

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k173857

B. Purpose for Submission:

New Device

C. Measurand:

Tacrolimus

D. Type of Test:

Quantitative immunoassay

E. Applicant:

Roche Diagnostics

F. Proprietary and Established Names:

Elecsys Tacrolimus

G. Regulatory Information:

1. Regulation section:

21 CFR 862.1678, Tacrolimus test system

2. Classification:

Class II

3. Product code:

MLM

4. Panel:

Toxicology (91)

H. Intended Use:

1. Intended use(s):

See Indications for Use below.

2. Indication(s) for use:

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

The electrochemiluminescence immunoassay “ECLIA” is intended for use on Elecsys and cobas e immunoassay analyzers.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Roche cobas e 411

I. Device Description:

The Elecsys Tacrolimus assay uses electrochemiluminescence for detection and measurement of tacrolimus. The reagent working solutions for this assay are packaged as a rackpack (kit placed on the analyzer) and include the following:

- Reagent M, containing streptavidin-coated microparticles (0.72 mg/mL in preservative)
- Reagent R1, containing biotinylated monoclonal anti-tacrolimus-antibody (sheep) (15 µg/L in phosphate buffer with preservative)
- Reagent R2 containing tacrolimus-derivative labeled with ruthenium complex (Tris(2,2'-bipyridyl)ruthenium(II)- complex (Ru(bpy) (4 µg /L in citrate buffer with preservative)

Samples must be treated with ISD Sample Pretreatment reagent before testing, which is available as a 30 mL bottle containing zinc sulfate solution in methanol and ethylene glycol.

J. Substantial Equivalence Information:

1. Predicate device name(s):

ARCHITECT Tacrolimus Assay

2. Predicate 510(k) number(s):

k070820

3. Comparison with predicate:

Similarities		
Item	Candidate Device Roche Elecsys Tacrolimus (k173857)	Predicate Device Abbott ARCHITECT Tacrolimus (k070820)
Intended Use/Indications for 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 liver and kidney transplant patients receiving tacrolimus therapy.	Same
Sample Matrix	EDTA anti-coagulated whole blood	Same
Sample Preparation	Manual Pretreatment	Same
Reagent format	Liquid, ready-to-use	Same

Differences		
Item	Candidate Device Roche Elecsys Tacrolimus (k173857)	Predicate Device Abbott ARCHITECT Tacrolimus (k070820)
Test Principle	Electrochemiluminescence using electrochemiluminescent immunoassay (ECLIA) technology	Delayed one-step immunoassay using chemiluminescent microparticle immunoassay (CMIA) technology
Traceability	The ARCHITECT Tacrolimus Calibrators are traceable to an internal reference standard gravimetrically prepared and confirmed via	This method has been standardized against reference standards traceable to tacrolimus reference material (USP = United States

Differences		
Item	Candidate Device Roche Elecsys Tacrolimus (k173857)	Predicate Device Abbott ARCHITECT Tacrolimus (k070820)
	HPLC.	Pharmacopeia) by weight.
Assay Range	0.75 to 30.0 ng/mL	2.0 to 30.0 ng/mL

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP05-A2 Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline Second Edition

CLSI EP06-A Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline First Edition

CLSI EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline Second Edition

CLSI EP15 User Verification of Precision and Estimation of Bias; Approved Guideline Third Edition

Class II Special Controls Guidance Document: Cyclosporine and Tacrolimus Assays; Guidance for Industry and FDA

L. Test Principle:

The Elecsys Tacrolimus assay uses the principle of electrochemiluminescence for detection and measurement. Before testing with the Elecsys Tacrolimus assay, the specimen, calibrators and controls are pretreated with the Elecsys ISD Pretreatment Reagent. The reagent lyses the cells, extracts Tacrolimus and precipitates blood proteins. The pretreated samples are centrifuged, and an aliquot of the resulting supernatant containing tacrolimus is then assayed using the Elecsys Tacrolimus reagent.

The Elecsys Tacrolimus is a competitive immunoassay.

- 1st incubation: 35 µL of pretreated sample is incubated with a tacrolimus-specific biotinylated antibody and a ruthenium complex-labelled tacrolimus-derivative. If there is no tacrolimus in the sample, the labelled antibody binding site is occupied only with ruthenium complex-labelled tacrolimus-derivative. As the concentration of tacrolimus in the sample increases, the labelled antibody binding site is occupied in part with sample tacrolimus and in part with ruthenium complex labelled tacrolimus-derivative, thus the amount of antibody bound ruthenium complex labelled tacrolimus-derivative is inversely proportional to the quantity of tacrolimus in the plasma specimen.
- 2nd incubation: After addition of streptavidin-coated microparticles 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. Application of a voltage to the electrode then induces electrochemiluminescent 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Internal Precision Study

An internal precision study was conducted using the CLSI guideline document EP05-A2 as a guide. Four pooled patient whole blood samples and single-donor spiked human whole blood samples (Human Sample Pool or HSP) and three controls (PeciControl ISD Level 1, 2 and 3) were measured using one lot of reagent. Each sample was tested in single determination in four separate aliquots (divided in two runs per day) for 21 operating days, and samples were randomized for each run. A separate extraction was performed with the ISD Sample Pretreatment reagent for each sample measurement.

Sample	n	Mean (ng/mL)	Repeatability		Intermediate precision	
			SD (ng/mL)	CV %	SD (ng/mL)	CV %
HSP 1	168	1.28	0.064	5.0	0.182	14.2
HSP 2	168	9.14	0.231	2.5	0.513	5.6
HSP 3	168	18.5	0.471	2.6	0.600	3.3
HSP 4	168	30.7	0.699	2.3	0.824	2.7
PC 1	168	2.49	0.107	4.3	0.213	8.6
PC 2	168	10.2	0.196	1.9	0.383	3.7
PC 3	168	19.6	0.532	2.7	0.571	2.9

HSP = Human Sample Pool

PC = PeciControl

External Precision Study

Imprecision of Elecsys Tacrolimus was determined at three external sites using CLSI guideline documents EP05-A2 and EP15 as guides. The study was conducted using six human sample pool samples (HSP) and three levels of quality control material (PeciControl ISD). The study was conducted using three reagent lots, three cobas e 411 analyzers, at three external sites. Reproducibility testing was conducted in two runs, per day, for five days.

			Repeatability		Between Day		Between Site		Reproducibility	
Sample	N	Mean (ng/mL)	SD	% CV	SD	% CV	SD	% CV	SD	% CV
HSP 01	90	2.67	0.19	7.3	0.18	6.8	0.21	7.8	0.34	12.6
HSP 02	90	6.23	0.52	8.3	0.28	4.4	0.25	4.0	0.64	10.2
HSP 03	90	11.38	0.69	6.1	0.55	4.8	0.44	3.9	0.99	8.7
HSP 04	90	20.66	0.74	3.6	0.86	4.2	0.63	3.0	1.30	6.3
HSP 05	90	27.76	1.19	4.3	2.09	7.5	1.52	5.5	2.85	10.2
HSP 06	90	34.85	1.27	3.6	2.19	6.3	1.05	3.0	2.74	7.9
PC 1	90	2.11	0.12	5.5	0.15	7.0	0.18	8.3	0.26	12.1
PC 2	90	9.49	0.28	3.0	0.51	5.4	0.37	3.9	0.69	7.3
PC 3	90	15.83	0.39	2.5	0.85	5.3	0.19	1.2	0.95	6.0

HSP = Human Sample Pool

PC = PreciControl

b. Linearity/assay reportable range:

The linearity of the tacrolimus assay was assessed on the cobas e 411 analyzer. Three dilution series were prepared from three different spiked human whole blood samples. Each dilution series included 22 dilutions and included concentrations between 0.5 to 32.5 ng/mL. Each sample was measured 4-fold within one run and the measured concentrations were plotted against the expected sample concentration. A separate extraction was performed with the ISD Sample Pretreatment reagent for each sample. The linearity data was determined in accordance with CLSI EP6-A. The results from a representative dilution series are as follows:

$$y=1.0942x-0.1099; r^2=0.9980$$

The sponsor's claimed measuring range is 0.75 to 30 ng/mL.

Recovery

The recovery of the tacrolimus assay was evaluated using pooled human whole blood (drug free) and patient samples.

Drug-free human whole blood pools were spiked with known concentrations of tacrolimus. The patient derived samples (from patients taking tacrolimus) were measured prior spiking, to determine the endogenous concentration. Each of these aliquots was spiked with a defined concentration of analyte (5 ng/mL, 10 ng/mL, 15 ng/mL and 20 ng/mL) to obtain at least four different spiking levels for each patient sample (five total samples). Each level was spiked independently to produce 5 replicates. For each measurement, a separate extraction was performed with the ISD

Sample Pretreatment reagent.

For the pooled human whole blood samples, the recovery was calculated using the mean of all five spiked samples compared to the expected concentration. For the spiked patient samples, the recovery was calculated using the mean spiked concentration.

The results are summarized in the tables below.

Pooled Human Whole Blood Samples:

Tacrolimus spiked amount (ng/mL)	Avg % Recovery	Range of % Recovery
1.80	98.3	86.7% to 108.9%
5.00	103	98% to 104.8%
10.0	100	96.9% to 106%
20.0	101	100% to 105%
30.0	98.3	96.0% to 101%

Patient Samples Recovery

Tacrolimus spiked amount (ng/mL)	Avg % Recovery	Range of % Recovery
0	-	-
5	106	102% to 109%
10	102	99% to 107%
15	100	94% to 100%
20	99	101% to 105%

Dilution Study

A dilution study was performed to validate the manual dilution protocol recommended by the manufacturer in the package insert (1:3 dilution on samples with results > 30.0 ng/mL). The labeling specifies that samples must be diluted manually prior to pretreatment.

Three pooled human whole blood samples were spiked separately with a known concentration of tacrolimus. These samples were used to prepare a dilution series of nine respective dilutions. Each sample was measured in duplicate. These samples were diluted with the Elecsys Diluent Universal prior to the manual pre-treatment procedure. A separate extraction was performed with the ISD Sample Pretreatment reagent for each sample measurement. The percent recoveries were calculated between the expected and the measured concentration, and ranged from 91.4% to 117%. *tc. Traceability, Stability, Expected values (controls, calibrators, or methods):*

Traceability:

Elecsys Tacrolimus is traceable to gravimetrically prepared tacrolimus reference material (USP), confirmed by LC-MS/MS.

Real-time stability studies were conducted on the Roche Tacrolimus CalSet test material to support the sponsor's storage claim for 15 months at 4°C.

The sponsor conducted sample stability studies to support the following claims:

- 5 days at 15-25°C
- 7 days at -20°C or lower for up to 3 months. Specimens should only be frozen once.
- Pretreated samples can be stored in closed tubes up to 4 hours at 20-25°C. Pretreated samples should be analyzed/measured within 30 minutes after opening the vials and loading the samples on the system.

d. Detection limit:

Limit of Blank (LoB)

LoB of the Elecsys Tacrolimus assay has been determined according to CLSI EP17-A2. The Limit of Blank was determined as the 95th percentile of the measurement of blank samples. The distribution of values for five analyte-free whole blood samples was determined with one reagent lot on two cobas e 411 analyzers with six runs distributed over a period of 5 days. The sample was measured in one-fold determination in each run. In summary, 30 measuring points were collected per instrument, for a total of 60 measured values. The LoB was determined to be 0.078 ng/mL.

Limit of Detection (LoD):

The distribution of values for five spiked human whole blood samples with low analyte has been determined with one reagent lot on two cobas e 411 analyzers with six runs distributed over a period of 5 days. Samples were measured in one-fold determination in each run. 30 measuring points were collected per instrument, for a total of 60 measured values. The sum of standard deviations (SD total) of the five samples was calculated. The LoD was determined according to the following EP17-A2 calculation:

$$\text{LoD} = \text{LoB} + 1.653 \times \text{SD total (of low analyte samples)}$$

A separate extraction was performed with the ISD Sample Pretreatment reagent for each sample measurement. LoD was determined to be 0.2209 ng/mL.

Limit of Quantitation (LoQ)

LoQ of the Elecsys Tacrolimus assay has been determined according to CLSI EP17-A2. The LoQ was determined as the lowest concentration of analyte that can be quantified with a total error of $\leq 25\%$. Testing was conducted using three reagent lots on two cobas e 411 analyzers with six runs distributed over six days. A separate extraction was performed with the ISD Sample Pretreatment reagent for each sample measurement. 84 measuring points were collected per instrument, for a total of 168 measured values per lot.

Reagent lot	LoQ (ng/mL)
1	0.629
2	0.597
3	0.580

The results support the sponsor's claimed LoQ of 0.75 ng/mL.

e. Analytical specificity:

Analytical Specificity/Cross Reactivity

Potential cross-reacting compounds were spiked into two human whole blood samples. For metabolites I – IV, the spiked samples were evaluated at four concentrations. A separate extraction was performed with the ISD Sample Pretreatment reagent for each sample measurement. For metabolites V- VIII, the spiked samples were evaluated at two concentrations. The percent cross reactivity was calculated using the following equation:

$$\% \text{ of cross reactivity} = 100 * [(\text{measured result from sample with spiked analyte} - \text{measured result from unspiked sample}) / \text{spiked concentration of cross-reactant}]$$

Metabolite	Metabolite Name	Purity	Max Concentration Added (ng/mL)	Max Cross-Reactivity (%)
M-I	13-O-desmethyl Tacrolimus	> 99.0	50	1
M-II	31-O-desmethyl Tacrolimus	> 99.0	50	71
M-III	15-O-desmethyl Tacrolimus	> 99.0	50	3
M-IV	12-hydroxy Tacrolimus	> 99.0	50	1

Metabolite	Metabolite Name	Purity	Max Concentration Added (ng/mL)	Max Cross-Reactivity (%)
M-V	15,31-di-O-desmethyl Tacrolimus	60/40*	6.6	52
M-VI	13,31-di-O-desmethyl Tacrolimus	99	50	1
M-VII	13,15-di-O-desmethyl Tacrolimus	99	50	0
M-VIII	N/A	80/20†	50	4

Endogenous Interferences

Low and high tacrolimus-containing whole blood samples (approximately 3 ng/mL and 10 ng/mL tacrolimus, respectively) were divided into two aliquots each. One aliquot was spiked with the interfering substance up to a concentration given in the table below, while the other was spiked with diluent to serve as a spike volume control. Each sample was tested in 10 replicates. The recovery for each sample was calculated based on the difference between the interferent-spiked and control-spiked results. The sponsor claims that no significant interference was observed based on the following specifications:

- LoQ to 3 ng/mL must be $\leq \pm 0.3$ ng/mL recovery to reference
- > 3 to 30 ng/mL must be $\leq \pm 10\%$ recovery to the reference

Compound	Highest Concentration Tested With No Significant Interference
Albumin	12.0 g/dL
Bilirubin	1026 μ mol/L or 60.0 mg/dL
Cholesterol	500 mg/dL
HASA ^{b)}	10 μ g/mL
Hematocrit	25-60%
IgG	12.0 g/dL
Intralipid	1500 mg/dL
Rheumatoid factors	500 IU/mL
Uric Acid	20 mg/dL

Specimens with biotin concentrations up to 100 ng/mL demonstrated $\leq 10\%$ bias in results. Biotin concentrations greater than 100 ng/mL led to higher positive bias Tacrolimus results. The following is a summary of the biotin interference testing conducted by the sponsor.

% Bias for samples containing various concentrations of biotin					
Samples (ng/mL)	Biotin concentration (ng/mL)				
	100	200	250	300	350
2.28	+5.1%	+11.2%	+20.2%	+27.4%	+30.8%
18.1	+1.8%	+6.1%	+8.6%	+12.4%	+14.7%

% Bias for samples containing various concentrations of biotin				
Samples (ng/mL)	Biotin concentration (ng/mL)			
	400	600	1000	1200
2.28	+41.7%	+87.8%	+165.6%	+203.7%
18.1	+17.8%	+34.4%	+76.5%	+93.3%

The sponsor's labeling states the following in the Limitations (Interference) section:

“Do not test samples from patients who are taking biotin.”

Exogenous Interferences

41 exogenous compounds were spiked into human whole blood samples containing high and low concentrations of tacrolimus (spiked with approximately 3 ng/mL and 10 ng/mL tacrolimus). The samples were tested in 10 replicates. The mean value was used to calculate the percent recovery of the drug sample to the reference. A separate extraction was performed with the ISD Sample Pretreatment reagent for each sample measurement. The sponsor claims that no significant interference was observed (based on recovery within $\pm 10\%$ of the control value) for the following compounds:

Compound	Highest Concentration Tested With No Significant Interference
Acetaminophen	200 mg/L
N-Acetylcysteine	1660 mg/L
Acetylsalicylic acid	1000 mg/L
Acyclovir	3.2 $\mu\text{g/mL}$
Amphotericin B	5.8 $\mu\text{g/mL}$
Ampicillin-Na	1000 mg/L
Ascorbic acid	300 mg/L
Cefoxitin	2500 mg/L
Ciprofloxacin	7.4 $\mu\text{g/mL}$
Cyclosporine	5000 ng/mL
Doxycycline	50 mg/L
Erythromycin	20 mg/mL
Everolimus	60 ng/mL
Fluconazole	30 $\mu\text{g/mL}$
Flucytosine	40 $\mu\text{g/mL}$

Compound	Highest Concentration Tested With No Significant Interference
Gancyclovir	1000 µg/mL
Gentamicin	12 mg/dL
Heparin	5000 U/L
Ibuprofen	500 mg/L
K2-EDTA	6 mg/mL
K3-EDTA	6 mg/mL
Kanamycin	100 µg/mL
Ketoconazole	50 µg/mL
Levodopa	20 mg/L
Lidocaine	6 mg/dL
Methyldopa+1.5	20 mg/L
Metronidazole	200 mg/L
MPA (mycophenolic	1800 µg/mL
Mycophenolic acid	500 µg/mL
Nitrofurantoin	6 µg/mL
Phenobarbital	15 mg/dL
Phenylbutazone	400 mg/L
Rifampicin	6 mg/dL
Sirolimus	60 ng/mL
Spectinomycin	100 µg/mL
Sulfomethoxazole	200 µg/mL
Theophylline	100 mg/L
Tobramycin	2 mg/dL
Trimethoprim	40 µg/mL
Vancomycin	6 mg/dL

Itraconazole was found to interfere (>10%) at 50 and 10 mg/L (ug/mL) (single daily drug dose for Itraconazole). The sponsor states the following in their labeling: “Itraconazole (INN international non-proprietary name) recovery was found to interfere at 10 µg/mL (114%).”

f. Assay cut-off:

Not applicable. This is a quantitative assay.

2. Comparison studies:

a. Method comparison with predicate device:

The sponsor conducted a method comparison study of the candidate device, compared against both the predicate immunoassay device (ARCHITECT Tacrolimus Assay, k070820) and a validated LC-MS/MS method. The candidate device was evaluated at

three sites, whereas the predicate device and LC-MS/MS comparator method were evaluated at a single site. Four reagent lots were evaluated for the candidate device. A total of 571 eligible samples were available for the study, 355 from kidney transplant patients and 216 from liver transplant patients. Each sample was drawn from an individual patient. The results of regression analyses are shown below.

Elecsys Tacrolimus vs Abbott ARCHITECT Tacrolimus				
Total # of samples		Slope (95% CI) (Deming)	Intercept (95% CI) (Deming)	Correlation (r)
Combined 553		0.99 (0.98, 1.01)	0.06 (-0.07, 0.18)	0.99
Kidney	346	1.01 (0.98, 1.03)	0.04 (-0.17, 0.24)	0.99
Liver	207	0.97 (0.95, 0.99)	0.13 (-0.01, 0.27)	0.99
Elecsys Tacrolimus vs. LC-MS/MS				
Total # of samples		Slope (95% CI) (Deming)	Intercept (95% CI) (Deming)	Correlation (r)
Combined 554		0.92 (0.90, 0.95)	-0.01 (-0.16, 0.14)	0.96
Kidney	344	0.93 (0.90, 0.96)	0.04 (-0.19, 0.27)	0.97
Liver	210	0.91 (0.87, 0.95)	-0.05 (-0.25, 0.16)	0.95

b. Matrix comparison:

The sponsor's labeling states that only K2-EDTA and K3 EDTA whole blood may be used.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

The following is stated in the package insert:

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. Values obtained with different assay methods cannot be used interchangeably due to differences in assay methods and cross-reactivity with metabolites, nor should correction factors be applied. Therefore, consistent use of one assay for individual patients is recommended. These ranges will vary according to the commercial in vitro diagnostic test used. Ranges must be established for each commercial test used.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.