November 6, 2018

Roche Diagnostics
Khoa Tran
Regulatory Affairs Principal
9115 Hague Road
Indianapolis, IN 46250

Re: K173857
Trade/Device Name: Elecsys Tacrolimus
Regulation Number: 21 CFR 862.1678
Regulation Name: Tacrolimus test system
Regulatory Class: Class II
Product Code: MLM
Dated: October 23, 2018
Received: October 24, 2018

Dear Khoa Tran:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's

requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/CombinationProducts/GuidanceRegulatoryInformation/ucm597488.htm); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/) and CDRH Learn (http://www.fda.gov/Training/CDRHLearn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (http://www.fda.gov/DICE) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Kellie B. Kelm -S

for Courtney H. Lias, Ph.D.
Director
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
k173857

Device Name
Elecsys Tacrolimus

Indications for Use *(Describe)*
Immunoassay for the in vitro quantitative determination of tacrolimus in EDTA 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 cobas e immunoassay analyzers.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

This section applies only to requirements of the Paperwork Reduction Act of 1995.

DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.

The burden time for this collection of information is estimated to average 79 hours per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."

510(k) Summary

k173857

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CFR 807.92.

Submitter Name	Roche Diagnostics
Address	9115 Hague Road Indianapolis, IN, 46250
Contact	Khoa Tran Phone: (317) -521-3409 FAX: (317) 521-2324 Email: khoa.tran@roche.com
Date Prepared	November 5th, 2018
Proprietary Name	Elecsys Tacrolimus
Common Name	Tacrolimus assay
Classification Name	Tacrolimus Test System
Product Codes	MLM, 21 CFR 862.1678
Predicate Devices	Abbott ARCHITECT Tacrolimus Assay k070820
Establishment Registration	Roche Diagnostics GmbH in Mannheim, Germany, is 9610126 Roche Diagnostics GmbH in Penzberg, Germany, is 9610529 Roche Diagnostics in the United States is 1823260

1. DEVICE DESCRIPTION

The Elecsys Tacrolimus immunoassay 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 virtually all 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.

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.

1.1. Reagents

The reagent working solution includes:

- 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) (black cap), 1 bottle, 8 mL: Tacrolimus‑derivative labeled with ruthenium complex 4 µg /L; citrate buffer 10 mmol/L, pH 3.3; preservative

1.2. ISD Sample Pretreatment reagent

ISD Sample Pretreatment is labeled as ISD Sample PT and it includes:

- 1 bottle containing 30 mL

Contents: zinc sulfate solution in methanol and ethylene glycol

2. INDICATIONS FOR USE

Immunoassay for the in vitro quantitative determination of tacrolimus in EDTA 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. TECHNOLOGICAL CHARACTERISTICS

Table 1: Assay Comparison

Assay Comparison		
Feature	**Predicate Device: Abbott ARCHITECT Tacrolimus (k070820)**	**Candidate Device: Elecsys Tacrolimus**
General Assay Features		
Intended Use/ Indications for Use	The ARCHITECT Tacrolimus assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of tacrolimus in human whole blood on the ARCHITECT i System. The ARCHITECT Tacrolimus assay is to be used as an aid in the management of liver and kidney allograft patients receiving tacrolimus therapy	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. The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.
Test Principle	The ARCHITECT Tacrolimus assay is a delayed one-step immunoassay for the quantitative determination of tacrolimus in human whole blood using CMIA technology with flexible assay protocols, referred to as Chemiflex. Prior to the initiation of the automated ARCHITECT sequence, a manual pretreatment step is performed in which the whole blood sample is extracted with a precipitation reagent and centrifuged. The supernatant is decanted into a Transplant Pretreatment Tube, which is placed onto the ARCHITECT System Sample, assay diluent, and anti-tacrolimus coated paramagnetic microparticles are combined to create a reaction mixture. Tacrolimus present in the sample binds to the anti-tacrolimus coated microparticles. After a delay, tacrolimus acridinium-labeled conjugate is added to the reaction mixture. The tacrolimus on the acridinium-labeled conjugate competes for the available binding sites on the microparticles. Following incubation, the microparticles are washed, and pre-trigger and trigger solutions are	The Elecsys Tacrolimus immunoassay 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 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 reagent. 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 (Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)) labeled Tacrolimus-derivative. Depending on the concentration of the analyte in the sample and the

Assay Comparison		
Feature	**Predicate Device: Abbott ARCHITECT Tacrolimus (k070820)**	**Candidate Device: Elecsys Tacrolimus**
	added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). An indirect relationship exists between the amount of tacrolimus in the sample and the RLUs detected by the ARCHITECT' System optics.	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 entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.

Assay Comparison		
Feature	**Predicate Device:** **Abbott ARCHITECT Tacrolimus (k070820)**	**Candidate Device:** **Elecsys Tacrolimus**
		• 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 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.
Sample Matrix	Human Whole Blood	Same
Sample Preparation	Manual Pretreatment	Same
Reagent	Liquid Ready-to-Use (Antibody coati microparticle reagent, Tacrolimus conjugate reagent, and Assay diluent)	Liquid Ready-to-Use (Streptavidin-coated microparticle reagent, biotinylated anti-Tacrolimus antibody reagent, tacrolimus conjugated reagent).
Assay Range	2.0 to 30.0 ng/mL	0.75 to 30.0 ng/mL
Detection Protocol	Chemiluminescent Microparticle immunoassay (CMIA)	Electrochemiluminescent Immunoassay (ECLIA)
Applications	Did not specified	18-minute application
Instrument Platform	ARCHITECT i System	**cobas e** 411
Sample Type	Human Whole Blood	Same
Reagents	Competition principle	Competition principle. Total duration of assay: 18 minutes
Calibration Interval	Once an ARCHITECT Tacrolimus calibration is accepted and stored, all subsequent samples may be tested without further calibration unless: • A reagent kit with a new lot number is used. • Controls are out of range.	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
Traceability / Standardization	The ARCHITECT Tacrolimus Calibrators are traceable to an internal reference standard gravimetrically prepared and confirmed via HPLC. Calibrators' values must be within ±1 % of the reference calibrator value.	This method has been standardized against reference standards traceable to tacrolimus reference material (USP = United States Pharmacopeia) by weight.

<table>
<tr><th colspan="3">Assay Comparison</th></tr>
<tr><th>Feature</th><th>Predicate Device:
Abbott ARCHITECT Tacrolimus (k070820)</th><th>Candidate Device:
Elecsys Tacrolimus</th></tr>
<tr><td>Reagent Stability</td><td>Unopened: 2-8°C - Up to the stated expiration date.
After Opening at 2-8°C – 30 days
On the Analyzers – 30 days</td><td>Unopened: 2-8°C - Up to the stated expiration date.
After Opening at 2-8°C – 84 days
On the Analyzers – 56 days</td></tr>
<tr><td>Measuring Range</td><td>2 -30 ng/mL</td><td>0.75 – 30 ng/mL</td></tr>
<tr><td>Precision</td><td>Within-run (results from two instruments)
<table>
<tr><th>Sample</th><th>Mean (ng/mL)</th><th>SD</th><th>CV (%)</th></tr>
<tr><td>Level 1</td><td></td><td></td><td></td></tr>
<tr><td>Instrument1</td><td>3.0</td><td>0.1</td><td>3.7</td></tr>
<tr><td>Instrument 2</td><td>2.9</td><td>0.2</td><td>5.8</td></tr>
<tr><td>Level 2</td><td></td><td></td><td></td></tr>
<tr><td>Instrument1</td><td>7.8</td><td>0.2</td><td>2.4</td></tr>
<tr><td>Instrument 2</td><td>8.5</td><td>0.2</td><td>2.7</td></tr>
<tr><td>Level 3</td><td></td><td></td><td></td></tr>
<tr><td>Instrument1</td><td>14.5</td><td>0.4</td><td>2.5</td></tr>
<tr><td>Instrument 2</td><td>15.7</td><td>0.5</td><td>2.9</td></tr>
<tr><td>Panel 1</td><td></td><td></td><td></td></tr>
<tr><td>Instrument1</td><td>5.5</td><td>0.2</td><td>3.6</td></tr>
<tr><td>Instrument 2</td><td>5.9</td><td>0.2</td><td>4.0</td></tr>
<tr><td>Panel 2</td><td></td><td></td><td></td></tr>
<tr><td>Instrument1</td><td>14.0</td><td>0.5</td><td>3.5</td></tr>
<tr><td>Instrument 2</td><td>15.3</td><td>0.6</td><td>4.1</td></tr>
<tr><td>Panel 3</td><td></td><td></td><td></td></tr>
<tr><td>Instrument1</td><td>4.8</td><td>0.2</td><td>4.4</td></tr>
<tr><td>Instrument 2</td><td>4.9</td><td>0.2</td><td>5.0</td></tr>
<tr><td>Panel 4</td><td></td><td></td><td></td></tr>
<tr><td>Instrument1</td><td>10.1</td><td>0.2</td><td>2.4</td></tr>
<tr><td>Instrument 2</td><td>11.2</td><td>0.5</td><td>4.1</td></tr>
<tr><td>Panel 5</td><td></td><td></td><td></td></tr>
<tr><td>Instrument1</td><td>21.2</td><td>0.7</td><td>3.3</td></tr>
<tr><td>Instrument 2</td><td>22.4</td><td>0.8</td><td>3.6</td></tr>
</table>
Abbott Immunosuppressant-MCC (levels 1, 2 and 3) and five whole blood panels were assayed, using two lots of reagents, on two instruments, in replicates of two at two separate times per day for 20 days.</td><td>cobas e 411:
Within-run (will be labeled Intermediate precision)
<table>
<tr><th>Sample</th><th>Mean (ng/mL)</th><th>SD</th><th>CV (%)</th></tr>
<tr><td>HSP 1</td><td>1.28</td><td>0.182</td><td>14.2</td></tr>
<tr><td>HSP 2</td><td>9.14</td><td>0.513</td><td>5.6</td></tr>
<tr><td>HSP 3</td><td>18.5</td><td>0.600</td><td>3.3</td></tr>
<tr><td>HPS 4</td><td>27.07</td><td>0.882</td><td>3.3</td></tr>
<tr><td>PC ISD1</td><td>2.49</td><td>0.213</td><td>8.6</td></tr>
<tr><td>PC ISD2</td><td>10.2</td><td>0.383</td><td>3.7</td></tr>
<tr><td>PC ISD 3</td><td>19.6</td><td>0.571</td><td>2.9</td></tr>
</table>
HSP = Human Sample Pool
PC = PreciControl</td></tr>
<tr><td>Analytical Sensitivity</td><td>Limit of detection ≤ 1.5 ng/mL</td><td>Limit of Blank (LoB): = 0.3 ng/mL
Limit of Detection (LoD): = 0.5 ng/mL
Limit of Quantitation (LoQ): = 0.75 ng/mL</td></tr>
</table>

<table>
<tr><th colspan="3">Assay Comparison</th></tr>
<tr><th>Feature</th><th>Predicate Device:
Abbott ARCHITECT Tacrolimus (k070820)</th><th>Candidate Device:
Elecsys Tacrolimus</th></tr>
<tr><td>Analytical Specificity</td><td>
<table>
<tr><th>Cross reactant (Metabolite)</th><th>Amount Added (ng/mL)</th><th>% Cross-reactivity</th></tr>
<tr><td>M I (13-O-demethyl)</td><td>10</td><td>8</td></tr>
<tr><td>M II (31-O-demethyl)</td><td>10</td><td>94</td></tr>
<tr><td>M III (15-O-demethyl)</td><td>10</td><td>45</td></tr>
<tr><td>M IV (12-O-hydroxy)</td><td>10</td><td>9</td></tr>
<tr><td>M V</td><td>N/A*</td><td>N/A*</td></tr>
<tr><td>M VI</td><td>N/A*</td><td>N/A*</td></tr>
<tr><td>M VII</td><td>N/A*</td><td>N/A*</td></tr>
<tr><td>M III</td><td>N/A*</td><td>N/A*</td></tr>
</table>
*Not available
</td><td>
<table>
<tr><th>Cross reactant (Metabolite)</th><th>Maximum Concentration of Metabolite Added (ng/mL)</th><th>% Cross-reactivity</th></tr>
<tr><td>M I (13-O-demethyl)</td><td>50</td><td>1</td></tr>
<tr><td>M II (31-O-demethyl)</td><td>50</td><td>71</td></tr>
<tr><td>M III (15-O-demethyl)</td><td>50</td><td>3</td></tr>
<tr><td>M IV (12-O-hydroxy)</td><td>50</td><td>1</td></tr>
<tr><td>M V</td><td>6.6</td><td>52</td></tr>
<tr><td>M VI</td><td>50</td><td>1</td></tr>
<tr><td>M VII</td><td>50</td><td>0</td></tr>
<tr><td>M VIII</td><td>50</td><td>4</td></tr>
</table>
</td></tr>
<tr><td>Linearity</td><td>2 to 30 ng/mL</td><td>0.75-30 ng/mL</td></tr>
<tr><td>Limitations</td><td>The assay is unaffected by:
Albumin 12 mg/dL
Bilirubin ≤ 40.0 mg/dL
Hematocrit ≤ 25%, ≥ 55 %
Cholesterol 500 mg/dL
HAMA 215 ng/mL
Rheumatoid Factor 245 IU
Triglycerides 800 mg/dL
Uric Acid 20 mg/dL</td><td>The assay is unaffected by:
Albumin ≤ 12.0 g/dL
Bilirubin ≤ 66.0 mg/dL
Cholesterol ≤ 500 mg/dL
HASA ≤ 10 µg/mL
Human IgG ≤ 12 g/dL
Hematocrit 25- 60 %
Intralipid ≤ 2000 mg/dL
Rheumatoid factors up to 1200 IU/mL
Uric acid ≤ 30 mg/dL
In vitro tests were performed on 16 commonly used pharmaceuticals and 25 special drugs. No significant interference with the assay was observed.
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.
For Itraconazole and biotin interference, see Sections 5.10 and 5.11 respectively.</td></tr>
</table>

<table>
<tr><th colspan="3">Assay Comparison</th></tr>
<tr><th>Feature</th><th>Predicate Device:
Abbott ARCHITECT Tacrolimus (k070820)</th><th>Candidate Device:
Elecsys Tacrolimus</th></tr>
<tr><td colspan="3">Labeled Performance Characteristics</td></tr>
<tr><td>Method Comparison</td><td colspan="2">
<table>
<tr><th colspan="2">Elecsys Tacrolimus vs. Abbott ARCHITECT Tacrolimus</th></tr>
<tr><td>n = 553</td><td>Weighted Deming Regression</td></tr>
<tr><td>Slope (95% CI)</td><td>0.99 (0.98, 1.01)</td></tr>
<tr><td>Intercept (95% CI)</td><td>0.06 (-0.07, 0.18</td></tr>
<tr><td>r</td><td>0.99</td></tr>
</table>
<table>
<tr><th colspan="2">Elecsys Tacrolimus vs. LC-MS/MS</th></tr>
<tr><td>n = 554</td><td>Weighted Deming Regression</td></tr>
<tr><td>Slope (95% CI)</td><td>0.92 (0.90, 0.95)</td></tr>
<tr><td>Intercept (95% CI</td><td>-0.01(-0.16, 0.14)</td></tr>
<tr><td>r</td><td>0.96</td></tr>
</table>
</td></tr>
</table>

4. TECHNOLOGICAL CHARACTERISTICS

The assay employs a competitive principle using electrochemiluminescence technology.

5. NON-CLINICAL PERFORMANCE EVALUATION

5.1. Precision

Non-clinical performance evaluations for the Elecsys Tacrolimus executed with the study briefly summarized.

Precision of the Tacrolimus assay was evaluated on the **cobas e** 411 analyzer. Within-run precision (repeatability) and total imprecision (intermediate precision) were determined according to the CLSI EP5-A2 precision evaluation experiment.

A seven-member panel consisting of four pooled patient and single donor spiked human whole blood samples and three controls (PreciControl ISD Level 1, 2 and 3) were measured. The protocol consisted of testing the samples in single determination in four separate aliquots (divided in two runs per day) for 21 operating days. The measurements were performed on the

cobas e 411 with one reagent lot, performing rack pack calibration according to instruction for use.

5.1.1. Specifications

	Repeatability	Intermediate Precision
LoQ – 3.5 ng/mL	SD ≤ 0.25 ng/mL	SD ≤ 0.35 ng/mL
> 3.5 – 12 ng/mL	CV ≤ 5 %	CV ≤ 6 %
> 12 – 30 ng/mL	CV ≤ 6 %	CV ≤ 7 %

All results met the pre-defined acceptance criteria for repeatability and intermediate precision.

5.2. Limit of Blank (LoB)

LoB of the 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.

Acceptance criterion: LoB ≤ 0.3 ng/mL

5.3. Limit of Detection (LoD)

LoD of the Tacrolimus assay has been determined according to CLSI EP17-A2. The LoD was determined as the lowest amount of analyte in a sample that can be detected with a 95% probability. 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. In summary, 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: LoD = LoB + 1.653 x SD_{total} (of low analyte samples).

Acceptance criterion: LoD ≤ 0.5 ng/mL

5.4. Limit of Quantitation (LoQ)

LoQ of the Tacrolimus assay has been determined according to CLSI EP17-A2. The LoQ was determined as the lowest concentration of analyte which can be quantified with a total error of no more than 25%. The distribution of values for 14 spiked human whole blood samples each diluted to concentrations which covered the range between LoB and 2x LoQ has been determined with three reagent lot on two **cobas e** 411 analyzers with six runs distributed over six days. Each run was calibrated separately using a two-point calibration. Samples were extracted separately and measured in one-fold determination in each run. In summary, a total of 84 measuring points were collected per instrument, for a total of 168 measured values per lot. A separate extraction was performed with the ISD Sample Pretreatment reagent for each sample measurement. The total error (TE) was calculated based on the following equation:

$$TE = \sqrt{s^2 + bias^2}$$

Since the % CV and % bias are a function of the concentration, LoQ was calculated individually for each member of the Low level sample set.

The LoQ samples are sorted according to the concentration of their measured mean value. LoQ is defined as the mean value of that sample which is the first that fulfills specifications for TE_{rel} and for which no sample with lower concentration exists that exceeds this specification.

A graphic is generated that plots the measured mean values of the LoQ samples (x) against the relative total error (TE_{rel}) (y).

Acceptance criterion: 25% total error at LoQ ≤ 0.75 ng/mL

5.5. Recovery (Accuracy)

The measure of accuracy study included the ISD free and patient samples. ISD-free human blood is spiked with known concentration of tacrolimus (USP or other suitable reference material). Each level will be spiked independently (no dilution series). The patient derived samples (from patients taking Tacrolimus) were measured prior spiking, to evaluate the 'endogenous' concentration. The samples were then divided into at least four aliquots. Each of this aliquot is 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. Each level will be spiked independently (no dilution series). The samples were measured on **cobas e** 411.

Acceptance criterion:

- ≤ ± 0.3 ng/mL for samples LoQ to 3 ng/mL
- ± 10% for samples > 3 ng/mL

Based on data, the recovery (accuracy) meets the specifications.

5.6. Linearity

The linearity of the Tacrolimus assay was assessed on the **cobas e** 411 immunoassay analyzer; three dilution series were prepared from three different spiked human whole blood samples. Each dilution series included 22 dilutions. 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 measurement. The linearity data was determined in accordance with CLSI EP6-A. In a first step, a linearity check was performed with a first order (linear) regression and then with higher order models (quadratic and cubic).

Acceptance criterion:

- ≤ ± 0.2 ng/mL for samples LoQ to 2 ng/mL
- ± 10% for samples > 2 ng/mL

Linearity was confirmed in the range from 0.75 to 30 ng/mL.

5.7. Dilution

To demonstrate the Tacrolimus assay dilution study, three ISD-free human whole blood samples were spiked separately with a known concentration of gravimetrically weight tacrolimus. These samples were used to prepare a dilution series of nine respective dilutions. Each sample was measured in duplicates (n=2).

The dilution factor for samples outside the measuring range is 1:3. The samples prepared above were diluted with the Elecsys Diluent Universal prior to the manual pre-treatment procedure.

Testing was performed on two **cobas e** 411 analyzers. The percent recoveries were calculated between the expected and the measured concentrations.

5.8. Analytical Specificity

The specificity was determined using the cross-reactant compound. Each cross-reactant compound was spiked into two human whole blood samples (analyte-free and slightly elevated analyte level). Testing was performed with one reagent lot in one run. The spiked samples were evaluated at four dilutions.

5.9. Endogenous Interferences

The effect on quantitation of analyte in the presence of endogenous interfering substances using the Tacrolimus was determined on the cobas e 411 immunoassay analyzer for the following 10 interfering substances Intralipid, Biotin, Bilirubin, Rheumatic Factor, Human Serum Albumin, IgG, Cholesterol, HASA, Uric Acid and Hematocrit using three spiked human whole blood samples (one low and one high concentration) to prepare dilution series of 10 dilutions that were tested with one reagent lot.

For each interfering substance the following protocol was followed:

One low and one high analyte containing sample were divided into two aliquots each. One aliquot was spiked with the interfering substance up to a concentration given in the table below (sample b) while the other was treated without interferent to mimic the dilution effect (sample a).

The recovery for each sample was calculated by comparison to the reference sample.

For Hemotocrit interfering substance the following protocol was followed:

ISD-free human EDTA blood is split into two equal volume fractions. A sample is taken to measure the initial hematocrit using a hematocrit centrifuge. Cellular constituents of each fraction are separated from the plasma either by centrifugation at approximately 1000 g for about 15 minutes or overnight sedimentation in the fridge. Blood plasma of the two fractions is then blended in a way that hematocrit concentrations of 15 % resp. 65 % are achieved. Afterwards the samples are homogenized for at least 30 min on a roller mixer at room temperature. After homogenization the two sample preparations are spiked with equal amounts of analyte (USP or other suitable reference material) to reach desired concentrations and again homogenized overnight on a roller mixer at 2-8 °C.

Thereafter the two stocks (15% and 65% hematocrit) are blended in a way to obtain 11 hematocrit dilutions ranging from 15% to 65%, roller mixed for at least 30 minutes at room temperature and then measured. The recovery for every single sample was calculated based on the mean result of the respective first 10 replicates. *Acceptance criterion:*

- LoQ to ≤ 3.0 ng/mL must be ≤ ± 0.3 ng/mL absolute deviation
- > 3 to 30 ng/mL must be ≤ ± 10% recovery to the reference

5.10. Exogenous Interferences — Drugs

The effect on quantitation of analyte in the presence of drugs was determined by using 16 common and 25 additional pharmaceutical compounds were spiked into each two spiked human tacrolimus containing human whole blood samples. The spiked samples (single donor samples spiked with 3 ng/mL and 10 ng/mL tacrolimus) were evaluated at drug concentrations greater than the daily dose and tested for interference by the Elecsys Tacrolimus assay on **cobas e** 411 immunoassay analyzer. Acceptance criterion is recovery within ± 10 % of initial value. All drugs met the predetermined criterion except for Itraconazole. The Itraconazole (INN international non‑proprietary name) recovery was found to interfere at 10 µg/mL (114%) .

5.11. Biotin interference

Biotin interference was tested up to 1,200 ng/mL in whole blood samples at low and high concentrations of Tacrolimus. Mean concentration of Biotin dilution sample divided by mean corresponding sample without Biotin. The results are summarized in the table below:

% Bias for samples containing various concentrations of biotin									
Samples (ng/mL)	Biotin Concentration (ng/mL)								
	100	200	250	300	350	400	600	1000	1200
2.28	+5.1%	+11.2%	+20.2%	+27.4%	+30.8%	+41.7%	+87.8%	+165.6%	+203.7%
18.1	+1.8%	+6.1%	+8.6%	+12.4%	+14.7%	+17.8%	+34.4%	+76.5%	+93.3%

The labeling states:

Specimens with biotin concentrations up to 100 ng/mL demonstrated ≤ 10 % bias in results. Biotin concentrations greater than 100 ng/mL led to higher positive bias Tacrolimus results. Some studies have shown that serum concentrations of biotin can reach up to 355 ng/mL within the first hour after biotin ingestion for subjects consuming supplements of 20 mg biotin per day and up to 1160 ng/mL for subjects after a single dose of 300 mg biotin.

Do not test samples from patients who are taking biotin.

6.1. Reagent Stability

To test reagent stability, the reagent stability was performed in three different studies.

6.1.1. Study 1: Reagent Stability after First Opening (2-8ºC)

Reagent stability after first opening for the Elecsys Tacrolimus assay was determined on a **cobas e** 411 immunoassay analyzer by comparing the reagent stability for four kits of the same lot. All reagent kits were opened on day 0. One kit was placed on the analyzer and calibrated and reference values for the samples tested were determined. The other three kits were stored at 2 to 8°C. After 36, 64 and 92 days, one of the stored kits was placed on the analyzer and calibrated, and the original test samples were measured. Samples tested include five human whole blood samples (pooled patient samples and single donor samples spiked with tacrolimus) and three controls. Acceptance criterion for recovery was compared to day 0 value.

6.1.2. Study 2: On-board Reagent Stability

On-board reagent stability for the Tacrolimus assay was tested on one **cobas e** 411 immunoassay analyzer. A fresh kit was placed on the analyzer and calibrated. Reference values for the samples tested were determined. After measurement, the kit was closed and kept at 20°C ± 3°C for 9 weeks (64 days) to simulate on-board conditions. Measurements were repeated every week for nine weeks (64 days). The kit was placed on the analyzer again utilizing the calibration curve from seven days earlier for determinations of stability, and the original test samples were measured. The recovery was compared to the measurements from day one. Samples were tested with one reagent lot in one run per day on one **cobas e** 411 analyzer in duplicate. Samples tested included five human whole blood samples (pooled patient samples and single donor samples spiked with tacrolimus) and three controls (PreciControl ISD). Acceptance criterion for recovery was compared to day 1 value.

6.1.3. Study 3: Shelf Life Stability

In the real-time stability study, the Tacrolimus assay material was stored at 2 to 8°C. The stored assay reagents were tested at time point T=0 and at specified intervals over the shelf life of the device up to the planned shelf life plus one month. Testing was performed using PreciControl ISD 1, 2 and 3 (stored at -20°C). For the production lots 171852, 172226 and 173556 data for the time-points at 0, 8, 10 and 16 months were tested in duplicates.

The average on-test recovery was calculated as percent recovery compared to the reference value (Assigned value for PreciControl ISD 1, 2 and 3).

The acceptance criterion for PreciControl ISD 1 is recovery of 75 – 125% of the reference value.

The acceptance criterion for PreciControl ISD 2 and 3 are recovery of 80 – 120% of the reference value.

6.2. Sample Stability

The sample stability was performed with six different studies and they are:

- Study 1. Sample stability before pre-treatment at room temperature (15 to 25°C)
- Study 2. Sample stability before pre-treatment at 2 to 8°C

- Study 3. Sample stability before pre-treatment at -20°C
- Study 4. Stability through freeze/thaw cycles
- Study 5. Sample stability after pre-treatment on-board in Hitachi sample cups
- Study 6. Sample stability after pre-treatment at room temperature in closed Hitachi sample cups

The stability claims to be tested are:

- 5 days storage at room temperature before pretreatment
- 7 days storage before pretreatment at 2-8°C
- 1 time freeze/thaw before pretreatment
- 4 hours storage at room temperature after pretreatment in closed tube
- 30 minutes on the analyzer after pretreatment
- 1 month storage before pretreatment at -15°C to -25°C

The percent recovery was calculated based on reference T0 (fresh sample) values.

The data collected in house supported the short stability claims. Literature reference is use to support 1 month stability claim.

7. CLINICAL PERFORMANCE EVALUATION

7.1. Reproducibility

Reproducibility was evaluated by testing human sample pools and PreciControl materials with the Elecsys Tacrolimus assay on the e 411 analyzer at three sites. Testing was conducted in accordance with requirements within CLSI EP5-A2 and EP15-A2. Table 3 below provides the summary of the clinical reproducibility study.

Table 2: Reproducibility

			Repeatability			Between Day		Between Lot		Between Site		System Reproducibility		
Sample	**N**	**Mean (ng/mL)**	**SD**	**UCL of SD 1-sided 95%**	**% CV**	**SD**	**% CV**	**SD**	**% CV**	**SD**	**% CV**	**SD**	**UCL of SD 1-sided 95%**	**% CV**
HSP 01	90	2.67	0.19	0.23	7.3	0.18	6.8	0.00	0.0	0.21	7.8	0.34	0.53	12.6
HSP 02	90	6.23	0.52	0.61	8.3	0.28	4.4	0.00	0.0	0.25	4.0	0.64	0.78	10.2
HSP 03	90	11.38	0.69	0.81	6.1	0.55	4.8	0.00	0.0	0.44	3.9	0.99	1.26	8.7
HSP 04	90	20.66	0.74	0.87	3.6	0.86	4.2	0.00	0.0	0.63	3.0	1.30	1.75	6.3
HSP 05	90	27.76	1.19	1.40	4.3	2.09	7.5	0.00	0.0	1.52	5.5	2.85	4.09	10.2
HSP 06	90	34.85	1.27	1.49	3.6	2.19	6.3	0.00	0.0	1.05	3.0	2.74	3.54	7.9
PC ISD_L1	90	2.11	0.12	0.14	5.5	0.15	7.0	0.00	0.0	0.18	8.3	0.26	0.45	12.1
PC ISD_L2	90	9.49	0.28	0.33	3.0	0.51	5.4	0.00	0.0	0.37	3.9	0.69	0.99	7.3
PC ISD_L3	90	15.83	0.39	0.46	2.5	0.85	5.3	0.00	0.0	0.19	1.2	0.95	1.18	6.0

Note: SD of zero due to variance contributed by particular component was below stated significant figure.

7.2. Method Comparison

Clinical test results for enrolled subjects were obtained using the Elecsys Tacrolimus Immunoassay and two comparative methods: Abbott ARCHITECT Tacrolimus, and LC-MS/MS, in order to validate performance of the Elecsys Tacrolimus Immunoassay. Table 4 below provides the summary of the clinical method comparison study.

Table 3: Summary of Clinical Method Comparison

Abbott ARCHITECT Tacrolimus vs. Elecsys 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

8. CONCLUSIONS

The information provided in this Premarket Notification [510(k)] will support a determination of substantial equivalence for the Elecsys Tacrolimus Assay.