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# Calcitonin

cobas®

**hCT - human calcitonin**

| REF | Σ | SYSTEM |
|---|---|---|
| **06445853** 190 | 100 | Elecsys 2010<br>MODULAR ANALYTICS E170<br>**cobas e** 411<br>**cobas e** 601<br>**cobas e** 602 |

## English

### 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 Elecsys and **cobas e** immunoassay analyzers.

### Summary

Human calcitonin (hCT) is a 32 amino acid peptide hormone with a molecular mass of 3418 daltons 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]

Elevated levels of hCT can be found in various pathological conditions e.g. medullary thyroid carcinoma, a tumor of the calcitonin secreting cells of the thyroid. It is also frequently elevated in leukemic and myeloproliferative disorders[7] or may be produced ectopically in tumors like lung (small cell lung cancer) or breast cancer.[8,9,10] Furthermore elevated levels can also be found in conjunction with hyperparathyroidism, hypergastrinemia, renal failure and chronic inflammatory disease.[3,11]

The most prominent clinical syndrome associated with a disordered hypersecretion of hCT is the medullary thyroid carcinoma (MTC), which comprises 5-10 % of all thyroid cancers. It may occur sporadically or in a familial form that is inherited as an autosomal dominant trait. Furthermore, calcitonin measurements can also be used to monitor the efficacy of therapy in patients with calcitonin producing tumors.[12,13] In a small but increasing percentage of patients, however, basal hormone levels are indistinguishable from normal. Some of these subjects represent the early stages of C-cell neoplasia or hyperplasia that are most amenable to surgical cure. To identify these patients in an early disease stage, provocative tests for hCT secretion are necessary to exclude false negative diagnosed patients. Most tumors respond with an increase of hCT levels after administration of calcium or pentagastrin or the combination of both.[14, 15]

The Elecsys Calcitonin assay employs monoclonal antibodies specifically directed against hCT. The antibodies labeled with ruthenium complex[a)] consist of mouse-specific components.

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

### Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 50 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/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.

### Reagents - working solutions

The reagent rackpack is labeled as hCT.

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

R1 Anti-hCT-Ab~biotin (gray cap), 1 bottle, 8 mL:
Biotinylated monoclonal anti-hCT antibody (mouse) 1.50 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

R2 Anti-hCT-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 8 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.
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, $K_2$-EDTA and $K_3$-EDTA plasma as well as Li-heparin plasma tubes containing separating gel.

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 ≤ ± 2x Limit of Blank (LoB) + coefficient of correlation ≥ 0.95.

Stable for 4 hours at 20-25 °C, 1 day at 2-8 °C, 3 months at -20 °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.

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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 06445861190, Calcitonin CalSet, for 4 x 1.0 mL
- REF 05618860190, PreciControl Varia, for 2 x 3 mL each of PreciControl Varia 1 and 2
- REF 03609987190, Diluent MultiAssay, 2 x 16 mL sample diluent
- 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 the IRP WHO Reference Standard 89/620.

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 2 months (56 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 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 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 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 assay is unaffected by icterus (bilirubin < 1128 µmol/L or < 66 mg/dL), hemolysis (Hb < 0.124 mmol/L or < 0.2 g/dL), lipemia (Intralipid < 2000 mg/dL), biotin (< 163 nmol/L or < 40 ng/mL), IgG < 4 g/dL, IgA < 1.6 g/dL and IgM < 0.7 g/dL.

Criterion: Recovery within ± 10 % of initial value for samples > 10 pg/mL or ± 1 pg/mL of initial value for samples ≤ 10 pg/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 1200 IU/mL.

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

In vitro tests were performed on 17 commonly used and 12 special pharmaceuticals. No interference with the assay was found.

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 |
| Levothyroxine | 0.25 |
| Carbimazol | 30 |
| Thiamazol | 80 |
| Propylthiouracil | 60 |
| Perchlorat | 2000 |
| Propranolol | 240 |
| Amiodaron | 200 |
| Prednisolon | 100 |
| Hydrocortison | 200 |
| Fluocortolon | 100 |
| Octreotid | 0.3 |

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.

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

Limit of Blank = 0.3 pg/mL

Limit of Detection = 0.5 pg/mL

Limit of Quantitation = 1 pg/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-A requirements.

The Limit of Blank is the 95$^{th}$ 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 relative error of ≤ 30 %.

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

**Expected values**

Upper limits of reference ranges are provided as the 97.5$^{th}$ percentile.

| Cohort | N | 97.5$^{th}$ percentile | Lower limit of 95 % confidence interval | Upper limit of 95 % confidence interval |
|---|---|---|---|---|
| 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 |

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 pg/mL | SD pg/mL | CV % | SD pg/mL | CV % |
| Human serum 1 | 1.01 | 0.034 | 3.4 | 0.052 | 5.2 |
| Human serum 2 | 11.5 | 0.345 | 3.0 | 0.413 | 3.6 |
| Human serum 3 | 48.5 | 1.24 | 2.5 | 1.71 | 3.5 |
| Human serum 4 | 482 | 13.8 | 2.9 | 19.2 | 4.0 |
| Human serum 5 | 1910 | 42.6 | 2.2 | 65.0 | 3.4 |
| PreciControl Varia 1 | 8.88 | 0.191 | 2.1 | 0.261 | 2.9 |
| PreciControl Varia 2 | 97.7 | 1.44 | 1.5 | 2.51 | 2.6 |

| MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers | | | | | |
|---|---|---|---|---|---|
| | | **Repeatability** | | **Intermediate precision** | |
| Sample | Mean pg/mL | SD pg/mL | CV % | SD pg/mL | CV % |
| Human serum 1 | 4.19 | 0.060 | 1.4 | 0.082 | 2.0 |
| Human serum 2 | 45.4 | 0.794 | 1.8 | 1.02 | 2.3 |
| Human serum 3 | 456 | 6.17 | 1.4 | 8.46 | 1.9 |
| Human serum 4 | 907 | 14.0 | 1.5 | 19.8 | 2.2 |
| Human serum 5 | 1613 | 25.9 | 1.6 | 34.7 | 2.2 |
| PreciControl Varia 1 | 8.87 | 0.128 | 1.4 | 0.146 | 1.6 |
| PreciControl Varia 2 | 93.7 | 1.67 | 1.8 | 1.87 | 2.0 |

**Method comparison**

A comparison of the Elecsys Calcitonin assay (y) with a commercially available method (x) using clinical samples gave the following correlations:

Number of samples measured: 248

| Passing/Bablok[16] | Linear regression |
|---|---|
| y = 0.970x - 0.133 | y = 1.12x - 1.91 |
| τ = 0.911 | r = 0.977 |

The sample concentrations were between 0.600 and 1866 pg/mL.

**Analytical specificity**

The following cross-reactivities were found, tested with hCT concentrations of 9.11 and 468 pg/mL:

| **Cross-reactant** | **Concentration tested ng/mL** | **Cross-reactivity %** |
|---|---|---|
| Salmon Calcitonin | 100 | 0.017 |
| Porcine Calcitonin | 500 | 0.007 |
| Chicken Calcitonin | 500 | 0.005 |
| ACTH (1-39) human | 100 | 0.037 |
| C-Peptide | 40000 | 0.000 |
| Calcitonin Gene Related Peptide | 1000 | 0.002 |
| PTH (1-84) human | 150 | 0.013 |
| TSH | 1000 µIU/mL | 0.009 |
| Insulin | 33500 | 0.000 |
| Prolactin | 1000 | 0.001 |
| Gastrin I | 2000 | 0.001 |
| Elcatonin | 100000 | 0.000 |

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| Cross-reactant | Concentration tested ng/mL | Cross-reactivity % |
|---|---|---|
| Katacalcin | 40000 | 0.055 |

**References**


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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.

| | |
|---|---|
| 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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