Elecsys Calcitonin

cobas®

REF		Σ	SYSTEM
09005676119	09005676500	100	**cobas e** 402 **cobas e** 801

English

System information

Short name	ACN (application code number)
HCT	10191

Intended use

Immunoassay for the in vitro quantitative determination of human calcitonin (thyrocalcitonin) in serum and plasma. The calcitonin determination is intended to be used as an aid in the diagnosis and treatment of diseases involving the thyroid and parathyroid glands, including carcinoma and hyperparathyroidism in conjunction with other clinical and laboratory findings.

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

Note: Please note that the catalogue number appearing on the package insert retains only the first 8 digits of the licensed 11-digit Catalogue Number: 09005676190 for the Elecsys Calcitonin assay. The last 3 digits -190 have been replaced by -119 for logistic purposes.

Summary

Human calcitonin (hCT) is a 32 amino acid peptide hormone with a molecular mass of 3418 Da which is secreted primarily by the parafollicular C cells of the thyroid gland.[1] It is metabolized in the liver and kidney and regulated by serum calcium levels. Physiologically hCT has effects on calcium and phosphorus metabolism. It is an inhibitor of bone resorption to prevent bone loss at times of calcium stress (e.g. pregnancy, lactation and growth).[2,3]

Serum hCT levels are relatively high in infants, decline rapidly and are relatively stable from childhood through adult life. In general hCT serum levels are higher in men than in women whereas smoking may lead to an additional increase in serum calcitonin levels.[4,5,6]

The most prominent clinical syndrome associated with a disordered hypersecretion of hCT is the medullary thyroid carcinoma (MTC), a tumor of the calcitonin secreting cells of the thyroid, which comprises 5-10 % of all thyroid cancers. 75-80 % of cases occur sporadically and the remaining occur in autosomal dominant diseases, in which MTC is one of the clinical features of the Multiple Endocrine Neoplasia (MEN) type 2 syndromes and familial MTC. MTC Management Guidelines were developed by the American Thyroid Association and recommend calcitonin measurements in the risk stratification / selection of treatment in MTC and in the evaluation and treatment post thyroidectomy.[7,8] These recommendations were endorsed by the European Thyroid Association and extended by an European Panel of Experts to routine measurement of serum calcitonin in patients with thyroid nodules.[9] Moderately elevated calcitonin levels can be falsely positive for either technical reasons or the presence of other rare pathological conditions (i.e. other neuroendocrine tumors, hyperparathyroidism, renal failure etc.). Therefore, the European Panel of Experts recommends that subjects with elevated basal calcitonin undergo a stimulation test, either by injection of pentagastrin or a rapid infusion of calcium. Most MTCs respond with a significant increase of hCT levels upon stimulation.[10,11]

The Elecsys Calcitonin assay employs monoclonal antibodies of mouse origin labeled with ruthenium complex[a)], specifically directed against hCT.

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

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 30 µL of sample, a biotinylated monoclonal hCT-specific antibody and a monoclonal hCT-specific antibody labeled with a ruthenium complex 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 II 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 **cobas** link.

Reagents - working solutions

The **cobas e** pack is labeled as HCT.

M Streptavidin-coated microparticles, 1 bottle, 5.8 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-hCT-Ab~biotin, 1 bottle, 7.2 mL:
Biotinylated monoclonal anti-hCT antibody (mouse) 1.50 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

R2 Anti-hCT-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 7.2 mL:
Monoclonal anti-hCT antibody (mouse) labeled with ruthenium complex 1.0 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

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

Warning

H317	May cause an allergic skin reaction.
Prevention:	
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.
Response:	
P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.
Disposal:	
P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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 available via the **cobas** link.

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Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **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
on the analyzers	16 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-EDTA and K_3-EDTA plasma.

Plasma tubes containing separating gel can be used.

Criterion: Recovery with a total deviation ≤ ± 2.0 pg/mL of initial value at concentrations ≤ 10 pg/mL; recovery within ± 20 % of initial value at concentrations > 10 pg/mL and slope 0.9-1.1 + intercept within ≤ ± 0.6 pg/mL + coefficient of correlation ≥ 0.95.

Stable for 4 hours at 20-25 °C, 1 day at 2-8 °C, 24 months at -20 °C (± 5 °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 and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators 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 09005684190, Calcitonin CalSet, for 4 x 1.0 mL
- REF 05618860190, PreciControl Varia, for 4 x 3.0 mL
- REF 07299010190, Diluent MultiAssay, 45.2 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/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.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: This method has been standardized against the IRP WHO Reference Standard 89/620.

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 **cobas e** pack 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 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Varia.

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 **cobas e** pack, 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 pg/mL or pmol/L (selectable).

Conversion factors: pg/mL x 0.2926 = pmol/L
pmol/L x 3.4176 = pg/mL

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1128 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.124 mmol/L or ≤ 200 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1200 IU/mL
IgG	≤ 4 g/dL
IgA	≤ 0.7 g/dL
IgM	≤ 0.4 g/dL

Criterion: For concentrations of 0.5-10 pg/mL the deviation is ≤ 1 pg/mL. For concentrations > 10 pg/mL the deviation is ≤ 10 %.

There is no high-dose hook effect at hCT concentrations up to 1 µg/mL.

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

In addition, the following special thyroid drugs were tested. No interference with the assay was found.

Special thyroid drugs

Drug	Concentration tested µg/mL
Iodide	0.2
Levothyroxine	0.25
Carbimazole	30
Thiamazole	16
Propylthiouracil	60
Perchlorate	2000
Propranolol	240
Amiodarone	200
Prednisolone	100
Hydrocortisone	200
Fluocortolone	100
Octreotide	0.3

Criterion: Recovery within ± 10 % of initial value.
In in vitro studies, the drug itraconazole caused decreased calcitonin findings at the daily therapeutic dosage level of 10 mg/L.

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

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

0.5-2000 pg/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.5 pg/mL. Values above the measuring range are reported as > 2000 pg/mL (or up to 200000 pg/mL for 100-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation
Limit of Blank = 0.3 pg/mL

Limit of Detection = 0.5 pg/mL

Limit of Quantitation = 1 pg/mL

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 the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

Dilution

Samples with hCT concentrations above the measuring range can be diluted with Diluent MultiAssay. The recommended dilution is 1:100 (either automatically by the analyzers or manually). The concentration of the diluted sample must be ≥ 20 pg/mL.

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

After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

Expected values

Upper limits of reference ranges are provided as the 97.5th percentile.

Cohort	N	97.5th percentile	Lower limit of 95 % CI[b)]	Upper limit of 95 % CI
Apparently healthy females	193	6.40 pg/mL	5.17 pg/mL	9.82 pg/mL
Apparently healthy males	162	9.52 pg/mL	8.31 pg/mL	14.3 pg/mL

b) CI = confidence interval

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 (EP05-A3) 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 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean pg/mL	SD pg/mL	CV %	SD pg/mL	CV %
Human serum 1	1.14	0.040	3.5	0.054	4.7
Human serum 2	10.3	0.200	1.9	0.253	2.5
Human serum 3	64.2	2.30	3.6	2.47	3.8
Human serum 4	1021	18.5	1.8	25.6	2.5
Human serum 5	1905	30.8	1.6	101	5.3
PreciControl Varia 1	9.79	0.139	1.4	0.173	1.8
PreciControl Varia 2	88.5	1.16	1.3	1.74	2.0

Method comparison

a) A comparison of the Elecsys Calcitonin assay, REF 09005676190 (**cobas e** 801 analyzer; y), with the Elecsys Calcitonin assay, REF 07027044190 (**cobas e** 801 analyzer; x), gave the following correlations (pg/mL):

Number of samples measured: 124

Passing/Bablok[12]	Linear regression
y = 1.02x + 0.430	y = 1.01x - 1.23
τ = 0.983	r = 0.999

The sample concentrations were between 0.572 and 1913 pg/mL.

b) A comparison of the Elecsys Calcitonin assay, REF 09005676190 (**cobas e** 402 analyzer; y), with the Elecsys Calcitonin assay, REF 09005676190 (**cobas e** 801 analyzer; x), gave the following correlations (pg/mL):

Number of samples measured: 127

Passing/Bablok[12]	Linear regression
y = 1.00x - 0.024	y = 0.995x + 2.15
τ = 0.984	r = 1.00

The sample concentrations were between 0.693 and 1996 pg/mL.

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Analytical specificity
The following cross-reactivities were found, tested with hCT concentrations of approximately 8 pg/mL:

Cross-reactant	Concentration tested ng/mL	Cross-reactivity %
Salmon Calcitonin	100	0.000
Porcine Calcitonin	500	0.000
Chicken Calcitonin	500	0.000
ACTH (1-39) human	100	0.000
C-Peptide	40000	0.000
Calcitonin Gene Related Peptide	1000	0.000
PTH (1-84) human	150	0.000
TSH	175	0.000
Insulin	33500	0.000
Prolactin	1000	0.000
Gastrin I	2000	0.000
Elcatonin	100000	0.000
Katacalcin	40000	0.000

References

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5 Machens A, Hoffmann F, Sekulla C, et al. Importance of gender-specific calcitonin thresholds in screening for occult sporadic medullary thyroid cancer. Endocr Relat Cancer 2009;16:1291-1298.

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10 Kratzsch J, Petzold A, Raue F, et al. Basal and stimulated calcitonin and procalcitonin by various assays in patients with and without medullary thyroid cancer. Clin Chem 2011;3:467-474.

11 Kudo T, Miyauchi A, Ito Y, et al. Serum calcitonin levels with calcium loading tests before and after total thyroidectomy in patients with thyroid diseases other than medullary thyroid carcinoma. Endocr J 2011;58(3):217-221.

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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

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 for reconstitution
GTIN	Global Trade Item Number

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