05889014500V4.0

Elecsys Cyclosporine

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REF		Σ	SYSTEM
			cobas e 411
05889014190	05889014500	100	**cobas e** 601
			cobas e 602

English

System information

For **cobas e** 411 analyzer: test number 1160
cobas e 601 and **cobas e** 602 analyzers: Application Code Number 705

Intended use

Immunoassay for the in vitro quantitative determination of cyclosporine in human whole blood. The assay is used as an aid in the management of heart, liver, kidney, lung and bone marrow transplant patients receiving cyclosporine therapy.

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

Summary

Cyclosporine is a cyclic undecapeptide of fungal origin and a potent immunosuppressive agent. The introduction of cyclosporine in human kidney transplantation in the late 1970s was a major step forward in transplantation medicine, and substantially improved patient and graft survival in patients receiving e.g. heart, kidney, liver, pancreas, lung or bone marrow transplants.[1,2,3]

Cyclosporine was the first drug identified to specifically and reversibly inhibit the activation and proliferation of lymphocytes and represents the prototype of a class of drugs called calcineurin inhibitors.[4]

The main mechanism through which cyclosporine exerts its immunosuppressive effect is believed to be via the inhibition of T cell activation and proliferation. Intracellular cyclosporine binds to both cyclophilin A and B and these complexes then inhibit the enzymatic activity of calcineurin.[3,5,6]

The inhibition of calcineurin restricts the dephosphorylation and nuclear translocation of nuclear factor of activated T cells (NFAT), which regulates transcription of several cytokines, including IL-2, IL-4, TNF-α, and interferon-γ, and therefore limits lymphocyte activation and proliferation.[7,8,9,10,11,12]

Cyclosporine is highly lipophilic and absorption from the gastrointestinal tract is incomplete and variable. Approximately 90 % of the cyclosporine within the plasma is bound to proteins.[13]

The bioavailability and metabolism of cyclosporine are predominantly influenced by the activity of the cytochrome P450 isozymes CYP3A4 and CYP3A5, as well as the efflux pump p-glycoprotein, which show significant inter- and intra-individual variability in expression and function.[14,15,16]

Cyclosporine displays significant inter- and intra-patient pharmacokinetic variability, as well as potentially severe side effects from doses that are either too low or too high. Inadequate cyclosporine concentrations might result in rejection of the transplanted organ. High levels may lead to severe adverse effects. The most significant and well recognized side effect of cyclosporine therapy is nephrotoxicity, which can manifest as both reversible acute manifestations and as irreversible chronic manifestations.[3,17] The use of cyclosporine is also associated with renal dysfunction, tremor, hirsutism, hypertension, and gum hyperplasia.[13]

Therefore the application of therapeutic drug monitoring (TDM) and concentration-controlled dosing in order to maintain each patient's drug exposure within a narrow therapeutic window is clearly required and part of standard clinical practice for many years.[18,19,20]

Monitoring is most effective when there is a measurement that is a good surrogate for total drug exposure (measured as area under the time-concentration curve AUC 0-12). Advantages of monitoring cyclosporine concentrations based on predose trough (C0) concentrations versus two hours after administration (C2) are still in discussion and more multicenter studies are required to demonstrate a clinical benefit of C2 monitoring.[18,21]

Test principle

Manual precipitation:

Before testing with the Elecsys Cyclosporine assay, samples, calibrators and controls must be **pretreated** with Elecsys IS Sample Pretreatment.

The reagent lyses the cells, extracts cyclosporine, and precipitates most of the blood proteins. The **pretreated** samples are centrifuged, and an aliquot of the resulting supernatant containing cyclosporine is then assayed using the Elecsys Cyclosporine assay.

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of pretreated sample is incubated with a cyclosporine-specific biotinylated antibody and a ruthenium complex[a)] labeled cyclosporine-derivate. Depending on the analyte concentration in the sample and the formation of the respective immune complex, the labeled antibody binding site is occupied in part with sample analyte and in part with ruthenylated hapten.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

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

Reagents - working solutions

The reagent rackpack is labeled as CSA.

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

R1 Anti-cyclosporine Ab~biotin (gray cap), 1 bottle, 9 mL:
Biotinylated monoclonal anti-cyclosporine antibody (mouse) 25 µg/L; phosphate buffer 50 mmol/L, pH 6.0; preservative.

R2 Cyclosporine~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 mL:
Cyclosporine labeled with ruthenium complex 5 µg/L; phosphate buffer 50 mmol/L, pH 6.0; 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:

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

EUH 208 May produce an allergic reaction.

Product safety labeling follows EU GHS guidance.

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.

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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	84 days
on the analyzers	56 days

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

K_2- and K_3-EDTA whole blood.

Specimens collected in EDTA tubes may be stored for up to 5 days at 15-25 °C or 7 days at 2-8 °C prior to being tested. If testing will be delayed by more than 7 days, store frozen at -20 °C (± 5 °C) or lower for up to 6 months. Freeze only once. Specimens must be mixed thoroughly after thawing to ensure consistency of the results.

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.

Mix thawed specimens thoroughly by hand or on a roller mixer or rocker. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.

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 pretreatment.

Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.

Pretreated samples can be stored in closed tubes for up to 4 hours at 20-25 °C.

Due to evaporation effects, pretreated samples should be analyzed/measured within 30 minutes after opening the vials and loading the samples on the analyzer. Avoid delays between loading and measurement to ensure the 30 minute stability of pretreated samples.

A re-run requires repeating of the manual pretreatment procedure.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 05889073190, ISD Sample Pretreatment, 1 x 30 mL
- REF 05889022190, Cyclosporine CalSet, for 6 x 1.0 mL
- REF 05889081190, PreciControl ISD, for 3 x 3.0 mL
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- Precision pipettes (use only positive displacement pipettes for ISD Sample Pretreatment reagent handling)
- Microcentrifuge tubes (2.0 mL capacity)
- Microcentrifuge (at least 10000 g)
- Vortex mixer
- Roller mixer or rocker
- **cobas e** analyzer

Additional materials for the **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Additional materials for **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 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 materials for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Manual specimen pretreatment

Follow the steps listed below to pretreat calibrators, controls and/or specimens. **The technical notes are an essential part of the instructions and must be read thoroughly before completing each step.** Follow Steps 1 through 7 to pretreat calibrators, controls and/or specimens.

Steps	Technical notes
1. Equilibrate all reagents, calibrators, controls and specimens to 20-25 °C. Mix all calibrators, controls and specimens gently but thoroughly just before use.	Do not vortex. The liquids may be mixed by hand or on a roller mixer or rocker. The calibrators and controls are a whole-blood hemolysate and may be slightly different in appearance from whole-blood samples.
2. Label one microcentrifuge tube for each calibrator, control and/or specimen to be pretreated.	none
3. Using a precision pipette, transfer 300 µL of each calibrator, control and/or specimen to the appropriately labeled micro-centrifuge tube.	Use a fresh pipette tip for each calibrator, control and/or specimen.
4. Using a precision pipette, add 300 µL of ISD Sample Pretreatment reagent to each microcentrifuge tube. Immediately cap each tube and immediately proceed to step 5.	Note: ISD Sample Pretreatment is highly volatile. Keep tightly closed when not in use to prevent evaporation.
5. Vortex each microcentrifuge tube for at least 10 seconds. Failure to perform this step may result in a supernatant that appears red. See Step 6, technical note.	Note: Failure to vortex each tube immediately after addition of the ISD Sample Pretreatment reagent will lead to erroneous assay results. Sample and reagent mixture should be completely homogeneous immediately after vortexing. Visual inspection is required.

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Steps	Technical notes
6. Centrifuge the samples for at least 4 minutes in a micro-centrifuge (≥ 10000 g).	The centrifuged samples should have well-defined pellets and clear supernatant. The supernatant should not appear cloudy or red. If the supernatant is red, discard and replace it with a newly extracted sample.
7. Transfer each supernatant directly into an appropriate vial and immediately cap each vial. The samples are ready to be assayed.	Pretreated samples can be stored in closed tubes for up to 4 hours at 20-25 °C. **Please note:** **Due to evaporation effects, pretreated samples should be analyzed/measured within 30 minutes after opening the vials and loading the samples on the system. Avoid delays between loading and measurement to ensure the 30 minutes stability of pretreated samples.** **This is supported by running the cyclosporine samples in batch mode:** **Based on average system sample processing time, no more than 35 cyclosporine samples may be loaded per calibrated measuring cell onto the analyzers at the same time.**

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

Calibration

Traceability: This method has been standardized against reference standards traceable to cyclosporine reference material by weight.

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.

Cyclosporine CalSet must be pretreated freshly before calibration.

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

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

Renewed calibration is recommended as follows:

- after 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

Quality control

For quality control, use PreciControl ISD.

PreciControl ISD must be pretreated freshly before measurement.

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, nmol/L, µg/L).

Conversion factors: ng/mL x 1.0 = µg/L

ng/mL x 0.832 = nmol/L

Limitations - interference

The effect of the following endogenous substances, pharmaceutical compounds and clinical conditions on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Criterion: Recovery within ± 18.0 ng/mL (concentration range ≤ 90.0 ng/mL) or within ± 20 % (concentration range > 90.0 ng/mL) of initial value.

Endogenous substances:

Compound	Concentration tested
Albumin	≤ 12.0 g/dL
Bilirubin	≤ 1026 µmol/L or ≤ 60.0 mg/dL
Biotin	< 30.0 ng/mL or < 123 nmol/L
Cholesterol	≤ 500 mg/dL
HAMA	≤ 50.0 µg/mL
Hematocrit	15-60 %
IgG	≤ 12.0 g/dL
Intralipid	≤ 1500 mg/dL
Rheumatoid factors	up to 500 IU/mL
Uric acid	≤ 20.0 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.

Pharmaceutical compounds:

In vitro tests were performed on 16 commonly used pharmaceutical compounds. No interference with the assay was found.

Criterion: Recovery within ± 18.0 ng/mL (concentration range ≤ 90.0 ng/mL) or within ± 20 % (concentration range > 90.0 ng/mL) of initial value.

26 special drugs were additionally tested. An interaction with Itraconazole (INN international nonproprietary name) was found. Do not use samples from patients under Itraconazole treatment.

Drug	Concentration tested
Acyclovir	3.2 µg/mL
Amphotericin B	5.8 µg/mL
Ciprofloxacin	7.4 µg/mL
K_2-EDTA	6 mg/mL
K_3-EDTA	6 mg/mL
Erythromycin	20 mg/dL
Everolimus	60 ng/mL
Fluconazile	30 µg/mL

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Drug	Concentration tested
Flucytosine	40 µg/mL
Gancyclovir	1000 µg/mL
Gentamicin	12 mg/dL
Itraconazole	50 µg/mL
Kanamycin	100 µg/mL
Ketoconazole	50 µg/mL
Lidocaine	6 mg/dL
MPA (mycophenolic acid) glucuronide	1800 µg/mL
Mycophenolic acid	500 µg/mL
Nitrofurantoin	6 µg/mL
Phenobarbital	15 mg/dL
Sirolimus	60 ng/mL
Spectinomycin	100 µg/mL
Sulfomethoxazole	200 µg/mL
Tacrolimus	60 ng/mL
Tobramycin	2 mg/dL
Trimethoprim	40 µg/mL
Vancomycin	6 mg/dL

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

30.0-2000 ng/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 30.0 ng/mL. Values above the measuring range are reported as > 2000 ng/mL.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 20.0 ng/mL

Limit of Detection = 30.0 ng/mL

Limit of Quantitation = 50.0 ng/mL

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

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 defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable error of ≤ 20 %.

Dilution

Samples with cyclosporine concentrations above the measuring range can be manually diluted 1:3 with Diluent Universal prior to the manual pretreatment procedure. The concentration of the diluted sample must be > 500 ng/mL.

After manual dilution, multiply the result by the dilution factor.

Expected values

No firm therapeutic range exists for cyclosporine in whole blood. The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic effects of cyclosporine, coadministration of other immunosuppressants, type of transplant, time post-transplant, and a number of other factors contribute to different requirements for optimal blood levels of cyclosporine. Individual cyclosporine values cannot be used as the sole indicator for making changes in the treatment regimen. Each patient should be thoroughly evaluated clinically before treatment adjustments are made, and each assay user must establish his or her ranges based on clinical experience.

These ranges will vary according to the commercial in vitro diagnostic test used. Ranges must be established for each commercial test used.

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 (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
HSP[b)] 1	63.3	2.45	3.9	5.80	9.2
HSP 2	146	4.57	3.1	10.2	7.0
HSP 3	391	13.9	3.5	23.3	6.0
HSP 4	951	29.4	3.1	44.8	4.7
HSP 5	1830	59.1	3.2	89.9	4.9
PC[c)] ISD1	65.0	1.96	3.0	4.87	7.5
PC ISD2	317	7.81	2.5	13.3	4.2
PC ISD3	1210	39.2	3.2	53.8	4.4

b) HSP = Human Sample Pool

c) PC = PreciControl

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
HSP 1	64.0	2.75	4.3	4.12	6.4
HSP 2	146	4.36	3.0	7.29	5.0
HSP 3	400	12.5	3.1	19.1	4.8
HSP 4	973	23.0	2.4	40.4	4.2
HSP 5	1820	48.2	2.6	105	5.8
PC ISD1	69.0	2.82	4.1	3.63	5.3
PC ISD2	326	6.45	2.0	10.1	3.1
PC ISD3	1230	38.3	3.1	53.8	4.4

Method comparison

a) A comparison of the Elecsys Cyclosporine assay (y) with an automated immunoassay (x) using clinical samples gave the following correlations:

Number of samples measured: 339

Passing/Bablok[22]	Weighted linear regression
y = 1.01x - 15.5	y = 0.946x - 8.95
τ = 0.857	r = 0.977

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The sample concentrations were between 30.7 and 1770 ng/mL.

b) A comparison of the Elecsys Cyclosporine assay (y) with an LC-MS-MS method (x) using clinical samples gave the following correlations:

Number of samples measured: 352

Passing/Bablok[22]	Weighted linear regression
y = 1.091x + 2.08	y = 1.092x + 1.87
τ = 0.900	r = 0.997

The sample concentrations were between 30.7 and 1912 ng/mL.

Analytical specificity

A study was performed with the Elecsys Cyclosporine assay based on guidance from the CLSI document EP7-A2.

Metabolite	Maximum concentration of metabolite added ng/mL	Cross-reactivity %
AM1	2000	2
AM19	2000	n. d.[d)]
AM1c	2000	n. d.
AM1c9	2000	n. d.
AM4n	2000	2
AM9	2000	6

d) n. d. = not detectable

Cross-reactivity was designated as „not detectable“ if the obtained value was less than the sensitivity of the assay.

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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 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

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

Roche

ms_06633188190V2.0

Elecsys Everolimus

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REF	Σ	SYSTEM
06633188 190	100	MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

System information

For **cobas e** 411 analyzer: test number 1030
For MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 486

Intended use

Immunoassay for the in vitro quantitative determination of everolimus in human whole blood. The assay is used as an aid in the management of kidney, liver and heart transplant patients receiving everolimus therapy.

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

Summary

Everolimus is a derivative of sirolimus often formulated for oral administration and is synthesized by the introduction of a 2-hydroxyethyl group at the carbon atom at position 40 of sirolimus.[1] The drug has many clinical applications, the most prominent being in organ transplantation, oncology and cardiology.[2] It has been shown that early replacement of calcineurin inhibitors such as cyclosporin with an everolimus based regimen might improve long-term outcomes in kidney transplant patients.[3]

Everolimus exerts its immunosuppressive and anti-proliferative effects through binding to FKBP-12 and subsequent inhibition of mTOR-mediated signaling, which is a mechanism identical to that of sirolimus. Everolimus displays the same activity in vivo as sirolimus.[2]

Upon entry into the cell, everolimus binds to the abundant immunophilin FKBP-12. The everolimus-FKBP-12 complex binds to mTOR which has the following two major functions: 1) activation of p70 S6 kinase, a key enzyme in signal transduction which leads to DNA synthesis, and 2) binding of the eukaryotic initiation factor 4E (eIF-4E) to phosphorylated heat- and acid-stable protein I (PHAS-I), a pathway that is more involved in protein synthesis. By binding to mTOR, everolimus blocks its function and thus inhibits activation of p70 S6 kinase, resulting in the arrest of the cell cycle at the G1 to S phase. Interleukin (IL)-2 receptor-dependent as well as CD28-dependent signaling pathways are inhibited by these effects on mTOR.[2,4,5]

The maximum concentration (c_{max}) of everolimus is reached within 1-2 hours following oral administration.[6] Similar to sirolimus, everolimus is a substrate of P-glycoprotein and CYP3A4. Therefore metabolism in the gastrointestinal tract and export back into the gut lumen can significantly affect overall bioavailability.[2] The parent drug is metabolized mainly in the liver and the gut by demethylation, hydroxylation, and ring degradation to form 6 main metabolites.[2] Approximately 75 % of circulating everolimus is bound to red blood cells and nearly 75 % of the remaining fraction is bound to plasma proteins.[2] The elimination half-life in renal transplant patients is 18-35 hours, which is approximately half that observed for sirolimus. The elimination half-life is slightly longer in liver transplant patients at 35-40 hours.[2]

When used in immunosuppressive therapy, the most common side effects associated with everolimus are peripheral edema, constipation, hypertension, nausea, anemia, urinary tract infection, and hyperlipidemia. Side effects also include increased risk of infection, development of lymphomas, graft thrombosis, delayed wound healing, nephrotoxicity, opportunistic infections and new onset diabetes after transplantation.[6]

Blood concentrations of everolimus in solid organ transplant patients correlate with therapeutic efficacy and frequency of adverse effects.[2] Due to the drug's narrow therapeutic range, the significant pharmacokinetic drug interactions and the high inter-patient variability, therapeutic drug monitoring (TDM) of everolimus within whole blood is therefore recommended for all solid organ transplant patients, and will likely result in an improved efficacy of the drug.[2,7,8,9]

Test principle

Manual precipitation:

Before testing with the Elecsys Everolimus assay, samples, calibrators and controls must be **pretreated** with Elecsys ISD Sample Pretreatment.

The reagent lyses the cells, extracts everolimus, and precipitates most of the blood proteins. The **pretreated** samples are centrifuged, and the resulting supernatant containing everolimus is then assayed using the Elecsys Everolimus assay.

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: By incubating 35 µL of the pretreated sample with an everolimus-specific biotinylated antibody, an immunocomplex is formed, the amount of which is dependent upon the analyte concentration in the sample.
- 2nd incubation: After addition of streptavidin-coated microparticles and an everolimus derivative labeled with a ruthenium complex[a)], the still-vacant sites of the biotinylated antibodies become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

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

Reagents - working solutions

The reagent rackpack is labeled as EVL.

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

R1 Anti-everolimus Ab~biotin (gray cap), 1 bottle, 9 mL:
Biotinylated monoclonal anti-everolimus antibody (rabbit) 35 µg/L; phosphate buffer 100 mmol/L, pH 7.8; preservative.

R2 Everolimus derivate~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 mL:
Everolimus derivate labeled with ruthenium complex 18 µg/L; citrate buffer 10 mmol/L, pH 6.0; 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.

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.

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Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	56 days
on the analyzers	14 days onboard or 56 days when stored alternatively in the refrigerator and on the analyzer, with the total time onboard on the analyzer not exceeding 10 x 8 hours

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

K_2- and K_3-EDTA whole blood.

Specimens collected in EDTA tubes may be stored for up to 5 days at 15-25 °C or 7 days at 2-8 °C prior to being tested. If testing will be delayed by more than 7 days, store frozen at -20 °C (± 5 °C) or lower for up to 6 months.

Freeze only once. Specimens must be mixed thoroughly after thawing to ensure consistency of the results.

Mix thawed specimens thoroughly by hand or on a roller mixer or rocker. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.

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.

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 pretreatment.

Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.

Pretreated samples can be stored in closed tubes for up to 4 hours at 20-25 °C.

Due to evaporation effects, pretreated samples should be analyzed/measured within 30 minutes after opening the vials and loading the samples on the analyzer. Avoid delays between loading and measurement to ensure the 30 minute stability of pretreated samples.

A re-run requires repeating of the manual pretreatment procedure.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 05889073190, ISD Sample Pretreatment, 1 x 30 mL
- REF 06633196190, Everolimus CalSet, for 6 x 1.0 mL
- REF 07294131190, PreciControl Everolimus, for 3 x 3.0 mL
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent or REF 05192943190, Diluent Universal 2, 2 x 36 mL sample diluent
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- Precision pipettes (use only positive displacement pipettes for ISD Sample Pretreatment reagent handling)
- Microcentrifuge tubes (2.0 mL capacity)
- Microcentrifuge (at least 10000 g)
- Vortex mixer
- Roller mixer or rocker
- 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

Manual specimen pretreatment

Follow the steps listed below to pretreat calibrators, controls and/or specimens. **The technical notes are an essential part of the instructions and must be read thoroughly before completing each step.** Follow steps 1 through 7 to pretreat calibrators, controls and/or specimens.

Steps	Technical notes
1. Equilibrate all reagents, calibrators, controls and specimens to 20-25 °C. Mix all calibrators, controls and specimens gently but thoroughly just before use.	Do not vortex. The liquids may be mixed by hand or on a roller mixer or rocker. The calibrators and controls are a whole-blood hemolysate and may be slightly different in appearance from whole-blood samples.
2. Label one microcentrifuge tube for each calibrator, control and/or specimen to be pretreated.	none
3. Using a precision pipette, transfer 300 µL of each calibrator, control and/or specimen to the appropriately labeled microcentrifuge tube.	Use a fresh pipette tip for each calibrator, control and/or specimen.
4. Using a precision pipette, add 300 µL of ISD Sample Pretreatment reagent to each microcentrifuge tube. Immediately cap each tube and immediately proceed to step 5.	Note: ISD Sample Pretreatment is highly volatile. Keep tightly closed when not in use to prevent evaporation.
5. Vortex each microcentrifuge tube for at least 10 seconds. Failure to perform this step may result in a supernatant that appears red. See step 6, technical note.	Note: Failure to vortex each tube immediately after addition of the ISD Sample Pretreatment reagent will lead to erroneous assay results. Sample and reagent mixture should be completely homogeneous immediately after vortexing. Visual inspection is required.

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Steps	Technical notes
6. Centrifuge the samples for at least 4 minutes in a microcentrifuge (≥ 10000 g).	The centrifuged samples should have well-defined pellets and clear supernatant. The supernatant should not appear cloudy or red. If the supernatant is red, discard and replace it with a newly extracted sample.
7. Transfer each supernatant directly into an appropriate vial and immediately cap each vial. The samples are ready to be assayed.	Pretreated samples can be stored in closed tubes for up to 4 hours at room temperature. **Please note:** **Due to evaporation effects, pretreated samples should be analyzed/measured within 30 minutes after opening the vials and loading the samples on the system. Avoid delays between loading and measurement to ensure the 30 minutes stability of pretreated samples.** **This is ensured by running the everolimus samples in batch mode:** **Based on average system sample processing time, no more than 35 everolimus samples may be loaded per calibrated measuring cell onto the analyzers at the same time.**

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

Calibration

Traceability: This method has been standardized against gravimetrically produced master calibrators consisting of exactly defined pure substance everolimus concentrations in human whole blood matrix.

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.

Everolimus CalSet must be pretreated freshly before calibration.

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

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

Renewed calibration is recommended as follows:

- after 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

Quality control

For quality control, use PreciControl Everolimus.

PreciControl Everolimus must be pretreated freshly before measurement.

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, nmol/L or µg/L).

Conversion factors: ng/mL x 1.0 = µg/L

ng/mL x 1.044 = nmol/L

Limitations - interference

The effect of the following endogenous substances, pharmaceutical compounds and clinical conditions on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Criterion: Recovery within ± 0.60 ng/mL (concentration range ≤ 3.0 ng/mL) or within ± 20 % (concentration range > 3.0 ng/mL) of initial value.

Endogenous substances:

Compound	Concentration tested
Albumin	≤ 7.0 g/dL
Bilirubin	≤ 1129 µmol/L or ≤ 66.0 mg/dL
Biotin	≤ 287 nmol/L or ≤ 70.0 ng/mL
Cholesterol	≤ 500 mg/dL
HARA (human anti-rabbit antibodies)	≤ 10.0 µg/mL
Hematocrit	15-60 %
IgG	≤ 7.0 g/dL
IgM	≤ 1.0 g/dL
IgA	≤ 1.6 g/dL
Intralipid	≤ 2000 mg/dL
Rheumatoid factors	up to 1200 IU/mL
Uric acid	≤ 30.0 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.

Pharmaceutical compounds:

In vitro tests were performed on 16 commonly used pharmaceutical compounds. No interference with the assay was found.

Criterion: Recovery within ± 0.60 ng/mL (concentration range ≤ 3.0 ng/mL) or within ± 20 % (concentration range > 3.0 ng/mL) of initial value.

25 special drugs were additionally tested.

Due to the cross-reactivity with sirolimus, a switch from one drug to the other might lead to overestimation of the blood levels of the currently administered immunosuppressant. Therefore, do not use samples from patients under sirolimus treatment or under transition from sirolimus to everolimus. The transition period may be approximated by the half-life of the eliminated drug where, for example, 12.5 % of a drug remains after 3 times the half-life.[6]

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Drug	Concentration tested
Acyclovir	3.2 µg/mL
Amphotericin B	5.8 µg/mL
Ciprofloxacin	7.4 µg/mL
K_2-EDTA	6 mg/mL
K_3-EDTA	6 mg/mL
Erythromycin	20 mg/dL
Fluconazole	30 µg/mL
Flucytosine	40 µg/mL
Gancyclovir	1000 µg/mL
Gentamicin	12 mg/dL
Itraconazole	10 µg/mL
Kanamycin	100 µg/mL
Ketoconazole	50 µg/mL
Lidocaine	6 mg/dL
MPA (mycophenolic acid) glucuronide	1800 µg/mL
Mycophenolic acid	500 µg/mL
Nitrofurantoin	6 µg/mL
Phenobarbital	15 mg/dL
Spectinomycin	100 µg/mL
Sulfomethoxazole	200 µg/mL
Tacrolimus	60 ng/mL
Tobramycin	2 mg/dL
Trimethoprim	40 µg/mL
Vancomycin	6 mg/dL

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

0.5-30 ng/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.5 ng/mL. Values above the measuring range are reported as > 30 ng/mL.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.4 ng/mL

Limit of Detection = 0.5 ng/mL

Limit of Quantitation = 1.0 ng/mL

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

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 defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable error of ≤ 25 %.

Dilution

Samples with everolimus concentrations above the measuring range can be manually diluted 1:2 with Diluent Universal or Diluent Universal 2 prior to the manual pretreatment procedure. The concentration of the diluted sample must be > 12 ng/mL.

After manual dilution, multiply the result by the dilution factor.

Expected values

No firm therapeutic range exists for everolimus in whole blood. The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic effects of everolimus, coadministration of other immunosuppressants, type of transplant, time post-transplant, and a number of other factors contribute to different requirements for optimal blood levels of everolimus. Individual everolimus values cannot be used as the sole indicator for making changes in the treatment regimen. Each patient should be thoroughly evaluated clinically before treatment adjustments are made, and each assay user must establish his or her ranges based on clinical experience.

These ranges will vary according to the commercial in vitro diagnostic test used. Ranges must be established for each commercial test used.

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		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
HSP[b)] 1	1.66	0.134	8.1	0.135	8.1
HSP 2	3.19	0.091	2.8	0.135	4.2
HSP 3	13.2	0.367	2.8	0.486	3.7
HSP 4	16.6	0.532	3.2	0.679	4.1
HSP 5	24.1	0.634	2.6	0.946	3.9
PC EVL[c)] 1	2.69	0.077	2.9	0.140	5.2
PC EVL 2	9.27	0.187	2.0	0.248	2.7
PC EVL 3	19.8	0.499	2.5	0.746	3.8

b) HSP = Human Sample Pool

c) PC EVL = PreciControl Everolimus

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
HSP 1	1.80	0.100	5.5	0.120	6.7
HSP 2	3.58	0.115	3.2	0.192	5.4
HSP 3	13.6	0.354	2.6	0.622	4.6
HSP 4	18.4	0.619	3.4	0.769	4.2
HSP 5	26.8	0.818	3.1	1.09	4.1
PC EVL 1	2.79	0.116	4.2	0.176	6.3
PC EVL 2	9.91	0.379	3.8	0.508	5.1
PC EVL 3	20.6	0.528	2.6	0.797	3.9

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Method comparison

a) A comparison of the Elecsys Everolimus assay (y) with an automated immunoassay (x) using clinical samples gave the following correlations:

Number of samples measured: 151

Passing/Bablok[10]	Weighted Deming regression
y = 0.939x + 1.69	y = 1.05x + 1.03
τ = 0.753	r = 0.910

The sample concentrations were between 1.0 and 19.6 ng/mL.

b) A comparison of the Elecsys Everolimus assay (y) with an LC-MS-MS method (x) using clinical samples gave the following correlations:

Number of samples measured: 184

Passing/Bablok[10]	Weighted Deming regression
y = 1.13x + 0.905	y = 1.20x + 0.580
τ = 0.840	r = 0.947

The sample concentrations were between 1.5 and 20.2 ng/mL.

Analytical specificity

Metabolite	Maximum concentration added ng/mL	Maximum cross-reactivity[d] %
24-Hydroxy everolimus	25	21.3
25-Hydroxy everolimus	25	15.4
45/46-Hydroxy everolimus	25	6.0
PKF 226-320 (RAD SA)	25	9.8
PKF 299-255 (RAD PSA)	25	10.1
RAD-PC	25	109.3

d) Representative data; results in individual laboratories may vary from these data.

References

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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

CE

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

Roche

05889057500V5.0

Elecsys Tacrolimus

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REF		Σ	SYSTEM
			cobas e 411
05889057190	05889057500	100	**cobas e** 601
			cobas e 602

English

System information

For **cobas e** 411 analyzer: test number 1070
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 556

Intended use

Immunoassay for the in vitro quantitative determination of tacrolimus in human whole blood. The assay is used as an aid in the management of heart, liver and kidney transplant patients receiving tacrolimus therapy.

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

Summary

Tacrolimus (also referred to as FK506) is a macrolide antibiotic identified as a product of the actinobacterium Streptomyces tsukubaensis in Japan in 1984.[1,2,3] Studies demonstrated that tacrolimus is 10-100 times more active than cyclosporine at inhibiting several immune responses.[4]

The main mechanism through which tacrolimus exerts its immunosuppressive effect is believed to be via the inhibition of T cell activation and proliferation. Intracellular tacrolimus binds an immunophilin called FK506-binding protein (FKBP-12) and these complexes then inhibit the enzymatic activity of calcineurin.[5] The inhibition of calcineurin restricts the dephosphorylation and nuclear translocation of nuclear factor of activated T cells (NFAT), which regulates transcription of several cytokines, including IL-2, IL-4, TNF-α, and interferon-γ, and therefore limits lymphocyte activation and proliferation.[6,7,8,9,10]

Tacrolimus is highly lipophilic and absorption is incomplete and variable. Following absorption, tacrolimus is highly bound to proteins and erythrocytes, with 99 % of the drug within the plasma being bound to albumin or α-1-glycoprotein.[11]

The bioavailability and metabolism of tacrolimus are predominantly influenced by the activity of the cytochrome P450 isozymes CYP3A4 and CYP3A5, as well as the efflux pump p-glycoprotein, which show significant inter- and intra-individual variability in expression and function.[12,13,14]

Tacrolimus displays a high degree of inter- and intra-patient variability, as well as potentially severe side effects from doses that are either too low or too high. Inadequate tacrolimus concentrations might result in rejection of the transplanted organ. High levels may lead to severe adverse effects. Principle adverse effects associated with tacrolimus include nephrotoxicity, neurotoxicity, gastrointestinal disturbances, diabetogenesis, hypertension and malignant complications.[15,16]

The application of therapeutic drug monitoring (TDM) and concentration-controlled dosing in order to maintain each patient's drug exposure within a narrow therapeutic window is part of standard clinical practice for many years and is a major support to patient management.[16,17] Trough concentration (C0) monitoring is still widely used as a guide to individualizing tacrolimus dose requirements, even though some controversies remain about the relationship between C0 and clinical outcome. Area under the concentration-time curve (AUC0-12) is generally considered the best marker of exposure but is expensive and impractical. To assess the efficacy of alternative strategies to C0, multicenter prospective trials are needed.[16]

Test principle

Manual precipitation:

Before testing with the Elecsys Tacrolimus assay, samples, calibrators and controls must be **pretreated** with Elecsys ISD Sample Pretreatment.

The reagent lyses the cells, extracts tacrolimus, and precipitates most of the blood proteins. The **pretreated** samples are centrifuged, and an aliquot of the resulting supernatant containing tacrolimus is then assayed using the Elecsys Tacrolimus assay

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 35 µL of pretreated sample is incubated with a tacrolimus-specific biotinylated antibody and a ruthenium complex[a)] labeled tacrolimus-derivate. Depending on the analyte concentration in the sample and the formation of the respective immune complex, the labeled antibody binding site is occupied in part with sample analyte and in part with ruthenylated hapten.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

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

Reagents - working solutions

The reagent rackpack is labeled as TCL.

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

R1 Anti-Tacrolimus-S-Ab~biotin (gray cap), 1 bottle, 10 mL:
Biotinylated monoclonal anti-tacrolimus-antibody (sheep) 15 µg/L; phosphate buffer 100 mmol/L, pH 7.8; preservative.

R2 Tacrolimus~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 8 mL:
Tacrolimus-derivative labeled with ruthenium complex 4 µg/L; citrate buffer 10 mmol/L, pH 3.3; 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:

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

EUH 208 May produce an allergic reaction.

Product safety labeling follows EU GHS guidance.

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	84 days

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Stability:	
on the analyzers	56 days

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

K_2-EDTA and K_3-EDTA whole blood.

Specimens collected in EDTA tubes may be stored for up to 5 days at 15-25 °C or 7 days at 2-8 °C prior to being tested. If testing will be delayed by more than 7 days, store frozen at -20 °C (± 5 °C) or lower for up to 6 months. Freeze only once. Specimens must be mixed thoroughly after thawing to ensure consistency of the results.

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.

Mix thawed specimens thoroughly by hand or on a roller mixer or rocker. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.

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 pretreatment.

Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.

Pretreated samples can be stored in closed tubes for up to 4 hours at 20-25 °C.

Due to evaporation effects, pretreated samples should be analyzed/measured within 30 minutes after opening the vials and loading the samples on the analyzer. Avoid delays between loading and measurement to ensure the 30 minute stability of pretreated samples.

A re-run requires repeating of the manual pretreatment procedure.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 05889073190, ISD Sample Pretreatment, 1 x 30 mL
- REF 05889065190, Tacrolimus CalSet, for 6 x 1.0 mL
- REF 05889081190, PreciControl ISD, for 3 x 3.0 mL
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- Precision pipettes (use only positive displacement pipettes for ISD Sample Pretreatment reagent handling)
- Microcentrifuge tubes (2.0 mL capacity)
- Microcentrifuge (at least 10000 g)
- Vortex mixer
- Roller mixer or rocker
- **cobas e** analyzer

Additional materials for the **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Additional materials for **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 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 materials for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Manual specimen pretreatment

Follow the steps listed below to pretreat calibrators, controls and/or specimens. **The technical notes are an essential part of the instructions and must be read thoroughly before completing each step.** Follow Steps 1 through 7 to pretreat calibrators, controls and/or specimens.

Steps	Technical notes
1. Equilibrate all reagents, calibrators, controls and specimens to 20-25 °C. Mix all calibrators, controls and specimens gently but thoroughly just before use.	Do not vortex. The liquids may be mixed by hand or on a roller mixer or rocker. The calibrators and controls are a whole-blood hemolysate and may be slightly different in appearance from whole-blood samples.
2. Label one microcentrifuge tube for each calibrator, control and/or specimen to be pretreated.	none
3. Using a precision pipette, transfer 300 µL of each calibrator, control and/or specimen to the appropriately labeled micro-centrifuge tube.	Use a fresh pipette tip for each calibrator, control and/or specimen.
4. Using a precision pipette, add 300 µL of ISD Sample Pretreatment reagent to each microcentrifuge tube. Immediately cap each tube and immediately proceed to step 5.	Note: ISD Sample Pretreatment is highly volatile. Keep tightly closed when not in use to prevent evaporation.
5. Vortex each microcentrifuge tube for at least 10 seconds. Failure to perform this step may result in a supernatant that appears red. See Step 6, technical note.	Note: Failure to vortex each tube immediately after addition of the ISD Sample Pretreatment reagent will lead to erroneous assay results. Sample and reagent mixture should be completely homogeneous immediately after vortexing. Visual inspection is required.
6. Centrifuge the samples for at least 4 minutes in a micro-centrifuge (≥ 10000 g).	The centrifuged samples should have well-defined pellets and clear supernatant. The supernatant should not appear cloudy or red. If the supernatant is red, discard and replace it with a newly extracted sample.

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Steps	Technical notes
7. Transfer each supernatant directly into an appropriate vial and immediately cap each vial. The samples are ready to be assayed.	Pretreated samples can be stored in closed tubes for up to 4 hours at 20-25 °C. **Please note:** **Due to evaporation effects, pretreated samples should be analyzed/measured within 30 minutes after opening the vials and loading the samples on the system. Avoid delays between loading and measurement to ensure the 30 minutes stability of pretreated samples.** **This is supported by running the tacrolimus samples in batch mode:** **Based on average system sample processing time, no more than 35 tacrolimus samples may be loaded per calibrated measuring cell onto the analyzers at the same time.**

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

Calibration

Traceability: This method has been standardized against reference standards traceable to tacrolimus reference material (USP = United States Pharmacopeia) by weight.

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.

Tacrolimus CalSet must be pretreated freshly before calibration.

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

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

Renewed calibration is recommended as follows:

- after 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

Quality control

For quality control, use PreciControl ISD.

PreciControl ISD must be pretreated freshly before measurement.

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, nmol/L, µg/L).

Conversion factors: ng/mL x 1.0 = µg/L

ng/mL x 1.2438 = nmol/L

Limitations - interference

The effect of the following endogenous substances, pharmaceutical compounds and clinical conditions on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Criterion: Recovery within ± 0.3 ng/mL (concentration range > 0.5-2 ng/mL) or within ± 20 % (concentration range > 2-40 ng/mL) of initial value.

Endogenous substances:

Compound	Concentration tested
Albumin	≤ 12.0 g/dL
Bilirubin	≤ 1026 µmol/L or ≤ 60.0 mg/dL
Biotin	< 30.0 ng/mL or < 123 nmol/L
Cholesterol	≤ 500 mg/dL
HASA	≤ 10.0 µg/mL
Hematocrit	15-60 %
IgG	≤ 12.0 g/dL
Intralipid	≤ 1500 mg/dL
Rheumatoid factors	up to 500 IU/mL
Uric acid	≤ 20.0 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.

Pharmaceutical compounds:

In vitro tests were performed on 16 commonly used pharmaceutical compounds. No interference with the assay was found. Criterion: Recovery within ± 20 % of initial value.

26 special drugs were additionally tested. An interaction with Itraconazole (INN international nonproprietary name) was found. Do not use samples from patients under Itraconazole treatment.

Drug	Concentration tested
Acyclovir	3.2 µg/mL
Amphotericin B	5.8 µg/mL
Ciprofloxacin	7.4 µg/mL
Cyclosporine	5000 ng/mL
K_2-EDTA	6 mg/mL
K_3-EDTA	6 mg/mL
Erythromycin	20 mg/dL
Everolimus	60 ng/mL
Fluconazole	30 µg/mL
Flucytosine	40 µg/mL
Gancyclovir	1000 µg/mL
Gentamicin	12 mg/dL
Itraconazole	50 µg/mL
Kanamycin	100 µg/mL

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Drug	Concentration tested
Ketoconazole	50 µg/mL
Lidocaine	6 mg/dL
MPA (mycophenolic acid) glucuronide	1800 µg/mL
Mycophenolic acid	500 µg/mL
Nitrofurantoin	6 µg/mL
Phenobarbital	15 mg/dL
Sirolimus	60 ng/mL
Spectinomycin	100 µg/mL
Sulfomethoxazole	200 µg/mL
Tobramycin	2 mg/dL
Trimethoprim	40 µg/mL
Vancomycin	6 mg/dL

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

0.5-40 ng/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.5 ng/mL. Values above the measuring range are reported as > 40 ng/mL.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.3 ng/mL

Limit of Detection = 0.5 ng/mL

Limit of Quantitation = 1.0 ng/mL

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

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 defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable error of ≤ 20 %.

Dilution

Samples with tacrolimus concentrations above the measuring range can be manually diluted 1:3 with Diluent Universal prior to the manual pretreatment procedure. The concentration of the diluted sample must be > 5 ng/mL.

After manual dilution, multiply the result by the dilution factor.

Expected values

No firm therapeutic range exists for tacrolimus in whole blood. The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic effects of tacrolimus, coadministration of other immunosuppressants, type of transplant, time post-transplant, and a number of other factors contribute to different requirements for optimal blood levels of tacrolimus. Individual tacrolimus values cannot be used as the sole indicator for making changes in the treatment regimen. Each patient should be thoroughly evaluated clinically before treatment adjustments are made, and each assay user must establish his or her ranges based on clinical experience.

These ranges will vary according to the commercial in vitro diagnostic test used. Ranges must be established for each commercial test used.

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 (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
HSP[b)] 1	1.28	0.064	5.0	0.182	14.2
HSP 2	9.14	0.231	2.5	0.513	5.6
HSP 3	18.5	0.471	2.6	0.600	3.3
HSP 4	30.7	0.699	2.3	0.824	2.7
PC[c)] ISD1	2.49	0.107	4.3	0.213	8.6
PC ISD2	10.2	0.196	1.9	0.383	3.7
PC ISD3	19.6	0.532	2.7	0.571	2.9

b) HSP = Human Sample Pool

c) PC = PreciControl

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
HSP 1	1.20	0.059	4.9	0.124	10.4
HSP 2	8.97	0.225	2.5	0.349	3.9
HSP 3	18.3	0.355	1.9	0.649	3.5
HSP 4	31.3	0.684	2.2	1.59	5.1
PC ISD1	2.41	0.095	3.9	0.169	7.0
PC ISD2	10.0	0.221	2.2	0.298	3.0
PC ISD3	19.4	0.404	2.1	0.600	3.1

Method comparison

a) A comparison of the Elecsys Tacrolimus assay (y) with an automated immunoassay (x) using clinical samples gave the following correlations:

Number of samples measured: 205

Passing/Bablok[18]	Weighted linear regression
y = 0.958x - 0.0521	y = 0.985x - 0.198
τ = 0.922	r = 0.981

The sample concentrations were between 0.6 and 37.2 ng/mL.

b) A comparison of the Elecsys Tacrolimus assay (y) with an LC-MS-MS method (x) using clinical samples gave the following correlations:

Number of samples measured: 206

Passing/Bablok[18]	Weighted linear regression
y = 1.052x - 0.184	y = 1.059x - 0.196
τ = 0.933	r = 0.996

The sample concentrations were between 0.6 and 37.2 ng/mL.

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Analytical specificity

A study was performed with the Elecsys Tacrolimus assay based on guidance from the CLSI document EP7-A2.

Metabolite	Maximum concentration of metabolite added ng/mL	Cross-reactivity %
M I	50	n. d.[d)]
M II	50	70
M III	50	n. d.
M IV	50	n. d.

d) n. d. = not detectable

Cross-reactivity was designated as „not detectable“ if the obtained value was less than the sensitivity of the assay.

References

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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 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

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

Roche

Elecsys Vitamin D total II

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REF		Σ	SYSTEM
			cobas e 411
07464215190	07464215500	100	**cobas e** 601
			cobas e 602

English

System information

For **cobas e** 411 analyzer: test number 1500
For **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.[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 (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/$(bpy)_3^{2+}$(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:

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:

Elecsys Vitamin D total II

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

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.[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 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_2- and K_3-EDTA plasma.

Plasma tubes containing separating gel can be used.

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

Serum, Li-heparin, K_2- and K_3-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 (± 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, 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
- **cobas e** analyzer

Additional materials for the **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Additional materials for **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 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

Additionals materials for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

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

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

cobas e 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

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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 using internal standards which are traceable to the ID-LC-MS/MS 25-hydroxyvitamin D Reference Measurement Procedure.[18,19] The ID-LC-MS/MS is traceable to the National Institute of Standards and Technology Standard Reference Material 2972.[20]

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

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

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

Renewed calibration is recommended as follows:

- after 3 months (12 weeks) when using the same reagent lot
- after 7 days when using the same reagent kit on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Vitamin D total II.

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

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

Criterion: ± 2.0 ng/mL of initial value for samples ≤ 20 ng/mL and within ± 10 % of initial value for samples > 20 ng/mL.

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 ng/mL (< 7.5 nmol/L). Values above the measuring range are reported as > 100 ng/mL (> 250 nmol/L).

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 manually diluted with Diluent Universal or a suitable human serum with a low analyte concentration. The recommended dilution is 1:2. The concentration of the diluted sample must be ≥ 40 ng/mL (≥ 100 nmol/L). After manual dilution, multiply the results by the dilution factor 2. The endogenous analyte concentration of the human serum used for dilution has to be taken into account.

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]

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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.[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

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[b)] 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[c)] 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

cobas e 601 and **cobas e** 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

cobas e 601 and **cobas e** 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[27,28]	Passing Bablok[29]
y = 0.954x - 0.707	y = 0.937x - 0.360
r = 0.982	r = 0.902

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

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Cross-reactant	Concentration added ng/mL	Mean cross-reactivity %
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

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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 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

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

Roche

ms_05894913190V8.0

Elecsys Vitamin D total

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REF	Σ	SYSTEM
05894913 190	100	MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

System information

For **cobas e** 411 analyzer: test number 950
For MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 459

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.[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 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_3 (25-OH) whereas vitamin D_2 (25-OH) reaches measurable levels only in patients taking vitamin D_2 supplements.[2,3,4] Vitamin D_2 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; in elderly, the risk of falling has been attributed to the effect of vitamin D on muscle function.[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 total assay employs a vitamin D binding protein (VDBP) as capture protein to bind vitamin D_3 (25-OH) and vitamin D_2 (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 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:

Elecsys Vitamin D total

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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.
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.[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 (8 weeks)
on **cobas e** 411	21 days (3 weeks)
on MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602	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_2- and K_3-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 < ± 2 x Limit of Blank + coefficient of correlation > 0.9.

Serum, Li-heparin, K_2- and K_3-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 total 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.

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 4 x 3 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 automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Elecsys Vitamin D total

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Calibration

Traceability: This method has been standardized against LC-MS/MS[15] 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).

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

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

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) and biotin (< 287 nmol/L or < 70 ng/mL).

Criterion: For concentrations from LoQ up to 15 ng/mL, deviation is ≤ 1.5 ng/mL; for concentrations > 15 ng/mL, 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.

In vitro tests were performed on 17 commonly used pharmaceuticals and 5 special therapeutic drugs (Bonviva (Ibandronate), EinsAlpha (Alfacalcidol), Fosamax (Alendronate), Pamidron HEXAL (Pamidronate) and Zometa (Zoledronate)). No interference with the assay was found.

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

3.00-70.0 ng/mL or 7.50-175 nmol/L (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 3.00 ng/mL (< 7.50 nmol/L). Values above the measuring range are reported as > 70.0 ng/mL (> 175 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 %.

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 defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable relative error of ≤ 30 %.

The total error concept describes the maximum possible error of a test result taking into account the imprecision (SD) and inaccuracy (bias) of the test system. The Total Error (TE) was determined using the RMS (Root Mean Square) model (CLSI EP17-A2). The relative allowable total error refers to the respective concentration of the sample.

Dilution

Samples with vitamin D (25-OH) concentrations above the measuring range can be manually diluted with Diluent Universal or a suitable human serum with a low analyte concentration. The recommended dilution is 1:2. The concentration of the diluted sample must be > 30.0 ng/mL (> 75.0 nmol/L). After manual dilution, multiply the results by the dilution factor 2. The endogenous analyte concentration of the human serum used for dilution has to be taken into account.

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 apparently healthy individuals of Caucasian heritage. The age range was 20-77 years. Samples were collected between November and July in northern Germany.

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

Elecsys Vitamin D total

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			Gender			
	All (n = 453)		Female (n = 252)		Male (n = 201)	
Unit	ng/mL	nmol/L	ng/mL	nmol/L	ng/mL	nmol/L
Mean	20.6	51.5	21.6	54.0	19.4	48.5
2.5th percentile	5.26	13.2	6.23	15.6	4.92	12.3
97.5th percentile	47.0	118	49.9	125	42.7	107

A lower recovery may be found in particular clinical cohorts, for example dialysis patients.[23]

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP5-A2) 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[a] 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[b] 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

cobas e 411 analyzer					
			Intermediate precision		
Sample	Mean		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, **cobas e** 601 and **cobas e** 602 analyzers					
			Repeatability		
Sample	Mean		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, **cobas e** 601 and **cobas e** 602 analyzers					
			Intermediate precision		
Sample	Mean		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[24] 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[24] 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_0 and the results are summarized in the following table:

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	non detectable
1,25-dihydroxyvitamin D_2	non detectable
Vitamin D_3	non detectable
Vitamin D_2	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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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

CE

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