (National Institute for Biological Standards and Control) 65/93 Standard.

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).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

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 ThyroAB.

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.

If necessary, repeat the measurement of the samples concerned.

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

Calculation

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

Limitations - interference

The assay is unaffected by icterus (bilirubin < 1129 µmol/L or < 66 mg/dL), hemolysis (Hb < 1.05 mmol/L or < 1.69 g/dL), lipemia (Intralipid < 2000 mg/dL) and biotin (< 246 nmol/L or < 60 ng/mL).

Criterion: Recovery within ± 15 % 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 300 IU/mL.

In vitro tests were performed on 24 commonly used pharmaceuticals. No interference with the assay was found.

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Tg concentrations > 2000 ng/mL may lead to falsely elevated anti-Tg concentrations. Accordingly anti-Tg values should not be reported for patient samples in that case.

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

10.0-4000 IU/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 10.0 IU/mL. Values above the measuring range are reported as > 4000 IU/mL.

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: < 10.0 IU/mL

The Lower Detection Limit represents the lowest 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

Sample dilution is not possible. The autoantibodies are heterogeneous and this gives rise to non-linear dilution phenomena.

Approximately 5 % of the pathological samples can have concentrations > 4000 IU/mL.

Expected values

Studies conducted with the Elecsys Anti-Tg assay in 5 clinical centers covering a total of 391 healthy subjects (MCE Elecsys Anti-Tg assay) confirmed the currently used threshold value of 115 IU/mL; this value corresponds to the 94th percentile.

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.

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 and pooled human sera according to a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 5 or 6 times daily for 10 days (n = 59 or 60); repeatability on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

cobas e 411 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean IU/mL	SD IU/mL	CV %	SD IU/mL	CV %
Human serum 1	62.8	3.07	4.9	5.44	8.7
Human serum 2	115	5.85	5.1	8.28	7.2
Human serum 3	290	13.2	4.6	17.3	5.9
Human serum 4	2894	161	5.6	183	6.3

cobas e 601 and **cobas e** 602 analyzers					
	Repeatability			**Intermediate precision**	
Sample	Mean IU/mL	SD IU/mL	CV %	SD IU/mL	CV %
Human serum 1	47.2	2.33	4.9	49.8	3.12	6.3
Human serum 2	588	7.42	1.3	597	12.5	2.1
Human serum 3	3289	42.0	1.3	3251	111	3.4

Precision was determined using Elecsys reagents and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean IU/mL	SD IU/mL	CV %	SD IU/mL	CV %
PC[b)] THYRO1	95.6	3.63	3.8	5.74	6.0
PC THYRO2	175	8.95	5.1	10.2	5.8

b) PC = PreciControl

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean IU/mL	SD IU/mL	CV %	SD IU/mL	CV %
PC THYRO1	88.7	1.55	1.8	4.52	5.1
PC THYRO2	166	3.39	2.1	7.63	4.6

Method comparison

The following are comparisons of values lying above and below the Elecsys Anti-Tg limit (115 IU/mL). These values were found with the Enzymun-Test Anti-Tg method (115 IU/mL) and two commercial Anti-Tg tests (60 IU/mL and 40 IU/mL). The concentrations measured with the Elecsys Anti-Tg assay were between < 10 IU/mL and up to > 4000 IU/mL.

a) Clinical routine samples

Comparison of results obtained with the Elecsys Anti-Tg assay and the Enzymun-Test Anti-Tg method in clinical routine samples:

		Enzymun-Test Anti-Tg method		
		< 115 IU/mL	> 115 IU/mL	Total
Elecsys Anti-Tg assay	> 115 IU/mL	8	32	40
	< 115 IU/mL	157	20	177
	Total	165	52	217
Percent agreement = 87 % (95 % confidence range 82-91 %)				

Comparison of results obtained with the Elecsys Anti-Tg assay and a commercial Anti-Tg test in clinical routine samples:

		Anti-Tg comparison test 1		
		< 60 IU/mL	> 60 IU/mL	Total
Elecsys Anti-Tg assay	> 115 IU/mL	16	24	40
	< 115 IU/mL	177	0	177
	Total	193	24	217
Percent agreement = 93 % (95 % confidence range 88-96 %)				

Comparison of results obtained with the Elecsys Anti-Tg assay and a second commercial Anti-Tg test in clinical routine samples:

	Anti-Tg comparison test 2			
Elecsys Anti-Tg assay		< 40 IU/mL	> 40 IU/mL	Total
	> 115 IU/mL	11	31	42
	< 115 IU/mL	100	5	105
	Total	111	36	147
Percent agreement = 89 % (95 % confidence range 83-94 %)				

b) Patient samples

Comparison of results obtained with the Elecsys Anti-Tg assay and the Enzymun-Test Anti-Tg method in samples from patients with Graves' disease (n = 39) and Hashimoto's thyroiditis (n = 43):

	Enzymun-Test Anti-Tg method			
Elecsys Anti-Tg assay		< 115 IU/mL	> 115 IU/mL	Total
	> 115 IU/mL	3	51	54
	< 115 IU/mL	21	7	28
	Total	24	58	82
Percent agreement = 88 % (95 % confidence range 79-94 %)				

Comparison of results obtained with the Elecsys Anti-Tg assay and a commercial Anti-Tg test in samples from patients with Graves' disease (n = 39) or Hashimoto's thyroiditis (n = 43):

	Anti-Tg comparison test 1			
Elecsys Anti-Tg assay		< 60 IU/mL	> 60 IU/mL	Total
	> 115 IU/mL	4	50	54
	< 115 IU/mL	25	3	28
	Total	29	53	82
Percent agreement = 91 % (95 % confidence range 83-97 %)				

Comparison of results obtained with the Elecsys Anti-Tg assay and a second commercial Anti-Tg test in samples from patients with Graves' disease (n = 27) or Hashimoto's thyroiditis (n = 24):

	Anti-Tg comparison test 2			
Elecsys Anti-Tg assay		< 40 IU/mL	> 40 IU/mL	Total
	> 115 IU/mL	5	27	32
	< 115 IU/mL	13	6	19
	Total	18	33	51
Percent agreement = 78 % (95 % confidence range 65-89 %)				

Analytical specificity

In samples containing up to approximately 1400 IU/mL anti-TPO (measured with the Elecsys Anti-TPO assay), a maximum anti-Tg value of 51 IU/mL was found.

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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 (for USA: see dialog.roche.com for definition of symbols used):

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

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FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

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