Elecsys PTH STAT

cobas®

REF	Σ	SYSTEM
04892470 160	100	**cobas e** 411 **cobas e** 601 **cobas e** 602

English

For use in the USA only

System information

For **cobas e** 411 analyzer: test number 840
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 221

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 formed in the parathyroid glands and secreted into the blood stream. Intact PTH consists of a single polypeptide chain containing 84 amino acids and has a molecular weight of approximately 9500 daltons. The biologically active N-terminal fragment has a half-life of only a few minutes. Selective measurement of the (mainly) intact parathyroid hormone permits direct ascertainment of the secretory activity of the parathyroid glands.[1,2] PTH, together with vitamin D and calcitonin, brings about mobilization of calcium and phosphate from the skeletal system and increases the uptake of calcium in the intestine and the excretion of phosphate via the kidneys. The constancy of the blood calcium level is ensured by the interaction of PTH and calcitonin. The secretion of PTH is inhibited by high calcium concentrations and promoted by low calcium concentrations. 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.[3,4]
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).
Today, great significance is attached to the determination of the PTH and calcium concentrations when assessing hyperparathyroidism. The determination of PTH intraoperatively during adenoma resection in the parathyroid glands has also been reported for primary hyperparathyroidism,[5,6,7] secondary hyperparathyroidism relating to renal failure,[8,9] and tertiary hyperparathyroidism post renal transplant surgery.[10] Because PTH has a reported half life of 3-5 minutes,[11] a significant drop in PTH levels after resection of the abnormal gland or glands enables the surgeon to assess the completeness of resection and whether all hyperfunctioning parathyroid tissue has been removed from the patient.[12] The NACB (National Academy of Clinical Biochemistry) guidelines recommend that baseline samples be obtained preoperation and pre-excision of the suspected hyperfunctioning gland.[13] Specimens for PTH testing should be drawn at 5 and 10 minutes post resection and that a > 50 % reduction in PTH levels from the highest baseline be used as criteria for surgical success. Additional samples may be necessary as it has been shown that sensitivity can increase with time.[14] Failure of PTH to drop below recommended levels indicates that either 1) residual hyperfunctioning tissue is still present and further exploration may be necessary, as was in the case of two patients, both with a fifth ectopic parathyroid gland requiring further surgery,[7] or 2) a spike in PTH levels during adenoma mobilization occurred.[15] Intraoperative PTH measurements offer fast, reliable assessment when all hyperfunctioning parathyroid tissue has been removed during the surgical process.
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: 9 minutes

cobas e 411 analyzer:

- 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.

cobas e 601 and **cobas e** 602 analyzers:

- During a 9 minute incubation, antigen in the sample (50 µL), a biotinylated monoclonal PTH-specific antibody, a monoclonal PTH-specific antibody labeled with a ruthenium complex and streptavidin-coated microparticles react to form a sandwich complex, which is bound to the solid phase.

All analyzers:

- 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 Elecys PTH-STAT.

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.

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

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.

Elecsys PTH STAT

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.
K_2-EDTA and K_3-EDTA plasma.

Because of the short half-life of PTH, it is recommended that, when serum is needed, the blood should be centrifuged immediately.

Preference should be given to EDTA plasma, as it is stable longer than serum.

Criterion: Method comparison serum versus plasma, slope 0.9-1.1 + intercept within < ± 2x analytical sensitivity (LDL) + 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).

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.

The claims, including those pertaining to sample stability made in the labeling of the cleared/approved reagents of Roche Diagnostics are part of the clearance of the overall IVD test system (assay). Sample stability was tested only for the temperatures/time frame as claimed by the manufacturer under the conditions claimed in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 05618860160, PreciControl Varia, for 6 x 3 mL
- REF 04894138190, PTH STAT CalSet, for 4 x 1 mL or
  REF 08243930190, CalSet PTH STAT, for 4 x 1 mL
- General laboratory equipment
- **cobas e** analyzer

Accessories for **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

Accessories 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

Accessories for all analyzers:

- REF 11298500160, 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 prior to use and the reading in of the test-specific parameters via the reagent barcode take place automatically. No manual input is necessary. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers (except for the **cobas e** 602 analyzer).

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 Elecsys PTH (REF 11972103). This in turn was 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).

Elecsys PTH STAT

cobas®

Conversion factors: pg/mL x 0.106 = pmol/L
pmol/L x 9.43 = pg/mL

Limitations - interference

Do not analyze samples that show visible signs of hemolysis.

The assay is affected by hemolysis ≥ 0.25 g/dL. The assay is unaffected by icterus (bilirubin < 1112 µmol/L or < 65 mg/dL), lipemia (Intralipid < 1500 mg/dL), and biotin (< 205 nmol/L or < 50 ng/mL).

Criterion: Recovery within ± 10 % 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 1500 IU/mL.

There is no high-dose hook effect at PTH concentrations of 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[16,17]

15.0-65.0 pg/mL (1.60-6.90 pmol/L)

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). 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	53.4	5.66	1.10	0.117	2.1	2.05	0.217	3.8
HS 2	215	22.8	3.56	0.377	1.7	5.93	0.628	2.8
HS 3	980	104	16.4	1.74	1.7	24.1	2.55	2.5

b) HS = human serum

Precision was determined using Elecsys reagents, pooled human sera 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 0	17.6	1.87	0.388	0.041	2.2	0.711	0.075	4.0
PC Varia 1	48.4	5.13	0.801	0.085	1.7	1.10	0.117	2.3
PC Varia 2	164	17.4	2.55	0.270	1.6	3.07	0.325	1.9

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	%
HS 1	2.47	0.262	0.243	0.025	9.8	0.406	0.043	16.5
HS 2	47.4	5.02	1.19	0.126	2.5	1.29	0.137	2.7
HS 3	255	27.0	4.26	0.452	1.7	5.61	0.595	2.2
HS 4	522	55.3	10.2	1.08	2.0	10.9	1.16	2.1
HS 5	3856	409	84.6	8.97	2.2	97.1	10.3	2.5
PC Varia 0	14.1	1.49	0.382	0.041	2.7	0.714	0.076	5.1
PC Varia 1	38.4	4.07	0.817	0.087	2.1	0.983	0.104	2.6
PC Varia 2	141	14.9	2.42	0.257	1.7	3.22	0.341	2.3

Method comparison

A comparison of the Elecsys PTH STAT assay (y) with the Elecsys PTH assay (x) - performed on the Elecsys 2010 analyzer - using clinical samples gave the following correlations (pg/mL):

Number of samples measured: 159

Passing/Bablok[18]	Linear regression
y = 1.047x + 0.314	y = 1.047x - 0.237
τ = 0.984	r = 0.998

The sample concentrations were between 1.97 and 1394 pg/mL (0.21 and 148 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.

Functional sensitivity

6.00 pg/mL (0.640 pmol/L)

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.[13] 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.[13]
PTH testing during parathyroid surgery was conducted by several groups of investigators using the Elecsys PTH immunoassay.[6,7,8,9,10] 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 Silverman R, Yalow RS. Heterogeneity of parathyroid hormone: Clinical and physiologic implications. J Clin Invest 1973;52:1958-1971.

2 Flentje D, Schmidt-Gayk H, Fischer S, et al. Intact parathyroid hormone in primary hyperparathyroidism. Br J Surg 1990;77:168-172.

3 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.

4 Berson SA, Yalow RS, Aurbach GD, et al. Immunoassay of bovine and human parathyroid hormone. Proc Natl Acad Sci USA 1963;49:613-617.

5 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.

6 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.

7 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.

8 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.

9 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.

10 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.

11 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.

12 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.

13 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.

14 Bergenfelz A, Isaksson A, Lindblom P, et al. Measurement of parathyroid hormone in patients with primary hyperparathyroidism undergoing first and reoperative surgery. Br J Surg 1998;85:1129-1132.

15 Yang GP, Levine S, Weigel RJ. A spike in parathyroid hormone during neck exploration may cause a false-negative intra-operative assay result. Arch Surg 2001;136:945-949.

16 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.

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

18 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).

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.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

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.

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

Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support 1-800-428-2336