

# Vitamin D total II

cobas®

| REF          |     | SYSTEM  |
|--------------|-----|---|
| 07464215 190 | 100 | MODULAR ANALYTICS E170<br>cobas e 411<br>cobas e 601<br>cobas e 602 |

## English

### System-Information

For **cobas e 411** analyzer: test number 1500  
For MODULAR ANALYTICS E170, **cobas e 601** and **cobas e 602** analyzers: Application Code Number 166

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

### 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.<sup>1</sup>

The two most important forms of vitamin D are vitamin D<sub>3</sub> (cholecalciferol) and vitamin D<sub>2</sub> (ergocalciferol). In contrast to vitamin D<sub>3</sub>, the human body cannot produce vitamin D<sub>2</sub> which is taken up with fortified food or given by supplements. In human plasma vitamin D<sub>3</sub> and D<sub>2</sub> 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<sub>3</sub> whereas 25-hydroxyvitamin D<sub>2</sub> reaches measurable levels only in patients taking vitamin D<sub>2</sub> supplements.<sup>2,3,4</sup> Vitamin D<sub>2</sub> is considered to be less effective.<sup>5</sup>

The most abundant product of 25-hydroxyvitamin D catabolism by 24-hydroxylase (CYP24A1) is 24,25-dihydroxyvitamin D.<sup>6</sup> 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.<sup>6,7,8</sup>

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.<sup>9</sup> Vitamin D deficiency causes muscle weakness; in elderly, the risk of falling has been attributed to the effect of vitamin D on muscle function.<sup>10</sup>

Vitamin D deficiency is a common cause of secondary hyperparathyroidism.<sup>11,12</sup> 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.<sup>13</sup> Low 25-hydroxyvitamin D concentrations are also associated with lower bone mineral density.<sup>14</sup> 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.<sup>2</sup>

The Elecsys Vitamin D total II assay employs a vitamin D binding protein (VDBP) labeled with a ruthenium complex<sup>a)</sup> as capture protein to bind 25-hydroxyvitamin D<sub>3</sub> and 25-hydroxyvitamin D<sub>2</sub>. Cross-reactivity to 24,25-dihydroxyvitamin D is blocked by a specific monoclonal antibody.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)<sub>3</sub>)<sup>2+</sup>

### Test principle

Competition principle. Total duration of assay: 27 minutes.

- 1st incubation: By incubating the sample (20 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 ruthenylated labeled vitamin D binding proteins become occupied. A complex consisting of the ruthenylated vitamin D binding protein 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/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 (M, R1, R2) and the pretreatment reagents (PT1, PT2) are labeled as VitDII.

PT1 Pretreatment reagent 1 (white cap), 1 bottle, 4 mL:

Dithiothreitol 1 g/L, pH 5.5.

PT2 Pretreatment reagent 2 (gray cap), 1 bottle, 4 mL:

Sodium hydroxide 28 g/L.

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

Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Vitamin D binding protein-Ru(bpy)<sub>3</sub><sup>2+</sup> (gray cap), 1 bottle, 6.5 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 (black cap), 1 bottle, 6.5 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.

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:



Danger

H290 May be corrosive to metals.

H314 Causes severe skin burns and eye damage.

### Prevention:

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

## Response:

P301 + P330 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.  
+ P331

P303 + P361 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.  
+ P353

P304 + P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing.  
+ P310 Immediately call a POISON CENTER/doctor.

P305 + P351 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.  
+ P338 Continue rinsing. Immediately call a POISON CENTER/doctor.  
+ P310

P390 Absorb spillage to prevent material damage.

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 used assays approved by the FDA or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

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.<sup>15,16</sup>

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 | 56 days (8 weeks)                |
| on the analyzers        | 28 days (4 weeks)                |

## Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

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

Li-heparin, K<sub>2</sub>- and K<sub>3</sub>-EDTA plasma.

Plasma tubes containing separating gel can be used.

Criterion: Method comparison serum versus plasma, slope 0.9-1.1 + intercept within  $< \pm 3$  ng/mL + coefficient of correlation  $\geq 0.95$ .

Serum, Li-heparin, K<sub>2</sub>- and K<sub>3</sub>-EDTA plasma: 25-hydroxyvitamin D is stable for 8 hours at 20-25 °C, 4 days at 2-8 °C, 24 weeks at -20 °C ( $\pm 5$  °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.<sup>17</sup>

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] 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] 11732277122, Diluent Universal, 2 x 16 mL sample diluent or [REF] 03183971122, Diluent Universal, 2 x 36 mL sample diluent

- General laboratory equipment
- MODULAR ANALYTICS E170 or **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 MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- [REF] 04880340190, ProCell M, 2 x 2 L system buffer
- [REF] 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- [REF] 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- [REF] 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- [REF] 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- [REF] 12102137001, AssayTip/AssayCup, 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] 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 (except for the **cobas e** 602 analyzer).

MODULAR ANALYTICS E170, **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



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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.<sup>22,23</sup>*

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 88 years.

The values given are for information only and may vary from other published data.

|                               | Season           |        |                     |        |                     |        |
|-------------------------------|------------------|--------|---------------------|--------|---------------------|--------|
|                               | All<br>(n = 400) |        | Summer<br>(n = 197) |        | Winter<br>(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   |
| 2.5 <sup>th</sup> percentile  | 7.61             | 19.0   | 11.1                | 27.8   | 5.65                | 14.1   |
| 97.5 <sup>th</sup> percentile | 55.5             | 139    | 60.3                | 151    | 52.3                | 131    |

## 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, samples 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 |        |     |
| Sample                                | Mean  |        | SD            |        | CV  |
|                                       | ng/mL | nmol/L | ng/mL         | nmol/L | %   |
| HS <sup>b)</sup> 1                    | 11.1  | 27.8   | 0.725         | 1.81   | 6.6 |
| HS 2                                  | 20.8  | 52.0   | 0.849         | 2.12   | 4.1 |
| HS 3                                  | 25.6  | 64.0   | 0.774         | 1.94   | 3.0 |
| HS 4                                  | 47.5  | 119    | 0.749         | 1.87   | 1.6 |
| HS 5                                  | 92.6  | 232    | 1.76          | 4.40   | 1.9 |
| PC <sup>c)</sup> Vitamin D total II 1 | 15.4  | 38.5   | 0.748         | 1.87   | 4.8 |
| PC Vitamin D total II 2               | 29.1  | 72.8   | 1.04          | 2.60   | 3.6 |

b) HS = human serum

c) PC = PreciControl

| cobas e 411 analyzer    |       |        |                        |        |     |
|-------------------------|-------|--------|------------------------|--------|-----|
|                         |       |        | Intermediate precision |        |     |
| Sample                  | Mean  |        | SD                     |        | CV  |
|                         | ng/mL | nmol/L | ng/mL                  | nmol/L | %   |
| HS 1                    | 11.1  | 27.8   | 0.965                  | 2.41   | 8.7 |
| HS 2                    | 20.8  | 52.0   | 1.09                   | 2.73   | 5.2 |
| HS 3                    | 25.6  | 64.0   | 1.43                   | 3.58   | 5.6 |
| HS 4                    | 47.5  | 119    | 1.77                   | 4.43   | 3.7 |
| HS 5                    | 92.6  | 232    | 2.40                   | 6.00   | 2.6 |
| PC Vitamin D total II 1 | 15.4  | 38.5   | 1.30                   | 3.25   | 8.4 |
| PC Vitamin D total II 2 | 29.1  | 72.8   | 1.56                   | 3.90   | 5.4 |

| MODULAR ANALYTICS E170, <b>cobas e</b> 601 and <b>cobas e</b> 602 analyzers |       |        |               |        |     |
|---|-------|--------|---------------|--------|-----|
|   |       |        | Repeatability |        |     |
| Sample  | Mean  |        | SD            |        | CV  |
|   | ng/mL | nmol/L | ng/mL         | nmol/L | %   |
| HS 1  | 10.5  | 26.3   | 0.783         | 1.96   | 7.4 |
| HS 2  | 21.1  | 52.8   | 0.968         | 2.42   | 4.6 |
| HS 3  | 24.9  | 62.3   | 0.973         | 2.43   | 3.9 |
| HS 4  | 54.9  | 137    | 1.72          | 4.30   | 3.1 |
| HS 5  | 94.3  | 236    | 2.65          | 6.63   | 2.8 |
| PC Vitamin D total II 1   | 15.9  | 39.8   | 0.919         | 2.30   | 5.8 |
| PC Vitamin D total II 2   | 29.4  | 73.5   | 1.24          | 3.10   | 4.2 |

| MODULAR ANALYTICS E170, <b>cobas e</b> 601 and <b>cobas e</b> 602 analyzers |       |        |                        |        |     |
|---|-------|--------|------------------------|--------|-----|
|   |       |        | Intermediate precision |        |     |
| Sample  | Mean  |        | SD                     |        | CV  |
|   | ng/mL | nmol/L | ng/mL                  | nmol/L | %   |
| HS 1  | 10.5  | 26.3   | 0.934                  | 2.34   | 8.9 |
| HS 2  | 21.1  | 52.8   | 1.24                   | 3.10   | 5.9 |
| HS 3  | 24.9  | 62.3   | 1.23                   | 3.08   | 4.9 |
| HS 4  | 54.9  | 137    | 2.09                   | 5.23   | 3.8 |
| HS 5  | 94.3  | 236    | 3.59                   | 8.98   | 3.8 |
| PC Vitamin D total II 1   | 15.9  | 39.8   | 1.15                   | 2.88   | 7.2 |
| PC Vitamin D total II 2   | 29.4  | 73.5   | 1.46                   | 3.65   | 5.0 |

## 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<sup>27,28</sup>

$$y = 0.954x - 0.707$$

$$r = 0.982$$

Passing Bablok<sup>29</sup>

$$y = 0.937x - 0.360$$

$$\tau = 0.902$$

The sample concentrations were between approximately 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<sub>3</sub>.<sup>30</sup>

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

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

| Cross-reactant                         | Concentration added ng/mL | Mean cross-reactivity % |
|--|---------------------------|-------------------------|
| 25-hydroxyvitamin D <sub>3</sub>       | 50                        | 100                     |
| 25-hydroxyvitamin D <sub>2</sub>       | 50                        | 93.7                    |
| 24,25-dihydroxyvitamin D <sub>3</sub>  | 100                       | 13.7                    |
| 3-epi-25-hydroxyvitamin D <sub>3</sub> | 50                        | 112.8                   |
| 3-epi-25-hydroxyvitamin D <sub>2</sub> | 50                        | 91.4                    |

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| Cross-reactant                       | Concentration added ng/mL | Mean cross-reactivity % |
|--------------------------------------|---------------------------|-------------------------|
| 1,25-dihydroxyvitamin D <sub>3</sub> | 100                       | n. d. <sup>d)</sup>     |
| 1,25-dihydroxyvitamin D <sub>2</sub> | 100                       | n. d.                   |
| Vitamin D <sub>3</sub>               | 1000                      | 0.7                     |
| Vitamin D <sub>2</sub>               | 1000                      | 0.3                     |

d) n. d. = not detectable

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

## Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (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                            |

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