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 for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

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:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

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P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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
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

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Steps	Technical notes
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.
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

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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
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

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cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
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.

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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

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

Elecsys Tacrolimus

CALIBRATOR Calibrator

→ Volume after reconstitution or mixing

GTIN Global Trade Item Number

CE 0123

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