Elecsys PTH

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REF	📖	∇ Σ	SYSTEM
			cobas e 411
11972103122	11972103500	100	**cobas e** 601
			cobas e 602

English

System information
For **cobas e** 411 analyzer: test number 680
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 126

Intended use
Immunoassay for the in vitro quantitative determination of intact parathyroid hormone in human serum and plasma for the differential diagnosis of hypercalcemia and hypocalcemia. This assay can be used intraoperatively.

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

Summary
Parathyroid hormone (PTH) is a single-chain 84-amino-acid peptide produced by the parathyroid glands in response to decreased extracellular concentrations of ionized calcium. Its main role is to increase serum calcium levels by stimulating the release of calcium from bone and its renal re-absorption in the distal tubule. In the proximal tubule, PTH stimulates the synthesis of calcitriol which in turn increases intestinal absorption of calcium and exerts an endocrine feed-back on the secretion of PTH at the parathyroid level. PTH also decreases the renal re-absorption of phosphate in the proximal tubule, thereby decreasing serum phosphate.[1]

Parathyroid gland disorders lead to elevated or depressed blood calcium levels (hypercalcemia or hypocalcemia) brought about by a change in the secretion of PTH.

Detection of subfunctioning parathyroid glands (hypoparathyroidism) requires the use of a highly sensitive test in order to be able to measure PTH levels well below normal. Hyperfunctioning of the parathyroid glands results in an increased secretion of PTH (hyperparathyroidism). Primary causes are adenomas of the parathyroid glands. In secondary hyperparathyroidism the blood calcium level is low as a result of other pathological states (e.g. vitamin D deficiency).[2]

The determination of PTH intraoperatively during adenoma resection in the parathyroid glands has been reported for primary hyperparathyroidism,[3,4] secondary hyperparathyroidism relating to renal failure,[5,6] and tertiary hyperparathyroidism post renal transplant surgery.[7] Because PTH has a reported half-life of 3-5 minutes,[8] a significant drop in PTH levels after resection of the abnormal gland or glands enables the surgeon to assess whether all hyperfunctioning parathyroid tissue has been removed from the patient.[9]

The National Academy of Clinical Biochemistry recommends routine use of intraoperative PTH testing for patients undergoing surgery for primary hyperparathyroidism, both in initial surgeries and in reoperative procedures.[10]

The Kidney Disease Outcomes Quality Initiative (KDOQI) and Kidney Disease Improving Global Outcomes (KDIGO) guidelines recommend that serum PTH concentration should be measured regularly in patients with Chronic Kidney Disease (CKD) and maintained within the target ranges that are defined according to the stage of CKD.[11,12]

The Elecsys assay for determining intact PTH employs a sandwich test principle in which a biotinylated monoclonal antibody reacts with the N-terminal fragment (1-37) and a monoclonal antibody labeled with a ruthenium complex[a] reacts with the C-terminal fragment (38-84).

The antibodies used in this assay are reactive with epitopes in the amino acid regions 26-32 and 37-42.

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 PTH-specific antibody, and monoclonal PTH-specific antibody labeled with a ruthenium complex 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 or e-barcode.

Reagents - working solutions
The reagent rackpack is labeled as PTH.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:

Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-PTH-Ab~biotin (gray cap), 1 bottle, 7 mL:

Biotinylated monoclonal anti-PTH antibody (mouse) 2.3 mg/L; phosphate buffer 100 mmol/L, pH 7.0; preservative.

R2 Anti-PTH-Ab~Ru(bpy)$_3^{2+}$ (black cap), 1 bottle, 7 mL:

Monoclonal anti-PTH antibody (mouse) labeled with ruthenium complex 2.0 mg/L; phosphate 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.

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.

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	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 or tubes containing separating gel.

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

Because of the instability of PTH in unseparated serum, serum tubes should be centrifuged immediately. In contrast, PTH was found to be stable for > 24 hours at room temperature in whole blood anticoagulated with EDTA. Therefore, preference should be given to EDTA plasma.[13,14]

Criterion: Method comparison serum versus plasma, slope 0.9-1.1 + intercept within ≤ ± 3 pg/mL + coefficient of correlation ≥ 0.95.

Serum: Stable for 8 hours at 15-25 °C, 2 days at 2-8 °C, 6 months at -20 °C (± 5 °C).

Plasma: Stable for 2 days at 15-25 °C, 3 days at 2-8 °C, 6 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 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 11972219122, PTH CalSet, for 4 x 1.0 mL
- REF 08243875190, CalSet PTH, for 4 x 1.0 mL
- REF 05618860190, PreciControl Varia, for 4 x 3 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 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- 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

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.

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 a commercial PTH test (RIA). The recovery of the NIBSC 95/646 (WHO) standard was assessed by testing dilutions in human serum covering the measuring range (40-4000 pg/mL) on 16 analyzers (**cobas e** 411 and **cobas e** 601 analyzers). The mean recovery was 100 % ± 4 %.

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 12 weeks 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.

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).

Conversion factors:

pg/mL x 0.106 = pmol/L

pmol/L x 9.43 = 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	≤ 1129 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.093 mmol/L or ≤ 150 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 205 nmol/L or ≤ 50 ng/mL
Rheumatoid factors	≤ 1200 IU/mL

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Compound	Concentration tested
Albumin	≤ 70 g/L

Criterion: Recovery of ± 1.5 pg/mL of initial value for samples ≤ 15 pg/mL and within ± 10 % of initial value for samples > 15 pg/mL.

The assay is affected by hemolysis > 150 mg/dL. Do not analyze samples that show visible signs of hemolysis.

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.

There is no high-dose hook effect at PTH concentrations up to 17000 pg/mL (1802 pmol/L).

In vitro tests were performed on 16 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.

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
1.20-5000 pg/mL or 0.127-530 pmol/L (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 1.20 pg/mL (< 0.127 pmol/L). Values above the measuring range are reported as > 5000 pg/mL (> 530 pmol/L).

Lower limits of measurement
Lower detection limit of the test

Lower detection limit: 1.20 pg/mL (0.127 pmol/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
Not necessary due to the broad measuring range.

Expected values
15-65 pg/mL (1.6-6.9 pmol/L)[15,16]

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 in 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:

cobas e 411 analyzer							
			Repeatability			Intermediate precision	
Sample	Mean		SD		CV	SD	CV
	pg/mL	pmol/L	pg/mL	pmol/L	%	pg/mL	pmol/L	%
HS[b] 1	26.7	2.83	0.711	0.075	2.7	1.73	0.184	6.5
HS 2	52.5	5.56	0.853	0.091	1.6	2.07	0.220	3.9
HS 3	261	27.7	4.0	0.424	1.5	7.81	0.829	3.0

b) HS = human serum

cobas e 601 and cobas e 602 analyzers					
	Repeatability				
Sample	Mean		SD		CV
	pg/mL	pmol/L	pg/mL	pmol/L	%
HS 1	21.9	2.32	0.44	0.05	2.0
HS 2	35.0	3.71	0.43	0.05	1.2
HS 3	123	13.04	1.31	0.14	1.1

cobas e 601 and cobas e 602 analyzers					
	Intermediate precision				
Sample	Mean		SD		CV
	pg/mL	pmol/L	pg/mL	pmol/L	%
HS 1	23.2	2.46	0.79	0.08	3.4
HS 2	80.9	8.58	2.01	0.21	2.5
HS 3	240	25.4	6.72	0.71	2.8

Precision was determined using Elecsys reagents and controls in a separate study according to 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		SD		CV	SD	CV
	pg/mL	pmol/L	pg/mL	pmol/L	%	pg/mL	pmol/L	%
PC[c] Varia 1	54.6	5.79	0.657	0.070	1.2	1.11	0.118	2.0
PC Varia 2	182	19.3	2.43	0.258	1.3	3.14	0.333	1.7

c) PC = PreciControl

cobas e 601 and cobas e 602 analyzers							
			Repeatability			Intermediate precision	
Sample	Mean		SD		CV	SD	CV
	pg/mL	pmol/L	pg/mL	pmol/L	%	pg/mL	pmol/L	%
PC Varia 1	54.7	5.80	0.943	0.100	1.7	0.977	0.104	1.8
PC Varia 2	184	19.5	2.65	0.281	1.4	3.11	0.330	1.7

Method comparison
A comparison of the Elecsys PTH assay (y) with a commercial PTH test (x) using clinical samples gave the following correlations (pg/mL):

Number of samples measured: 152

Passing/Bablok[17]	Linear regression
y = 1.01x + 4.86	y = 0.83x + 19.4
τ = 0.886	r = 0.991

The sample concentrations were between 1.4 and 1880 pg/mL (0.15 and 199 pmol/L).

Analytical specificity
No cross-reactivities were found for: Osteocalcin, PTH fragment 1-37, PTH-related protein (1-86), bone-specific alkaline phosphatase, and β-CrossLaps.

The assay has a 99 % cross-reactivity to the PTH fragment 7-84.

Functional sensitivity
6.0 pg/mL (0.64 pmol/L)

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

Clinical investigations in intraoperative use

In 2006, the National Academy of Clinical Biochemistry published their Laboratory Medicine Practice Guidelines for point of care testing, entitled Evidence Based Practice for Point of Care Testing.[10] The guidelines recommend the use of intraoperative parathyroid hormone testing 1) for patients undergoing surgery for hyperparathyroidism, especially in minimally invasive or directed procedures, 2) for patients undergoing reoperation, and 3) as a replacement for traditional laboratory measurements of PTH during venous localization in order to help the angiography team guide sampling.

The guidelines further recommend for patients undergoing parathyroidectomy for hyperparathyroidism that baseline samples be obtained preoperation exploration and pre-excision of the gland, and that post-excision sampling be drawn at 5 and 10 minutes post resection with a 50 % reduction in PTH concentrations from the highest baseline level. The guidelines also caution that additional samples may be necessary.[10]

PTH testing during parathyroid surgery was conducted by several groups of investigators using the Elecsys PTH immunoassay.[4,5,6,7,18]

The overall sensitivity and specificity of the assay to demonstrate successful surgery as defined by postoperative reduction of calcium levels was 99.6 % and 93.7 %, respectively.

References

1 Souberbielle JC, Roth H, Fouque D. Parathyroid hormone measurement in CKD. Kidney Int 2010;77:93–100.

2 Nussbaum S, Potts JT. Advances in Immunoassays for Parathyroid Hormone. Clinical Applications to Skeletal Disorders of Bone and Mineral Metabolism. In Bilezikian JP, Levine MA, Marcus R (eds). The Parathyroids: Basic an Clinical Concepts. Raven Press, New York 1994:157-169.

3 Bergenfelz A, Nordén NE, Ahrén B. Intraoperative fall in plasma levels of intact parathyroid hormone after removal of one enlarged parathyroid gland in hyperthyroid patients. Eur J Surg 1991;157:109-112.

4 Ohe MN, Santos RO, Kunii IS, et al. Usefulness of a rapid immunometric assay for intra-operative parathyroid hormone measurements. Braz J Med Biol Res 2003;36(6):715-721.

5 Seehofer D, Rayes N, Ulrich F, et al. Intra-operative measurement of intact parathyroid hormone in renal hyperparathyroidism by an inexpensive routine assay. Langenbecks Arch Surg 2001;386(6):440-443.

6 Seehofer D, Rayes N, Klupp J, et al. Predictive value of intact parathyroid hormone measurement during surgery for renal hyperparathyroidism. Langenbecks Arch Surg 2005;390(3):222-229.

7 Haustein SV, Mack E, Starling JR, et al. The role of intra-operative parathyroid hormone testing in patients with tertiary hyperparathyroidism after renal transplantation. Surgery 2005;138(6):1066-1071.

8 Maier GW, Kreis ME, Renn W, et al. Parathyroid hormone after adenectomy for primary hyperparathyroidism: A study of peptide hormone elimination kinetics in humans. Jour Clin Endocrinol Metab 1998;83(11):3853-3856.

9 Carter AB, Howanitz TJ. Intra-operative testing for parathyroid hormone: a comprehensive review of the use of the assay and the relevant literature. Arch Pathol Lab Med 2003;127:1424-1442.

10 Nichols JH, Christenson RH, Clarke W, et al. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines: Evidence Based Practice for Point of Care Testing. AACC Press:2006.

11 National Kidney Foundation. K/DOQI clinical practice guidelines for bone metabolism and diseases in chronic kidney disease. Am J Kidney Dis 2003;42:S1-201.

12 KDIGO – Kidney Disease Improving Global Outcomes KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder (CKDMBD). Kidney International 2009;76(Suppl 113).

13 Hanon EA, Sturgeon CM, Lamb EJ. Sampling and storage conditions influencing the measurement of parathyroid hormone in blood samples: a systematic review. Clin Chem Lab Med. 2013;51(10):1925-1941.

14 Stokes FJ, Ivanov P, Bailey LM, et al. The Effects of Sampling Procedures and Storage Conditions on Short-term Stability of Blood-Based Biochemical Markers of Bone Metabolism. Clin Chem 2011;57(1):138-140.

15 Blind E. Measurement of Intact Parathyroid Hormone by an Extracting Two-Site Immunometric Assay. In: Schmidt-Gayk H, Armbruster FP, Bouillon R, (eds). Calcium regulating hormones, vitamin D metabolites, an cyclic AMP. Heidelberg: Springer 1990:151.

16 Thomas L. Parathyroid hormone (PTH). Clinical Laboratory Diagnosis. TH-Books, Frankfurt. 1st english edition 1998:248-250.

17 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.

18 Ohe MN, Santos RO, Kunii IS, et al. Usefulness of intra-operative PTH measurement in primary and secondary hyperparathyroidism: experience with 109 patients. Arq Bras Endocrinol Metab 2006;50(5):869-875.

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

CE

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