

Elecsys Vitamin D

cobas®

REF



SYSTEM

06506780160

06506780501

100

cobas e 411
cobas e 601
cobas e 602

English

For use in the USA only

System information

For **cobas e 411** analyzer: test number 990

For **cobas e 601** and **cobas e 602** analyzers: Application Code Number 141

Intended use

This Vitamin D 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 in adults.

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.¹

The two most important forms of vitamin D are vitamin D₃ (cholecalciferol) and vitamin D₂ (ergocalciferol). In contrast to vitamin D₃, the human body cannot produce vitamin D₂ which is taken up with fortified food or given by supplements. In human plasma vitamin D₃ and D₂ 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 D₃ (25-OH) whereas vitamin D₂ (25-OH) reaches measurable levels only in patients taking vitamin D₂ supplements.^{2,3,4} Vitamin D₂ is considered to be less effective.⁵

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.⁶ Vitamin D deficiency causes muscle weakness; reduced Vitamin D has also been associated with falls and fractures not explained by reduced bone density.⁷ Vitamin D deficiency is a common cause of secondary hyperparathyroidism.^{8,9} 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.¹⁰ Low vitamin D (25-OH) concentrations are also associated with lower bone mineral density.¹¹ 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 assay employs a vitamin D binding protein (VDBP) as capture protein to bind vitamin D₃ (25-OH) and vitamin D₂ (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 or e-barcode.

Reagents - working solutions

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

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.

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

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

2-methyl-2H-isothiazol-3-one hydrochloride

EUH 208 May produce an allergic reaction.



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:

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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.
+ 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.

Contact phone: 1-800-428-2336

Product safety labeling follows EU GHS guidance.

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.^{12,13}

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
on cobas e 411	21 days
on cobas e 601 and cobas e 602	28 days

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, K₂-EDTA and K₃-EDTA plasma.

Li-heparin plasma tubes containing separating gel can be used.

Criterion: Method comparison serum versus plasma, slope 0.9-1.1 + intercept within $\pm 2 \times$ Limit of Blank (LoB) + coefficient of correlation > 0.95 .

Serum, Li-heparin, K₂- and K₃-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 (± 5 °C).

The stability of vitamin D (25-OH) found with the Elecsys Vitamin D assay is in line with earlier studies using a vitamin D binding protein assay and mass spectrometry.¹⁴

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.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- [REF](#) 06506798160, Vitamin D CalSet, for 4 x 1.0 mL
- [REF](#) 05618860160, PreciControl Varia, for 6 x 3.0 mL
- [REF](#) 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **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, 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](#) 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

Additional material for all analyzers:

- [REF](#) 11298500160, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

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

Resuspension of the microparticles 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.

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

Calibration

Traceability: This method has been standardized against LC-MS/MS¹⁵ which in turn has been standardized to the NIST standard.¹⁶

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). Renewed calibration is recommended as follows:

- after 1 month (28 days) 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

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

Quality control

For quality control, use PreciControl Varia.

In addition, other suitable control material can be used.

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

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

If necessary, repeat the measurement of the samples concerned.

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

Calculation

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

Conversion factors: nmol/L x 0.40 = ng/mL
 ng/mL x 2.50 = nmol/L

Limitations - interference

Samples showing visible signs of hemolysis may cause interference.

Hemoglobin concentrations > 2 g/L (> 0.124 mmol/L) may lead to elevated results.

The assay is unaffected by icterus (bilirubin < 1129 µmol/L or < 66 mg/dL), lipemia (Intralipid < 400 mg/dL), biotin (< 287 nmol/L or < 70 ng/mL), serum albumin (< 7 g/dL), IgG (< 3.5 g/dL) and cholesterol (< 380 mg/dL).

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.

In vitro tests were performed on 16 commonly used pharmaceuticals and 6 special therapeutic drugs (see table) at low (approximately 10 ng/mL) and medium (approximately 25 ng/mL) concentration of Vitamin D (25-OH).

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

Criterion: For concentrations from Limit of Quantitation up to 15 ng/mL, deviation is ≤ 1.5 ng/mL; for concentrations > 15 ng/mL, deviation is ≤ 10 %. No interference was found.

Drug interference (general drug panel) Elecsys Vitamin D assay	
Compound	Drug concentration mg/L
Acetylcystein	1663
Ampicillin-Na	1000
Ascorbic Acid	300

Drug interference (general drug panel) Elecsys Vitamin D assay	
Compound	Drug concentration mg/L
Cyclosporine	105
Cefoxitin	660
Heparin	5000 U
Levodopa	140
Methyldopa+1.5	15
Metronidazole	200
Phenylbutazone	400
Doxycyclin	30
Acetylsalicylic acid	1000
Rifampicin	64.2
Acetaminophen	200
Ibuprofen	500
Theophyllin	100

Drug interference (special drug panel) Elecsys Vitamin D assay	
Compound	Drug concentration mg/L
Bonviva (Bondronate)	50
EinsAlpha (α-calcidol)	0.003
Fosamax (Alendronate)	350
Panidron (Pamidronate)	90
Zometa (Zolendronate)	4
PTH (1-34)	0.02

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

5.00-60.0 ng/mL or 12.5-150 nmol/L (defined by the Limit of Quantitation and the maximum of the master curve). Values below the Limit of Quantitation are reported as < 5.00 ng/mL (< 12.5 nmol/L). Values above the measuring range are reported as > 60.0 ng/mL (> 150 nmol/L).

Linear range

Based on guidance from the CLSI Protocol EP6-A a study was performed to establish the linear range of the Elecsys Vitamin D assay. Parameters of the linear regression line showed an intercept of -1.14 ng/mL and a slope of 1.07. A maximum deviation of 1.56 ng/mL (3.4 %) was observed at 46.2 ng/mL. Linearity was determined from 0.0-60 ng/mL (0.0-150 nmol/L).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 2.00 ng/mL (5.00 nmol/L)

Limit of Detection = 3.00 ng/mL (7.50 nmol/L)

Limit of Quantitation = 5.00 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-A 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 %.

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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 (functional sensitivity) is defined as the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

Dilution

Samples with vitamin D (25-OH) concentrations above the measuring range can be manually diluted with Diluent Universal. The recommended dilution is 1:2. The concentration of the diluted sample must be > 25.0 ng/mL (> 62.5 nmol/L). After manual dilution, multiply the results by the dilution factor 2.

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.¹⁷

Most experts agree that vitamin D deficiency should be defined as vitamin D (25-OH) of ≤ 20 ng/mL (≤ 50 nmol/L).¹⁸ Vitamin D insufficiency is recognized as 21-29 ng/mL.¹⁸ Similarly, the US National Kidney Foundation considers levels < 30 ng/mL to be insufficient or deficient.¹⁹

The preferred level for vitamin D (25-OH) by many experts is now recommended to be ≥ 30 ng/mL (≥ 75 nmol/L).^{18,20,21,22}

Reference values measured in an apparently healthy population:

It should be taken into consideration that differences in vitamin D (25-OH) levels may exist with respect to gender, age, season, geographical latitude and ethnic groups.^{18,20}

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.^{18,19}

A reference range study was conducted with samples from 560 apparently healthy individuals. The age range was 21-79 years. Samples were collected between November and December from centers in the United States.

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

N	Mean	2.5 th percentile	97.5 th percentile	
560	25.5	6.36	49.5	ng/mL
	63.8	15.9	124	nmol/L

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 modified protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate and 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	%
Human serum 1	6.20	15.5	0.445	1.11	7.2
Human serum 2	11.2	28.0	0.566	1.42	5.1
Human serum 3	23.2	58.0	0.666	1.67	2.9

cobas e 411 analyzer					
Sample			Repeatability		
	Mean		SD		CV
	ng/mL	nmol/L	ng/mL	nmol/L	
Human serum 4	43.5	109	1.04	2.60	2.4
Human serum 5	52.6	132	0.858	2.15	1.6
PC ^{a)} Varia 0	11.6	29.0	0.529	1.32	4.6
PC Varia 1	17.0	42.5	0.745	1.86	4.4
PC Varia 2	31.1	77.8	0.802	2.01	2.6

a) PC = PreciControl

cobas e 411 analyzer					
Sample			Intermediate precision		
	Mean		SD		CV
	ng/mL	nmol/L	ng/mL	nmol/L	
Human serum 1	6.20	15.5	0.640	1.60	10.3
Human serum 2	11.2	28.0	0.849	2.12	7.6
Human serum 3	23.2	58.0	1.24	3.10	5.3
Human serum 4	43.5	109	1.46	3.65	3.4
Human serum 5	52.6	132	1.50	3.75	2.9
PC Varia 0	11.6	29.0	0.705	1.76	6.1
PC Varia 1	17.0	42.5	0.964	2.41	5.7
PC Varia 2	31.1	77.8	1.08	2.70	3.5

cobas e 601 and cobas e 602 analyzers					
Sample			Repeatability		
	Mean		SD		CV
	ng/mL	nmol/L	ng/mL	nmol/L	
Human serum 1	7.60	19.0	0.496	1.24	6.5
Human serum 2	11.6	29.0	0.695	1.74	6.0
Human serum 3	24.1	60.3	0.875	2.19	3.6
Human serum 4	43.8	110	1.04	2.60	2.4
Human serum 5	54.0	135	1.01	2.53	1.9
PC Varia 0	11.1	27.8	0.545	1.36	4.9
PC Varia 1	16.7	41.8	0.751	1.88	4.5
PC Varia 2	31.8	79.5	0.784	1.96	2.5

cobas e 601 and cobas e 602 analyzers					
Sample			Intermediate precision		
	Mean		SD		CV
	ng/mL	nmol/L	ng/mL	nmol/L	
Human serum 1	7.60	19.0	0.959	2.40	12.6
Human serum 2	11.6	29.0	1.07	2.68	9.2
Human serum 3	24.1	60.3	1.44	3.60	6.0
Human serum 4	43.8	110	1.55	3.88	3.5
Human serum 5	54.0	135	1.60	4.00	3.0
PC Varia 0	11.1	27.8	0.678	1.70	6.1
PC Varia 1	16.7	41.8	0.786	1.97	4.7
PC Varia 2	31.8	79.5	1.03	2.58	3.2

Method comparison

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

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Number of samples measured: 290

Deming²³ $y = 1.03x - 3.07$

Pearson $r = 0.85$

The sample concentrations were between 6 ng/mL (15 nmol/L) and 65 ng/mL (163 nmol/L).

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

Number of samples measured: 165

Deming²³ $y = 0.99x + 1.20$

Pearson $r = 0.91$

The 95 % confidence interval of the slope and intercept were 0.92 to 1.07, and -0.74 to 3.14 ng/mL, respectively. The sample concentrations were between 13 ng/mL (32.5 nmol/L) and 63 ng/mL (158 nmol/L).

Analytical specificity

The specificity was assessed with the following analytes at 50 % binding of B₀.

Cross-reactant	Cross-reactivity (%)
25-hydroxyvitamin D ₃	100
25-hydroxyvitamin D ₂	92
24,25-dihydroxyvitamin D ₃	149
C3-epimer of 25-hydroxyvitamin D ₃	91
1,25-dihydroxyvitamin D ₃	not detectable
1,25-dihydroxyvitamin D ₂	not detectable
Vitamin D ₃	not detectable
Vitamin D ₂	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).

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):

	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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Elecsys Vitamin D



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