Elecsys β-CrossLaps/serum

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REF		Σ	SYSTEM
			cobas e 411
09005773190	09005773500	100	**cobas e** 601
			cobas e 602

English

System information

For **cobas e** 411 analyzer: test number 670
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 127

Intended use

Immunoassay for the in vitro quantitative determination of degradation products of type I collagen in human serum and plasma as an aid in assessing bone resorption. The test may be used as an aid in monitoring antiresorptive therapies in osteoporotic patients.

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

Summary

Type I collagen is an important component of the bone matrix and its degradation products are the most commonly used bone resorption markers.[1]

During normal bone metabolism, mature type I collagen is degraded and small fragments pass into the circulation and are excreted via the kidneys.

In physiologically or pathologically elevated bone resorption (e.g. in old age or as a result of osteoporosis), type I collagen is degraded to an increased extent, and there is a commensurate rise in the level of collagen fragments in the blood.

Especially relevant fragments are the β-isomerized C (carboxy)-terminal cross-linking telopeptides (β-CTx), produced by osteoclastic hydrolysis of type I collagen.[1,2,3]

Elevated serum levels of isomerized C-terminal telopeptides of type I collagen have been reported for patients with increased bone resorption. The serum levels return to normal during anti-resorptive therapy.[4,5,6,7]

Determination of the C-terminal telopeptides in serum is recommended for monitoring the efficacy of antiresorptive therapy (e.g. bisphosphonates or hormone replacement therapy - HRT) in osteoporosis or other bone diseases. By these means, therapy-induced changes can be demonstrated after just a few months.[8,9]

Serum CTx has been selected by the IOF-IFCC Bone Marker Standards Working Group as marker for bone resorption, mainly based on the following criteria:

- It has been evaluated both for fracture prediction and monitoring osteoporosis therapies.
- The assay is widely available, for serum or plasma samples, with well documented requirements for sample handling and stability.
- The analyte is well characterized and allows the development of clearly defined reference standard.[1]

The Elecsys β-CrossLaps/serum assay is specific for crosslinked β-isomerized type I collagen fragments, independent of the nature of the crosslink (e.g. pyrrole, pyridinolines, etc.).[10] The assay specificity is guaranteed through the use of two monoclonal antibodies each recognizing linear β-8AA octapeptides (EKAHD-β-GGR). The Elecsys β-CrossLaps/serum assay therefore quantifies all type I collagen degradation fragments that contain the isomerized octapeptide β-8AA twice (β-CTx).[6,7]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 50 µL of sample and a biotinylated monoclonal anti-β-CrossLaps antibody are incubated together; the antigen in the sample is liberated from the serum components.
- 2nd incubation: Following addition of streptavidin-coated microparticles and a monoclonal β-CrossLaps-specific antibody labeled with a ruthenium complex[a)], a sandwich complex is formed which 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 CROSSL.

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

R1 Anti-β-CrossLaps-Ab~biotin (gray cap), 1 bottle, 10 mL:
Biotinylated monoclonal anti-β-CrossLaps antibody (mouse) 2.5 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

R2 Anti-β-CrossLaps-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 8 mL:
Monoclonal anti-β-CrossLaps antibody (mouse) labeled with ruthenium complex 2.4 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.

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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 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.

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

Li heparin plasma tubes containing separating gel can be used.

Criterion: Slope 1.00 ± 0.10 + intercept within ≤ ± 0.02 ng/mL + coefficient of correlation ≥ 0.95.

It is recommended to draw blood as fasting, morning samples. For long-term investigations, the samples should always be taken under same conditions as the baseline sample, as the serum β-CTx concentration is to some extent subject to a circadian rhythm.

Preference should be given to K_2- or K_3-EDTA plasma, as it is stable longer than serum.

Stability of serum: 6 hours at 20-25 °C, 8 hours at 2-8 °C.

Stability of Li-heparin plasma: 4 hours at 20-25 °C, 8 hours at 2-8 °C.

Stability of K_2- and K_3-EDTA plasma: 24 hours at 20-25 °C, 8 days at 2-8 °C.

Serum, heparinized and EDTA plasma are stable for 3 months at -20 °C (± 5 °C). For longer periods, store at -80 °C (± 10 °C). Freeze only once.

Hemolyzed samples (Hb > 0.5 g/dL) elicit a decrease in the β-CTx concentration.

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 11972316122, β-CrossLaps CalSet, for 4 x 1.0 mL
- REF 05618860190, PreciControl Varia, for 4 x 3.0 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

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 reference standards precisely defined by weighing out synthetic peptide.

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 8 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.

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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 ng/mL or 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	≤ 1112 µmol/L or ≤ 65 mg/dL
Hemoglobin	≤ 0.3 mmol/L or ≤ 500 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1000 IU/mL

Criterion: For concentrations ≤ 0.50 ng/mL the deviation is ≤ 0.05 ng/mL. For concentrations > 0.50 ng/mL the deviation is ≤ ± 10 %.

There is no high-dose hook effect at β-CTx concentrations up to 150 ng/mL (150000 pg/mL).

Pharmaceutical substances

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

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

Special drugs

Drug	Concentration tested mg/L
Ibandronate	6
Actonel (Risedronate)	150
Vitamin D3	0.075
Calcium Carbonate	2500
Vitamin D (25-OH)	1
17-β-Estradiol	2.5
β-Estradiol-17-valerate	2.5
β-Estradiol-3-sulfate	2.5

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.

Results may be confounded by clinical conditions known to affect bone resorption, e.g. hyperparathyroidism or hyperthyroidism.

Caution should be exercised when measuring serum β-CTx levels in patients with reduced renal function as this may lead to reduced excretion of serum β-CTx and a consequent increase in the apparent serum CTx levels is seen.[11]

There is evidence that β-CTx can predict loss of bone density.[12] However, a correlation with increased fracture risk was not yet demonstrated. The properties of β-CTx in case of hyperparathyroidism or hyperthyroidism have not yet been unequivocally described, either.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

They should not be used as a sole determinant for executing or modifying an existing treatment regimen.

Limits and ranges

Measuring range

0.01-6.00 ng/mL or 10-6000 pg/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.01 ng/mL (< 10 pg/mL). Values above the measuring range are reported as > 6.00 ng/mL (> 6000 pg/mL).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.008 ng/mL

Limit of Detection = 0.01 ng/mL

Limit of Quantitation = 0.05 ng/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

Not necessary due to the broad measuring range.

Expected values

1. Healthy subjects

The following 95 % reference intervals (RI) for serum βCTx in 1039 healthy men, and in 1029 healthy premenopausal and postmenopausal women (age range 24-76 years) were obtained from a Danish study with the Elecsys β-CrossLaps/serum assay. Subjects included were characterized by their history of osteoporosis and lifestyle, and women by their menopausal state and by taking no anti-osteoporotic medication. Based on the patterns in the sex-, age-, and menopause-stratified 95 % RIs, subjects were grouped into age intervals for each gender, and into pre-menopausal and postmenopausal.[13] Other studies have demonstrated, that ranges can differ between ethnicities and geographical locations.[14,15] Thus, measurements should be compared to reference intervals established on material from similar geographical regions and should reflect the same gender, age, and pre-/post-menopausal status.

The table shows the geometric means and 95% reference intervals of healthy male and female subjects after stratification for age, and for menopause of female subjects from a Danish study.[13]

Age range (years)	Men			Women		
	N	GM[b)] (pg/mL)	95 % RI (pg/mL)	N	GM (pg/mL)	95 % RI (pg/mL)
< 29.9	39	492	238-1019	58	378	148-967
30-39.9	80	459	225-936	111	308	150-635
40-49.9	234	382	182-801	257	296	131-670
50-59.9	248	345	161-737	281	440	183-1060
60-69.9	303	316	132-752	234	408	171-970
>70	135	302	118-776	88	362	152-858
Pre-menopause	-	-	-	449	306	136-689
Post-menopause	-	-	-	578	424	177-1015

b) Geometric mean

Intra-individual variance and least significant change (LSC)

The intra-individual variance of βCTx was determined in a subset of 18 healthy postmenopausal women (mean βCTx at baseline 0.516 ± 0.217 ng/mL) at 5 time points over 3 months. The median intra-

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individual variability as expressed by the mean CVi (intra-individual coefficient of variation) for serum βCTx values was 9.4 % (range, 4.1-27 %). On the basis of this CVi, the least significant change (LSC) was determined to be 27 %, meaning that an individual should display a ≥ 27 % decrease of serum βCTx concentrations when receiving antiresorptive therapy to have a < 5 % chance ($p < 0.05$) of the decrease being the result of random variation in marker concentration.[16]

2. Monitoring during antiresorptive therapy

Detecting changes of serum βCTx concentrations is valuable in the monitoring of antiresorptive therapies with bisphosphonates and in the assessment of therapy adherence of patients.[17] Bisphosphonates including alendronate, risedronate, ibandronate and zoledronic acid are commonly used medications to treat osteoporosis. They reduce bone resorption by inhibiting osteoclasts and thereby increasing bone mineral density (BMD). BMD is widely used to monitor response to treatment; however, treatment-induced increments in BMD are modest (typically 2 % per year). Taking a repeat error of 1-2 % into account true changes in BMD are observed only several years after starting treatment. Treatment-induced changes in bone turnover markers are much more rapid and occur at 3-6 months[18] or earlier.

a. Ibandronate therapy

The DIVA (Dosing IntraVenous Administration) study enrolled 1395 women aged 55-80 years, who were > 5 years menopausal, with osteoporosis diagnosed by lumbar spine [L2-L4] bone mineral density T score less than -2.5. Participants received a daily calcium dose of 500 mg and 400 IU vitamin D. A dosing scheme of oral 2.5 mg ibandronate daily, which has proven antifracture efficacy was compared with an i.v. 3 mg every 3 months dosing scheme and investigated for non-inferiority.

The table shows the median (%) change from baseline in serum βCTx levels after 2, 3, 4, 6 and 12 months[9] and after 24 months.[19]

The following values have been obtained from studies with the Elecsys β-CrossLaps/serum assay in healthy test subjects:

	Oral ibandronate 2.5 mg/daily		I.v. ibandronate 3 mg every 3 months	
Month	N	Median (%) change (95 % CI)[c)]	N	Median (%) change (95 % CI)
2	181	-45.0 (-48.7, -40.5)	-	-
3	192	-54.1 (-57.8, -48.7)	356	-43.2 (-45.9, -40.8)
4	180	-57.6 (-66.7, -50.0)	-	-
6	372	-62.5 (-65.3, -60.0)	353	-58.4 (-61.5, -55.2)
12	368	-62.6 (-66,0, -58.9)	352	-58.6 (-61.5, -55.4)
24	310	-59.9 (no CI available)	298	-53.4 (no CI available)

c) Confidence interval

b. Other anti-osteoporotic medications

Studies with different anti-osteoporotic medications (alendronate, risedronate, zoledronic acid and other drugs) at licensed doses revealed that βCTx reductions from baseline varied between the treatments, but serum βCTx was clinically useful in monitoring all anti-resorptive therapies. In a placebo-controlled clinical study with healthy postmenopausal women comparing the changes of different bone turnover markers, serum βCTx levels showed the highest decrease with 63.7 % in the alendronate group (N = 75) and 21.6 % in the placebo group (N = 73) after 12 months. Serum βCTx showed the highest correlation ($r = 0.60$, $p < 0.0001$) with changes in lumbar spine bone mineral density after 12 months.[20] In a clinical study performed in 54 study centers worldwide, the effectiveness of 5 mg i.v. zoledronic acid on the increase of lumbar spine bone mineral density was compared with 5 mg oral risedronate and monitored with bone turnover markers, e.g. serum βCTx.[21] The strong decrease of βCTx levels occurring within 9-11 days after onset of both treatments was maintained during the 12 months of the study. The reduction of βCTx levels after 6 and 12 months reflected the efficacy of both medications, i.e. the changes in lumbar bone mineral density.

Comparison of the least significant change (LSC) with the observed change in serum βCTx is a commonly proposed approach to determine its physiological relevance. A reduction in serum βCTx of smaller than the LSC of 27 % in a treated patient after 3 months from treatment initiation can thus be used as indicator of poor adherence or poor response of the patient to the anti-osteoporotic therapy.[17]

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 411 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	0.041	0.003	6.5	0.004	10.7
Human serum 2	0.403	0.008	2.1	0.011	2.8
Human serum 3	3.14	0.040	1.3	0.064	2.0
Human serum 4	4.90	0.068	1.4	0.100	2.0
Human serum 5	5.69	0.097	1.7	0.120	2.1
PreciControl Varia 1	0.295	0.005	1.8	0.009	2.9
PreciControl Varia 2	0.708	0.012	1.7	0.017	2.5

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	0.052	0.002	3.8	0.002	4.1
Human serum 2	0.438	0.008	1.8	0.009	2.0
Human serum 3	3.05	0.045	1.5	0.050	1.7
Human serum 4	4.80	0.061	1.3	0.065	1.4
Human serum 5	5.54	0.067	1.2	0.082	1.5
PreciControl Varia 1	0.315	0.006	1.9	0.007	2.2
PreciControl Varia 2	0.723	0.010	1.3	0.012	1.6

Method comparison

A comparison of the Elecsys β-CrossLaps/serum assay, REF 09005773190 (**cobas e** 601 analyzer; y) with the Elecsys β-CrossLaps/serum assay, REF 11972308122 (**cobas e** 601 analyzer; x) gave the following correlations (ng/mL):

Number of samples measured: 142

Passing/Bablok[22]	Linear regression
$y = 0.947x - 0.006$	$y = 0.938x + 0.003$
$\tau = 0.980$	$r = 1.000$

The sample concentrations were between 0.035 and 5.99 ng/mL.

Analytical specificity

The monoclonal antibodies used in the Elecsys β-CrossLaps/serum assay recognize all fragments of type I collagen containing the β-8AA octapeptide twice. No cross-reactivity detectable with osteocalcin, PTH or bone ALP.

References

1 Vasikaran S, Eastell R, Bruyère O, et al. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. Osteoporos Int 2011;22:391-420.

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2 Bonde M, Qvist P, Fledelius C, et al. Immunoassay for Quantifying Type I Collagen Degradation Products in Urine Evaluated. Clin Chem 1994;40(11):2022-2025.

3 Fledelius C, Johnsen A, Cloos P, et al. Identification of a β-isomerized aspartyl residue within the c-terminal telopeptide α1 chain of type I collagen. Possible relation to aging of bone. J Bone Miner Res 1996;11(Suppl1)AbstractNo.113.

4 Bonde M, Qvist P, Fledelius C, et al. Applications of an Enzyme Immunoassay for a New Marker of Bone Resorption (CrossLaps): Follow-up on Hormone Replacement Therapy and Osteoporosis Risk Assessment. J Clin Endocrinol Metab 1995;80:864-868.

5 Ravn P, Clemmesen B, Riis BJ, et al. The Effect on Bone Mass and Bone Markers of Different Doses of Ibandronate: A New Bisphosphonate for Prevention and Treatment of Postmenopausal Osteoporosis. A 1-year, Randomized, Double-Blind, Placebo-Controlled Dose-Finding Study. Bone 1996;19(5):527-533.

6 Rosenquist C, Fledelius C, Christgau S, et al. Serum CrossLaps One Step ELISA. First application of monoclonal antibodies for measurement in serum of bone-related degradation products from C-terminal telopetides of type I collagen. Clin Chem 1998;44(11):2281-2289.

7 Christgau S, Rosenquist C, Alexandersen P, et al. Clinical evaluation of the Serum CrossLaps One Step ELISA, a new assay measuring the serum concentration of bone-derived degradation products from type I collagen C-telopetides. Clin Chem 1998;44(11):2290-2300.

8 Grey A, Bolland M, Wattie D, et al. Prolonged antiresorptive activity of zoledronate: a randomized, controlled trial. JBMR 2010;25(10):2251-2255.

9 Delmas PD, Adami S, Strugula C, et al. Intravenous Ibandronate Injections in Postmenopausal Women With Osteoporosis. Arthritis & Rheumatism 2006;54(6):1838-1846.

10 Te Koppele JM. European patent application, EP 0829724A1. Europäisches Patentamt, Bulletin 1998/12.

11 Pagani F, Bonetti G, Stefini F, et al. Evaluation of a Fully Automated Assay to Measure C-Telopeptide of Type 1 Collagen in Serum. Clin Chem Lab Med 2000;38(11):1111-1113.

12 Garnero P, Sornay-Rendu E, Duboeuf F, et al. Markers of Bone Turnover Predict Postmenopausal Forearm Bone Loss Over 4 Years: The OFELY Study. J Bone Miner Res 1999;14:1614-1621.

13 Jorgensen NR, Mollehave LT, Hansen YBL, et al. Comparison of two automated assays of BTM (βCTx and P1NP) and reference intervals in a Danish population. Osteoporos Int 2017;28:2103-2113.

14 Glover SJ, Gall M, Schoenborn-Kellenberger O, et al. Establishing a Reference Interval for Bone Turnover Markers in 637 Healthy, Young, Premenopausal Women From the United Kingdom, France, Belgium, and the United States. Bone Miner Res 2009;24:389-397.

15 Hu WW, Zhang Z, He JW, et al. Establishing Reference Intervals for Bone Turnover Markers in the Healthy Shanghai Population and the Relationship with Bone Mineral Density in Postmenopausal Women. Int J Endocrinol 2013;2013:513925.

16 Ganero P, Borel O, Delmas PD. Evaluation of a Fully Automated Serum Assay for C-Terminal Cross-Linking Telopeptide of Type I Collagen in Osteoporosis. Clin Chem 2001;47(4):694-702.

17 Diez-Perez A, Naylor KE, Abrahamsen B, et al. International Osteoporosis Foundation and European Calcified Tissue Society Working Group. Recommendations for the screening of adherence to oral bisphosphonates. Osteoporos Int. 2017;28:767-74.

18 Kanis JA, McCloskey EV, Johansson H, et al.European guidance for the diagnosis and management of osteoporosis in postmenopausal women Osteoporos Int 2013;24:23–57.

19 Eisman JA, Civitelli R, Adami S, et al. Efficacy and Tolerability of Intravenous Ibandronate Injections in Postmenopausal Osteoporosis: 2-Year Results from the DIVA Stud. J Rheumatol 2008;35:488-497.

20 Nenonen A, Cheng S, Ivaska KK, et al. Serum TRACP 5b Is a Useful Marker for Monitoring Alendronate Treatment: Comparison with other markers of bone turnover. J Bone Miner Res 2005;20:1804-1812.

21 Reid DM, Devogelaer J-P, Saag K, et al. Zoledronic acid and risedronate in the prevention and treatment of glucocorticoid-induced osteoporosis (HORIZON): a multicentre, double-blind, double-dummy, randomised controlled trial. Lancet 2009;373: 1253-63.

22 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.

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

CE 0123

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