

ms\_05894913190V7.0

# Vitamin D total

## 25-Hydroxyvitamin D

| REF          |     | SYSTEM  |
|--------------|-----|---|
| 05894913 190 | 100 | Elecsys 2010<br>MODULAR ANALYTICS E170<br>cobas e 411<br>cobas e 601<br>cobas e 602 |

### English

#### 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 vitamin D (25-OH), i.e. 25-hydroxyvitamin D. It is commonly agreed that vitamin D (25-OH) 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 vitamin D (25-OH) is 2-3 weeks.

Most of the vitamin D (25-OH), measurable in serum, is vitamin D<sub>3</sub> (25-OH) whereas vitamin D<sub>2</sub> (25-OH) 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>

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>6</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>7</sup> Vitamin D deficiency is a common cause of secondary hyperparathyroidism.<sup>8,9</sup> Elevations of PTH levels, especially in elderly vitamin D deficient adults can result in osteomalacia, increased bone turnover, reduced bone mass and risk of bone fractures.<sup>10</sup> Low vitamin D (25-OH) concentrations are also associated with lower bone mineral density.<sup>11</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 assay employs a vitamin D binding protein (VDBP) as capture protein to bind vitamin D<sub>3</sub> (25-OH) and vitamin D<sub>2</sub> (25-OH).

#### Test principle

Competition principle. Total duration of assay: 27 minutes.

- 1st incubation: By incubating the sample (15 µL) with pretreatment reagent 1 and 2, bound vitamin D (25-OH) is released from the vitamin D binding protein.
- 2nd incubation: By incubating the pretreated sample with the ruthenium labeled vitamin D binding protein, a complex between the vitamin D (25-OH) and the ruthenylated vitamin D binding protein is formed.

- 3rd incubation: After addition of streptavidin-coated microparticles and vitamin D (25-OH) labeled with biotin, unbound ruthenium labeled vitamin D binding proteins become occupied. A complex consisting of the ruthenylated vitamin D binding protein and the biotinylated vitamin D (25-OH) 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.

#### Reagents - working solutions

The reagent rackpack (M, R1, R2) and the pretreatment reagents (PT1, PT2) are labeled as VITD-T.

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 55 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-BPRu (gray cap), 1 bottle, 9 mL:

Ruthenium labeled vitamin D binding protein 150 µg/L; bis-tris propane buffer 200 mmol/L; albumin (human) 25 g/L; pH 7.5; preservative.

R2 25-hydroxyvitamin D-biotin (black cap), 1 bottle, 8.5 mL:

Biotinylated vitamin D (25-OH) 14 µg/L; bis-tris propane buffer 200 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:

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

#### Response:

ms\_05894913190V7.0

# Vitamin D total

## 25-Hydroxyvitamin D



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

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

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

Product safety labeling primarily 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 applied were FDA-approved 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>12,13</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 Elecsys 2010 and <b>cobas e 411</b>                               | 21 days (3 weeks)                |
| on MODULAR ANALYTICS E170, <b>cobas e 601</b> and <b>cobas e 602</b> | 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 as well as Li-heparin plasma tubes containing separating gel.

Criterion: Method comparison serum versus plasma, slope 0.9-1.1 + intercept within  $< \pm 2 \times \text{LoB} + \text{coefficient of correlation} > 0.9$ .

Serum, Li-heparin, K<sub>2</sub>- and K<sub>3</sub>-EDTA plasma: Vitamin D (25-OH) is stable for 8 hours at 18-25 °C, 4 days at 2-8 °C, 24 weeks at -20 °C.

The stability of vitamin D (25-OH) found with the Elecsys Vitamin D total assay is in line with earlier studies using a vitamin D binding protein assay and mass spectrometry.<sup>14</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] 05894921190, Vitamin D total CalSet, for 4 x 1 mL
  - [REF] 05618860190, PreciControl Varia, for 2 x 3 mL each of PreciControl Varia 1 and 2
  - [REF] 11732277122, Diluent Universal, 2 x 16 mL sample diluent or [REF] 03183971122, Diluent Universal, 2 x 36 mL sample diluent
  - General laboratory equipment
  - Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer
- Accessories for Elecsys 2010 and **cobas e 411** analyzers:
- [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, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
  - [REF] 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips
- 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 Combimagazine M, 48 magazines x 84 reaction vessels 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.

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 automatically regulates the temperature of the reagents and the opening/closing of the bottles.

### Calibration

Traceability: This method has been standardized against LC-MS/MS<sup>15</sup> which in turn has been standardized to the NIST standard.<sup>16</sup>

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.



ms\_05894913190V7.0

# Vitamin D total

## 25-Hydroxyvitamin D



### Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). The following results were obtained:

| Elecsys 2010 and <b>cobas e 411</b> analyzers |       |        |               |        |     |
|---|-------|--------|---------------|--------|-----|
| Sample  | Mean  |        | Repeatability |        |     |
|   |       |        | SD            |        | CV  |
|   | ng/mL | nmol/L | ng/mL         | nmol/L | %   |
| HS <sup>a)</sup> 1                            | 6.76  | 16.9   | 0.525         | 1.31   | 7.8 |
| HS 2  | 15.0  | 37.5   | 0.770         | 1.93   | 5.1 |
| HS 3  | 28.0  | 70.0   | 0.860         | 2.15   | 3.1 |
| HS 4  | 67.0  | 168    | 1.15          | 2.88   | 1.7 |
| PC <sup>b)</sup> Varia 1                      | 19.9  | 49.8   | 0.948         | 2.37   | 4.8 |
| PC Varia 2                                    | 38.3  | 95.8   | 1.05          | 2.63   | 2.7 |

a) HS = human serum

b) PC = PreciControl

| Elecsys 2010 and <b>cobas e 411</b> analyzers |       |        |                        |        |      |
|---|-------|--------|------------------------|--------|------|
| Sample  | Mean  |        | Intermediate precision |        |      |
|   |       |        | SD                     |        | CV   |
|   | ng/mL | nmol/L | ng/mL                  | nmol/L | %    |
| HS 1  | 6.76  | 16.9   | 0.724                  | 1.81   | 10.7 |
| HS 2  | 15.0  | 37.5   | 1.28                   | 3.20   | 8.5  |
| HS 3  | 28.0  | 70.0   | 1.46                   | 3.65   | 5.2  |
| HS 4  | 67.0  | 168    | 1.46                   | 3.65   | 2.2  |
| PC Varia 1                                    | 19.9  | 49.8   | 1.23                   | 3.08   | 6.2  |
| PC Varia 2                                    | 38.3  | 95.8   | 1.41                   | 3.53   | 3.7  |

| MODULAR ANALYTICS E170, <b>cobas e 601</b> and <b>cobas e 602</b> analyzers |       |        |               |        |     |
|---|-------|--------|---------------|--------|-----|
| Sample  | Mean  |        | Repeatability |        |     |
|   |       |        | SD            |        | CV  |
|   | ng/mL | nmol/L | ng/mL         | nmol/L | %   |
| HS 1  | 8.35  | 20.9   | 0.567         | 1.42   | 6.8 |
| HS 2  | 15.8  | 39.5   | 0.824         | 2.06   | 5.2 |
| HS 3  | 28.3  | 70.8   | 1.11          | 2.78   | 3.9 |
| HS 4  | 69.6  | 174    | 1.50          | 3.75   | 2.2 |
| PC Varia 1  | 20.2  | 50.5   | 0.924         | 2.31   | 4.6 |
| PC Varia 2  | 39.6  | 99.0   | 1.06          | 2.65   | 2.7 |

| MODULAR ANALYTICS E170, <b>cobas e 601</b> and <b>cobas e 602</b> analyzers |       |        |                        |        |      |
|---|-------|--------|------------------------|--------|------|
| Sample  | Mean  |        | Intermediate precision |        |      |
|   |       |        | SD                     |        | CV   |
|   | ng/mL | nmol/L | ng/mL                  | nmol/L | %    |
| HS 1  | 8.35  | 20.9   | 1.10                   | 2.75   | 13.1 |
| HS 2  | 15.8  | 39.5   | 1.18                   | 2.95   | 7.5  |
| HS 3  | 28.3  | 70.8   | 1.83                   | 4.58   | 6.5  |
| HS 4  | 69.6  | 174    | 2.37                   | 5.93   | 3.4  |
| PC Varia 1  | 20.2  | 50.5   | 0.954                  | 2.39   | 4.7  |
| PC Varia 2  | 39.6  | 99.0   | 1.38                   | 3.45   | 3.5  |

### Method comparison

1) A comparison of the Elecsys Vitamin D total assay (y) using samples measured with LC-MS/MS (x) gave the following correlations (ng/mL):

Number of samples measured: 903

Passing/Bablok<sup>24</sup>  $y = 1.09x - 0.510$ Pearson  $r = 0.894$ 

The sample concentrations were between approximately 3 ng/mL (7.5 nmol/L) and 81 ng/mL (203 nmol/L).

2) A comparison of the Elecsys Vitamin D total assay (y) using samples measured with a commercially available vitamin D (25-OH) immunoassay (x) gave the following correlations (ng/mL):

Number of samples measured: 451

Passing/Bablok<sup>24</sup>  $y = 1.29x + 1.71$ Pearson  $r = 0.803$ 

The sample concentrations were between approximately 5 ng/mL (12.5 nmol/L) and 81 ng/mL (203 nmol/L).

### Analytical specificity

The specificity was assessed at 50 % B<sub>0</sub> and the results are summarized in the following table:

| Cross-reactant                                | Cross-reactivity (%) |
|---|----------------------|
| 25-hydroxyvitamin D <sub>3</sub>              | 100                  |
| 25-hydroxyvitamin D <sub>2</sub>              | 92                   |
| 24,25-dihydroxyvitamin D <sub>3</sub>         | 149                  |
| C3-epimer of 25-hydroxyvitamin D <sub>3</sub> | 91                   |
| 1,25-dihydroxyvitamin D <sub>3</sub>          | non detectable       |
| 1,25-dihydroxyvitamin D <sub>2</sub>          | non detectable       |
| Vitamin D <sub>3</sub>                        | non detectable       |
| Vitamin D <sub>2</sub>                        | non detectable       |

### Functional sensitivity

The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %. 8 samples with concentrations between 0.722 ng/mL and 10.1 ng/mL were measured on several days. The functional sensitivity was determined to be 4.01 ng/mL (CV 18.5 %).

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ms\_05894913190V7.0

# Vitamin D total

## 25-Hydroxyvitamin D



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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.

|   |   |
|---|---|
|  | Contents of kit                                     |
|  | Analyzers/Instruments on which reagents can be used |
|  | Reagent   |
|  | Calibrator  |
|  | Volume after reconstitution or mixing               |
|  | Global Trade Item Number                            |

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