Elecsys Vitamin D total II

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REF		Σ	SYSTEM
07028148190	07028148500	300	**cobas e** 801

English

System information

Short name	ACN (application code number)
VITDT 2	10145

Intended use

This assay is intended for the quantitative determination of total 25-hydroxyvitamin D in human serum and plasma. This assay is to be used as an aid in the assessment of vitamin D sufficiency.

The electrochemiluminescence binding assay is intended for use on the **cobas e** 801 immunoassay analyzer.

Summary

Vitamin D is a fat-soluble steroid hormone precursor that is mainly produced in the skin by exposure to sunlight. Vitamin D is biologically inert and must undergo two successive hydroxylations in the liver and kidney to become the biologically active 1,25-dihydroxyvitamin D.[1]

The two most important forms of vitamin D are vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). In contrast to vitamin D_3, the human body cannot produce vitamin D_2 which is taken up with fortified food or given by supplements. In human plasma vitamin D_3 and D_2 are bound to the vitamin D binding protein and transported to the liver where both are hydroxylated to form 25-hydroxyvitamin D. It is commonly agreed that 25-hydroxyvitamin D is the metabolite to determine the overall vitamin D status as it is the major storage form of vitamin D in the human body. This primary circulating form of vitamin D is biologically inactive with levels approximately 1000-fold greater than the circulating 1,25-dihydroxyvitamin D. The half-life of circulating 25-hydroxyvitamin D is 2-3 weeks.

Most of the 25-hydroxyvitamin D, measurable in serum, is 25-hydroxyvitamin D_3 whereas 25-hydroxyvitamin D_2 reaches measurable levels only in patients taking vitamin D_2 supplements.[2,3,4] Vitamin D_2 is considered to be less effective.[5]

The most abundant product of 25-hydroxyvitamin D catabolism by 24-hydroxylase (CYP24A1) is 24,25-dihydroxyvitamin D.[6] It accounts for 2-20 % of the total circulating 25-hydroxyvitamin D, has a half-life of approximately 7 days and is present in serum at concentrations of up to approximately 10 nmol/L.[6,7,8]

Vitamin D is essential for bone health. In children, severe deficiency leads to bone-malformation, known as rickets. Milder degrees of insufficiency are believed to cause reduced efficiency in the utilization of dietary calcium.[9] Vitamin D deficiency causes muscle weakness; in elderly, the risk of falling has been attributed to the effect of vitamin D on muscle function.[10] Vitamin D deficiency is a common cause of secondary hyperparathyroidism.[11,12] Elevations of parathyroid hormone levels, especially in elderly vitamin D deficient adults can result in osteomalacia, increased bone turnover, reduced bone mass and risk of bone fractures.[13] Low 25-hydroxyvitamin D concentrations are also associated with lower bone mineral density.[14] In conjunction with other clinical data, the results may be used as an aid in the assessment of bone metabolism.

So far, vitamin D has been shown to affect expression of over 200 different genes. Insufficiency has been linked to diabetes, different forms of cancer, cardiovascular disease, autoimmune diseases and innate immunity.[2]

The Elecsys Vitamin D total II assay employs a vitamin D binding protein (VDBP) labeled with a ruthenium complex[a)] as capture protein to bind 25-hydroxyvitamin D_3 and 25-hydroxyvitamin D_2. Cross-reactivity to 24,25-dihydroxyvitamin D is blocked by a specific monoclonal antibody.

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

Test principle

Competition principle. Total duration of assay: 27 minutes.

- 1st incubation: By incubating the sample (12 µL) with pretreatment reagent 1 and 2, bound 25-hydroxyvitamin D is released from the VDBP.
- 2nd incubation: By incubating the pretreated sample with the ruthenium labeled vitamin D binding protein, a complex between the 25-hydroxyvitamin D and the ruthenylated VDBP is formed.

  A specific unlabeled antibody binds to 24,25-dihydroxyvitamin D present in the sample and inhibits cross-reactivity to this vitamin D metabolite.
- 3rd incubation: After addition of streptavidin-coated microparticles and 25-hydroxyvitamin D labeled with biotin, unbound ruthenium labeled VDBPs become occupied. A complex consisting of the ruthenylated VDBP and the biotinylated 25-hydroxyvitamin D is formed and becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas** link.

Reagents - working solutions

The **cobas e** pack (M, R1, R2) and the pretreatment reagents (PT1, PT2) are labeled as VITDT 2.

PT1 Pretreatment reagent 1, 1 bottle, 9.1 mL:
Dithiothreitol 1 g/L, pH 5.5.

PT2 Pretreatment reagent 2, 1 bottle, 9.1 mL:
Sodium hydroxide 28 g/L.

M Streptavidin-coated microparticles, 1 bottle, 12.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Vitamin D binding protein-Ru/$(bpy)_3^{2+}$ (gray cap), 1 bottle, 13.9 mL:
Ruthenium labeled vitamin D binding protein 100 µg/L; bis-tris propane buffer 100 mmol/L; albumin (human) 40 g/L; pH 6.4; preservative.

R2 25-hydroxyvitamin D~biotin, 1 bottle, 13.9 mL:
Biotinylated 25-hydroxyvitamin D 140 µg/L; bis-tris propane buffer 100 mmol/L; pH 8.6; 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:

Danger

H290	May be corrosive to metals.
H314	Causes severe skin burns and eye damage.
H317	May cause an allergic skin reaction.

Prevention:

P261	Avoid breathing dust/fume/gas/mist/vapours/spray.

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P280	Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.

Response:

P301 + P330 + P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.
P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor.
P305 + P351 + P338 + P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.

Product safety labeling follows EU GHS guidance.

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

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods use assays that have been approved by the FDA or that are in compliance with the legal rules applicable to placing in vitro diagnostic medical devices for human use on the market in the European Union.
However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[15,16]

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the **cobas e** 801 analyzer	12 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

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

Plasma tubes containing separating gel can be used.

Criterion: Slope 0.9-1.1 + intercept within ≤ ± 3 ng/mL + coefficient of correlation ≥ 0.95.

Stable for 8 hours at 20-25 °C, 4 days at 2-8 °C, 24 weeks at -20 °C (± 5 °C).

Freeze only once.

The stability of 25-hydroxyvitamin D found with the Elecsys Vitamin D total II assay is in line with earlier studies using a vitamin D binding protein assay and mass spectrometry.[17]

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.

Re-centrifuge plasma samples in a secondary tube for 10 min at 2000 x g prior to measurement.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 07464240190, Vitamin D total II CalSet, for 4 x 1.0 mL
- REF 07464266190, PreciControl Vitamin D total II, for 6 x 1.0 mL
- REF 07299001190, Diluent Universal, 45.2 mL sample diluent
- General laboratory equipment
- **cobas e** 801 analyzer

Accessories for the **cobas e** 801 analyzer:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cups, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

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

Calibration

Traceability: This method has been standardized using internal standards which are traceable to the ID-LC-MS/MS 25-hydroxyvitamin D RMP.[18,19]

The ID-LC-MS/MS is traceable to the National Institute of Standards and Technology Standard Reference Material 2972.[20]

The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

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

Quality control

For quality control, use PreciControl Vitamin D total II.

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In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in ng/mL or nmol/L).

Conversion factors: nmol/L x 0.40 = ng/mL

ng/mL x 2.50 = nmol/L

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	≤ 0.373 mmol/L or ≤ 600 mg/dL
Intralipid	≤ 300 mg/dL
Biotin	≤ 123 nmol/L or ≤ 30 ng/mL

For concentrations ≤ 20 ng/mL the deviation is ≤ 2.0 ng/mL. For concentrations > 20 ng/mL the deviation is ≤ 10 %.

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.

Pharmaceutical substances

In vitro tests were performed on 16 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
EinsAlpha (alfacalcidol)	0.003
ZEMPLAR (paricalcitol)	0.002
Rocaltrol (calcitriol)	0.0017

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

3-100 ng/mL or 7.5-250 nmol/L (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 3.0 ng/mL (< 7.5 nmol/L). Values above the measuring range are reported as > 100 ng/mL (> 250 nmol/L) or up to 200 ng/mL (500 nmol/L) for 2-fold diluted samples.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 2 ng/mL (5 nmol/L)

Limit of Detection = 3 ng/mL (7.5 nmol/L)

Limit of Quantitation = 5 ng/mL (12.5 nmol/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

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

Dilution

Samples with 25-hydroxyvitamin D concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:2. The concentration of the diluted sample must be ≥ 40 ng/mL (≥ 100 nmol/L).

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

Expected values

Due to different standardizations between methods, result variation may arise. Clinical assessment should be taken into consideration when interpreting results.

Health based reference values (recommended for use):

Currently there is no standard definition of the optimal vitamin D status. Many specialists consider the commonly used population based reference values too low. Health based reference values are recommended to replace population based reference values.[21]

Most experts agree that vitamin D deficiency should be defined as 25-hydroxyvitamin D of ≤ 20 ng/mL (≤ 50 nmol/L).[22] Vitamin D insufficiency is recognized as 21-29 ng/mL.[22] Similarly, the US National Kidney Foundation considers levels < 30 ng/mL to be insufficient or deficient.[23]

The preferred level for 25-hydroxyvitamin D by many experts is now recommended to be ≥ 30 ng/mL (≥ 75 nmol/L).[22,24,25,26]

Reference values measured in an apparently healthy population:

It should be taken into consideration that differences in 25-hydroxyvitamin D levels may exist with respect to gender, age, season, geographical latitude and ethnic groups.[22,24]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Population based reference ranges should not be taken as clinical cutoff to recommend or dissuade from vitamin D supplementation. Guidance for supplementation should be taken from recent literature.[22,23]

A reference range study was conducted with samples from apparently healthy donors from the United States. Samples were collected from southern, middle and northern sites in summer and winter. There were approximately equal numbers of males and females, and approximately 30 % of the donors had dark complexion. The age range was 21 to 83 years.
The values given are for information only and may vary from other published data.

			Season			
	All (n = 400)		Summer (n = 197)		Winter (n = 203)	
Unit	ng/mL	nmol/L	ng/mL	nmol/L	ng/mL	nmol/L
Mean	25.7	64.3	28.9	72.3	22.6	56.5
2.5th percentile	7.61	19.0	11.1	27.8	5.65	14.1
97.5th percentile	55.5	139	60.3	151	52.3	131

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Specific performance data

Representative performance data on the analyzer is 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 801 analyzer					
			Repeatability		
Sample	Mean		SD		CV
	ng/mL	nmol/L	ng/mL	nmol/L	%
HS[b] 1	9.83	24.6	0.877	2.19	8.9
HS 2	29.2	73.0	0.899	2.25	3.1
HS 3	49.5	124	1.13	2.83	2.3
HS 4	88.4	221	1.40	3.50	1.6
HS 5	93.6	234	1.02	2.55	1.1
PC[c] Vitamin D total II1	10.6	26.5	0.899	2.25	8.5
PC Vitamin D total II2	29.0	72.5	0.961	2.40	3.3

b) HS = human serum

c) PC = PreciControl

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			Intermediate precision		
Sample	Mean		SD		CV
	ng/mL	nmol/L	ng/mL	nmol/L	%
HS 1	9.83	24.6	1.06	2.65	10.8
HS 2	29.2	73.0	1.26	3.15	4.3
HS 3	49.5	124	1.44	3.60	2.9
HS 4	88.4	221	2.12	5.30	2.4
HS 5	93.6	234	2.03	5.08	2.2
PC Vitamin D total II1	10.6	26.5	0.972	2.43	9.2
PC Vitamin D total II2	29.0	72.5	1.30	3.25	4.5

Method comparison

A comparison of the Elecsys Vitamin D total II assay (y) using the CDC Verification Samples with concentrations assigned by the CDC Vitamin D Reference Laboratory by ID-LC-MS/MS (x) gave the following correlations (ng/mL):

Number of samples measured: 111

Deming[27,28]	Passing Bablok[29]
y = 0.985x + 0.606	y = 0.979x + 0.555
r = 0.980	r = 0.921

The sample concentrations were between 5.6 ng/mL (14 nmol/L) and 93 ng/mL (233 nmol/L).

Analytical specificity

A study was performed based on guidance from CLSI EP07-A2 to evaluate the cross-reactivity of the assay with other vitamin D metabolites. Samples containing the cross-reactants were prepared at three 25-hydroxyvitamin D concentrations (25, 40 and 60 ng/mL). The % cross-reactivity was calculated for each sample using the equation below and normalized to the cross-reactivity of 25-hydroxyvitamin D_3.[30]

$$\% \text{ cross-reactivity} = \frac{(\text{mean conc. of spiked sample} - \text{mean conc. of unspiked sample})}{\text{spiked concentration}} \times 100\%$$

The mean results from this study are summarized in the following table:

Cross-reactant	**Concentration added ng/mL**	**Mean cross-reactivity %**
25-hydroxyvitamin D_3	50	100
25-hydroxyvitamin D_2	50	93.7
24,25-dihydroxyvitamin D_3	100	13.7
3-epi-25-hydroxyvitamin D_3	50	112.8
3-epi-25-hydroxyvitamin D_2	50	91.4
1,25-dihydroxyvitamin D_3	100	n. d.[d]
1,25-dihydroxyvitamin D_2	100	n. d.
Vitamin D_3	1000	0.7
Vitamin D_2	1000	0.3

d) n. d. = not detectable

References

1 Holick M. Vitamin D: the underappreciated D-lightful hormone that is important for skeletal and cellular health. Curr Opin Endocrinol Diabetes 2002;9(1)87-98.

2 Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266-281.

3 Houghton LA, Vieth R. The case against ergocalciferol (vitamin D2) as a vitamin supplement. Am J Clin Nutr 2006;84:694-697.

4 Hart GR, Furniss JL, Laurie D, et al. Measurement of vitamin D Status: background, clinical use and methodologies. Clin Lab 2006;52(7-8):335-343.

5 Armas LAG, Hollis BW, Heaney RP. Vitamin D2 is much less effective than Vitamin D3 in humans. J Clin Endocrinol Metab 2004;89(11):5387-5391.

6 Bosworth CR, Levin G, Robinson-Cohen C, et al. The serum 24,25-dihydroxyvitamin D concentration, a marker of vitamin D catabolism, is reduced in chronic kidney disease. Kidney Int 2012;82(6),693-700.

7 Glendenning P, Inderjeeth CA. Controversy and consensus regarding vitamin D: Recent methodological changes and the risks and benefits of vitamin D supplementation. Crit Rev Clin Lab Sci, 2015;1-16.

8 Berg AH, Powe CE, Evans MK, et al. 24,25-Dihydroxyvitamin d3 and vitamin D status of community-dwelling black and white Americans. Clin Chem 2015;61(6):877-884.

9 Steingrimsdottir L, Gunnarsson O, Indridason OS, et al. Relationship between serum parathyroid hormone levels, vitamin D sufficiency, and calcium intake. JAMA 2005 Nov 9;294(18):2336-2341.

10 Venning G. Recent developments in vitamin D deficiency and muscle weakness among elderly people. BMJ 2005;330:524-526.

11 Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocr Rev 2001 Aug;22(4):447-501.

12 Souberbielle JC, Lawson-Body E, Hammadi B, et al. The use in clinical practice of parathyroid hormone normative values established in vitamin D-sufficient subjects. J Clin Endocrinol Metab 2003 Aug;88(8):3501-3504.

13 Willett AM. Vitamin D status and its relationship with parathyroid hormone and bone mineral status in older adolescents. Proceeding of the Nutrition Society 2005;64:193-203.

14 Kuchukk NO, van Schoor NM, Pluijm SM, et al. Vitamin D status, parathyroid function, bone turnover, and BMD in postmenopausal women with osteoporosis: global perspective. J Bone Miner Res 2009;24:693-701.

15 Occupational Safety and Health Standards: Bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.

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16 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

17 Lewis JG, Elder PA. Serum 25-OH Vitamin D2 and D3 are Stable under Exaggerated Conditions. Clin Chem 2008;54:1931-1932.

18 Sempos CT, Vesper HW, Phinney KW, et al. The Vitamin D Standardization Program (VDSP). Vitamin D Status as an International Issue: National Surveys and the Problem of Standardization. Scandinavian Journal of Clinical & Laboratory Investigation, 2012;72(Suppl 243):32-40.

19 Thienpont LM, Stepman HCM, Vesper HW. Standardization of Measurements of 25-Hydroxyvitamin D3 and D2. Scandinavian Journal of Clinical & Laboratory Investigation, 2012;72(Suppl 243):41-49.

20 Phinney KW. Development of a standard reference material for vitamin D in serum. Am J Clin Nutr 2008;88(suppl):511-512.

21 Bischoff-Ferrari HA, Giovannucci E, Willett WC, et al. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 2006;84:18-28.

22 Holick MF. Vitamin D status: measurement, interpretation, and clinical application. Ann Epidemiol 2009;19(2):73-78.

23 KDOQI Clinical Practice Guidelines for Bone Metabolism and Disease in Children With Chronic Kidney Disease. http://www.kidney.org/PROFESSIONALS/kdoqi/guidelines_pedbone/guide8.htm

24 Souberbielle JC, Body JJ, Lappe JM, et al. Vitamin D and musculoskeletal health, cardiocascular disease, autoimmunity and cancer: Recommendations for clinical practice. Autoimmun Rev 2010;9:709-715.

25 Dawson-Hughes B, Heaney RP, Holick MF, et al. Estimates of optimal vitamin D status. Osteoporos Int 2005;16:713-716.

26 Vieth R. Why the minimum desirable serum 25-hydroxyvitamin D level should be 75 nmol/L (30 ng/mL). Best Pract Res Clin Endocrinol Metab 2011;25(4):681-691.

27 Linnet K. Evaluation of Regression Procedures for Methods Comparison Studies. Clin Chem 1993;39(3):424-432.

28 Linnet K. Estimation of the Linear Relationship between the Measurements of two Methods with Proportional Errors. Statistics in Medicine 1990;9(12):1463-1473.

29 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.

30 Carter GD, Jones JC, Berry JL. The anomalous behaviour of exogenous 25-hydroxyvitamin D in competitive binding assays. J Steroid Biochem 2007;103(3-5): 480-482.

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 after reconstitution or mixing
GTIN	Global Trade Item Number

CE 0123

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