Sirolimus

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REF	Σ	SYSTEM
06327974 190	100	MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

Intended use

Immunoassay for the in vitro quantitative determination of sirolimus in human whole blood. The assay is used as an aid in the management of kidney transplant patients receiving sirolimus 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

Sirolimus (also called Rapamycin) is a macrocyclic antibiotic produced by the bacterium Streptomyces hygroscopicus. It was first discovered in soil samples taken from Easter Island (Rapa Nui). Sirolimus was initially developed as an antifungal agent. In 1988 its immunosuppressive properties were identified. FDA approved Sirolimus for use in the prevention of kidney transplant rejection in 1999. mTOR inhibitors such as sirolimus and everolimus provide a means of reducing the exposure of transplant patients to calcineurin inhibitors thus potentially limiting renal toxicity in non-renal transplant patients and improving long-term allograft survival in kidney transplant patients.[1,2]

Sirolimus is a proliferation signal inhibitor and blocks growth factor-induced transduction signals that mediate cellular division in response to alloantigens. Upon entry into the cell, sirolimus binds to the abundant immunophilin, FKBP-12, which also serves as a cytosolic receptor for tacrolimus. The sirolimus-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, sirolimus 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.[1,3]

The maximum concentration (c_{max}) is reached approximately 2 hours after administration of sirolimus. After absorption, sirolimus in circulation is extensively bound (approximately 92 %) to plasma proteins. Sirolimus is a substrate for both CYP3A4 and P-glycoprotein and is extensively metabolized in the liver and the intestinal wall, as well as being transported from enterocytes of the small intestine back into the gut lumen. Sirolimus is metabolized via O-demethylation and/or hydroxylation into 7 major metabolites that are identifiable within whole blood; the parent compound contributes > 90 % of the immunosuppressive activity. The elimination half-life of sirolimus after multiple dosing in stable renal transplant patients was estimated to be approximately 60 hours.[4]

Common side effects include hypertension, hyperlipidemia, anemia, thrombocytopenia, electrolyte disturbances (hypokalemia and hypophosphatemia), peripheral edema, abdominal pain, arthralgia, skin disorders, pyrexia, headache, nausea, diarrhea or constipation, and a higher incidence of lymphoceles.[5]

Therapeutic drug monitoring (TDM) of sirolimus trough concentrations (C0) is necessary, especially due to the wide inter- and intra-individual variability in the pharmacokinetic behavior of the drug.[2,6] There has been general agreement that the predose (trough or C0) sample is a good reflection of total exposure, as measured by area under the time-concentration curve (AUC). Good correlation has been shown between sirolimus C0 concentrations and AUC. This is also the case when the drug is used in combination with cyclosporine or tacrolimus.[7,8]

A key reason to monitor the drug concentration is that the dosage is a poor predictor of drug exposure. As the clearance of sirolimus approximates liver blood flow, dose reductions guided by measured concentrations of sirolimus should be made in patients with impaired liver function. Close drug monitoring is also necessary as sirolimus is metabolized by the cytochrome P450-3A4 isoenzyme that metabolizes many other drugs and is thus responsive to drug-drug interactions. There are numerous data on drug interactions that might cause an increase or decrease in sirolimus blood concentrations, such as those reported for cyclosporine and tacrolimus.[7]

Test principle

Manual precipitation:

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

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

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: By incubating 35 µL of the pretreated sample with a sirolimus-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 sirolimus 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.

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

Reagents - working solutions

The reagent rackpack is labeled as SRL.

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

Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-sirolimus Ab~biotin (gray cap), 1 bottle, 9 mL:

Biotinylated monoclonal anti-sirolimus antibody (rabbit) 35 µg/L; phosphate buffer 100 mmol/L, pH 7.8; preservative.

R2 Sirolimus derivate~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 mL:

Sirolimus 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.

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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	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 in a sufficient number 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 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 06327982190, Sirolimus CalSet, for 6 x 1.0 mL
- REF 05889081190, PreciControl ISD, 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, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

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

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.

Steps	Technical notes
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 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 sirolimus samples in batch mode:** **Based on average system sample processing time, no more than 35 sirolimus 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.

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 sirolimus 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.

Sirolimus 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). 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.

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 1.0939 = 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 everolimus, 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 everolimus treatment or under transition from everolimus to sirolimus. 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.[3]

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 = 1.5 ng/mL with a total allowable error of ≤ 25 %

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 sirolimus 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 sirolimus in whole blood. The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic effects of sirolimus, 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 sirolimus. Individual sirolimus 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-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
HSP[b)] 1	1.19	0.123	10.3	0.130	10.9
HSP 2	3.96	0.145	3.7	0.167	4.2
HSP 3	13.2	0.314	2.4	0.436	3.3
HSP 4	23.3	0.955	4.1	0.959	4.1
HSP 5	29.6	0.716	2.4	0.838	2.8
PC ISD[c)] 1	2.81	0.149	5.3	0.173	6.2
PC ISD 2	9.57	0.265	2.8	0.347	3.6
PC ISD 3	17.3	0.432	2.5	0.494	2.9

b) HSP = Human Sample Pool

c) PC ISD = PreciControl ISD

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	1.03	0.086	8.3	0.098	9.5
HSP 2	4.11	0.171	4.2	0.220	5.4
HSP 3	13.8	0.383	2.8	0.550	4.0
HSP 4	23.3	0.670	2.9	0.970	4.2
HSP 5	28.9	0.870	3.0	1.20	4.1
PC ISD 1	2.62	0.133	5.1	0.181	6.9
PC ISD 2	9.52	0.313	3.3	0.464	4.9
PC ISD 3	16.9	0.358	2.1	0.584	3.4

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Method comparison

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

Number of samples measured: 119

Passing/Bablok[9]	Weighted Deming regression
y = 0.916x + 0.743	y = 0.992x + 0.319
τ = 0.828	r = 0.967

The sample concentrations were between 2.2 and 20.5 ng/mL.

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

Number of samples measured: 120

Passing/Bablok[9]	Weighted Deming regression
y = 1.03x + 0.625	y = 1.13x + 0.058
τ = 0.832	r = 0.977

The sample concentrations were between 1.1 and 18.1 ng/mL.

Analytical specificity

Metabolite	Maximum concentration added ng/mL	Maximum cross-reactivity[d] %
11-Hydroxy sirolimus	25	2.9
12-Hydroxy sirolimus	25	10.4
16-O-Desmethyl sirolimus	25	1.8
27-O-Desmethyl sirolimus	25	65.0
39-O-Desmethyl sirolimus	25	108.6

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

References

1 Sehgal SN. Sirolimus: its discovery, biological properties, and mechanism of action. Transplant Proc 2003;35(Suppl 3A):7S-14S. Review.

2 Halleck F, Duerr M, Waiser J, et al. An evaluation of sirolimus in renal transplantation. Expert Opin Drug Metab Toxicol 2012;8(10):1337-1356.

3 Augustine JJ, Bodziak KA, Hricik DE. Use of sirolimus in solid organ transplantation. Drugs 2007;67(3):369-391.

4 Wyeth Pharms INC. Rapamune Package Insert. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/021083s044s047,021083s044s047lbl.pdf [Last accessed 06-Jun-2014].

5 Merkel S, Mogilevskaja N, Mengel M, et al. Side effects of sirolimus. Transplant Proc 2006;38:714-715.

6 Aspeslet LJ, Yatscoff RW. Requirements for therapeutic drug monitoring of sirolimus, an immunosuppressive agent used in renal transplantation. Clin Ther 2000;22 Suppl B, B86-B92.

7 Holt DW, Denny K, Lee TD, et al. Therapeutic monitoring of sirolimus: its contribution to optimal prescription. Transplant Proc 2003;35(Suppl 3A):157S-161S.

8 Kahan BD, Napoli KL, Kelly PA, et al. Therapeutic drug monitoring of sirolimus: correlations with efficacy and toxicity. Clin Transplantation 2000;14:97-109.

9 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

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.

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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