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

A. 510(k) Number:
k070820

B. Purpose for Submission:
New device

C. Measurand:
Tacrolimus

D. Type of Test:
Quantitative immunoassay

E. Applicant:
Fujirebio Diagnostics Inc.

F. Proprietary and Established Names:
ARCHITECT Tacrolimus Assay
ARCHITECT Tacrolimus Calibrators

G. Regulatory Information:
1. Regulation section:
   21 CFR 862.1678, Tacrolimus test system
   21 CFR 862.3200, Calibrator
2. Classification:
   Class II (special controls)
   Class II
3. Product code:
   MLM
   JIT
4. Panel:
   Clinical Toxicology (91)

H. Intended Use:
1. Intended use(s):
   See Indications for Use below.
2. Indication(s) for use:

   Reagent Kit
   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.

Calibrator Kit
The ARCHITECT Tacrolimus Calibrators are for the calibration of the ARCHITECT *i* System when used for the quantitative determination of tacrolimus in human whole blood.

Whole Blood Precipitation Reagent
The ARCHITECT Tacrolimus Whole Blood Precipitation Reagent is for the extraction of tacrolimus from samples (human whole blood patient specimens, controls, and ARCHITECT Tacrolimus Calibrators) to be tested on the ARCHITECT *i* System.

3. Special conditions for use statement(s):
   For prescription use only; see Expected Values Section M.5 for further limitations.

4. Special instrument requirements:

   The Fujirebio ARCHITECT Tacrolimus assay is for use on ARCHITECT *i* System.

I. Device Description:
The assay reagent kit consists of a bottle of anti-tacrolimus microparticles in buffer with preservatives and stabilizers, a bottle of tacrolimus-acridinium conjugate in buffer with preservatives and stabilizers, and assay diluent. The calibrator kit consists of six bottles of calibrators ranging from zero to 30 ng/mL tacrolimus; Calibrator A (zero) contains additional volume so it can be used as a diluent for out of range specimens.

J. Substantial Equivalence Information:
1. Predicate device name(s):
   ABBOTT IMx Tacrolimus II Microparticle Enzyme Immunoassay

2. Predicate 510(k) number(s):
   P970007 (note: tacrolimus test systems have been reclassified into Class II since the predicate was approved)

3. Comparison with predicate:
   The predicate and the proposed device have similar intended uses, assay ranges, sample types, sample preparation steps, and both are mouse monoclonal antibody-based assays.

   The assays differ in their instrument platform (ARCHITECT vs. IMx) and enzymatic detection methodology (acridinium conjugate vs. alkaline phosphatase).

K. Standard/Guidance Document Referenced (if applicable):
CLSI Document EP5-A – "Evaluation of Precision Performance of Clinical Chemistry Devices"

L. 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. 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 i 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 solutions that initiate the chemiluminesence reaction are 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 i System optics.

M. Performance Characteristics (if/when applicable):
1. Analytical performance:
    a. *Precision/Reproducibility:*
       Precision was assessed at three different sites (the manufacturer's site and two clinical sites). Studies were modeled after CLSI EP5-A2 "Evaluation of Precision Performance of Clinical Chemistry Devices". The manual pretreatment extraction step was performed for each replicate to verify the effect of pretreatment procedure on precision estimates. Each site tested two pooled patient samples and one in-house quality control sample. In addition, the manufacturer's site tested two additional in-house quality control samples and Abbott Immunosuppressant-Multi-Constituent Controls (MCC). Each sample was analyzed in duplicate using two lots tested twice per day for 20 days (n=80).

       The results of the manufacturer's in-house testing for within run and total precision are shown in the table below; n= 80 for all samples, reagent lot 1 was tested on Instrument 1 while reagent lot 2 was tested on Instrument 2. Precision at the clinical sites was comparable.

In-house Precision Study: Fujirebio Architect Tacrolimus Assay

Sample Description	Instrument	Mean (ng/mL)	WithinRun		Total	
			SD	%CV	SD	%CV
MCC Level 1	1	3.0	0.1	3.7	0.1	4.9
(sub therapeutic)	2	2.9	0.2	5.8	0.2	6.7
MCC Level 2	1	7.8	0.2	2.4	0.3	3.6
(therapeutic)	2	8.5	0.2	2.7	0.4	4.2
MCC Level 3	1	14.5	0.4	2.5	0.5	3.5
(toxic)	2	15.7	0.5	2.9	0.6	4.0
Pooled Patient Samples–	1	5.5	0.2	3.6	0.2	4.4
sub-therapeutic range	2	5.9	0.2	4.0	0.3	5.2
Pooled Patient Samples	1	14.0	0.5	3.5	0.6	4.2
–therapeutic range	2	15.3	0.6	4.1	0.7	4.7
In-house Control	1	4.8	0.2	4.4	0.2	5.2
(sub-therapeutic range)	2	4.9	0.2	5.0	0.3	6.3
In-house Control	1	10.1	0.2	2.4	0.4	4.4
(therapeutic range)	2	11.2	0.5	4.1	0.6	5.3
In-house Control	1	21.2	0.7	3.3	0.9	4.4
(toxic range)	2	22.4	0.8	3.6	1.3	5.7

b. *Linearity/assay reportable range:*
Linearity across the claimed assay range (2 – 30 ng/mL) was demonstrated by diluting five whole blood samples that had been spiked to achieve tacrolimus concentrations in the upper end of the assay range. Architect Tacrolimus Calibrator A (0 ng/mL) was used as the diluent. Samples were extracted as per instructions and each sample was tested in quadruplicate and averaged. Linearity was defined as 90 – 110% recovery compared to the calculated value. Recovery of individual samples ranged from 94% to 109% of the expected values; the grand mean recovery was 102%. The linear regression equation that represented all samples tested was $y = 0.98x + 0.36$ with a correlation coefficient of 0.99.

Recovery of tacrolimus was assessed by spiking aliquots of ten (10) normal human EDTA whole blood samples with four concentrations of tacrolimus, pre-treating the sample according to manufacturer's directions, and testing in duplicate. The percent recovery was calculated as follows by using the mean of the two samples: % Recovery = (Mean observed concentration)/ (Mean Endogenous concentration + Added Concentration) * 100. A summary of the results is shown in the following table:

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Recovery Study: Fujirebio Architect Tacrolimus Assay

Spike Level (ng/mL)	Ave % Recovery n = 10	Range of % Recoveries n = 10
6.9	101%	94 - 115%
9.3	101%	93 - 104%
15.2	106%	103 - 109%
18.8	101%	98 - 106%

A high sample dilution study was conducted to evaluate the accuracy of results when a high sample is diluted with Architect Tacrolimus Calibrator A. Five patient negative samples with tacrolimus concentrations ranging from 0 to 6 ng/mL were spiked with an additional 45 ng/mL tacrolimus solution then diluted 1:2 and 1:4, extracted and assayed in quadruplicate. The percent recovery for the samples diluted 1:2 ranged from 91 to 99%. The percent recoveries for the samples diluted 1:4 ranged from 91 to 99%.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
   Accelerated stability data currently supports a shelf life claim of 8 months for the ARCHITECT Tacrolimus Calibrators; real time testing is underway.

   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.

   The sponsor presented a study that supports the claim that specimens collected in EDTA may be stored at 2 – 8 °C for up to 7 days before testing.

   The sponsor conducted a freeze-thaw study using twelve whole blood samples spiked with different levels of tacrolimus. Aliquots of the spiked samples were frozen and thawed one to ten times then assayed and compared to an unfrozen aliquot. Individual sample recovery was between 86 – 107% while average recoveries for each freeze-thaw cycle ranged between 96 – 104%.

d. *Detection limit:*
   An analytical sensitivity study was conducted by assaying the 0 ng/mL tacrolimus calibrator in replicates of 10 on three different instruments over four separate runs. The sponsor defined analytical sensitivity as the mean of the 0 ng/mL calibrator plus 2 SD - the lowest concentration of tacrolimus that can be distinguished from zero with a 95% confidence. The mean was 0.2 ng/mL and the SD was 0.05. Thus, the limit of blank was calculated as 0.3 ng/mL. The sensitivity results support the sponsors' lower limit of the range claim of 2 ng/mL.

   A functional sensitivity study was conducted to determine the lowest concentration of tacrolimus at which the sample coefficient of variation (CV)

is 20% for the ARCHITECT Tacrolimus assay. Human whole blood was spiked with tacrolimus to obtain concentrations at the following concentrations: 0.1, 0.2, 0.5, 1, 2, 3, and 5 ng/mL. Ten replicates of each sample were run twice a day for five days. The mean tacrolimus concentration and total % CV was calculated for each concentration. Regression analysis of the resulting values showed that the analyte concentration corresponding to a 20% CV is 0.8 ng/mL, below the sponsors' lower limit of the range of 2 ng/mL.

e. *Analytical specificity:*
   Potential interferences were evaluated by comparing the concentration of samples with known amounts of endogenous substances and tacrolimus to samples containing only tacrolimus. All interferents were tested at two or more concentrations of tacrolimus. All samples were pretreated and assayed in duplicate and the mean was calculated. Results, expressed as percent recovery compared to the control, are summarized in the chart below:

Endogenous Interferent Study: Fujirebio Architect Tacrolimus Assay

Endogenous Interferent	Highest Concentration Tested	Percent Recovery
Albumin	12 mg/dL	101 %
Bilirubin	40 mg/dL	101 %
Cholesterol	500 mg/dL	102 %
HAMA	215 ng/mL	97 %
Hematocrit	25 %	105 %
Hematocrit	55 %	106 %
Rheumatoid Factor	245 IU	99 %
Triglycerides	800 mg/dL	96 %
Uric Acid	20 mg/dL	98 %

Potential cross-reactivity of the assay with some tacrolimus metabolites was evaluated by adding 10 ng/mL of each metabolite to whole blood samples spiked with one of five different levels of tacrolimus across the assay range. Duplicates of each metabolite/tacrolimus concentration were assayed; the difference between the tacrolimus only sample and the tacrolimus and metabolite sample was used to calculate cross-reactivity.

Total cross-reactivity was calculated as the mean of each of the differences divided by the total concentration of the metabolite added. The results are shown in the chart below:

Tacrolimus Metabolite Interference Study:
Fujirebio Architect Tacrolimus Assay

Metabolite	Mean Conc. Difference	% Cross Reactivity
M-I (13-O-demethyl tacrolimus)	0.6 ng/mL	6 %
M-II (31-O-demethyl tacrolimus)	9.4 ng/mL	94%
M-III (15-O-demethyl tacrolimus)	4.5 ng/mL	45 %
M-IV (12-hydroxy tacrolimus)	0.8 ng/mL	8 %
* M-V, M-VI, M-VII, and M-VIII were not tested		

The sponsor conducted an interference study on 54 commonly co-administered drugs, including several immunosuppressive drugs. Samples containing known amounts of co-administered drug and tacrolimus were compared to samples containing only tacrolimus. All co-administered drugs were tested at two levels tacrolimus (therapeutic and toxic range). All samples were pretreated and assayed in duplicate and the mean was calculated. Recovery of tacrolimus from the samples containing co-administered drug were between 95% and 104 % of the control sample. A complete list of the compounds tested can be found in the assay package insert.

f. Assay cut-off:
Not applicable for this assay.

2. Comparison studies:
    a. Method comparison with predicate device:
    The ARCHITECT Tacrolimus assay was compared against two methods: liquid chromatography/tandem mass spectrometry (LC/MS/MS) and the Abbott IMx® Tacrolimus II Assay. Two external sites compared the two assay methods while samples were also assessed by LC/MS/MS in the manufacturer's laboratory. Trough samples were obtained from liver and kidney transplant patients. The samples were prepared as per manufacturer's instructions. Samples below the measurable range were excluded from analysis.

Method Comparison Study: Fujirebio Architect Tacrolimus Assay

Study	n	Regression Analysis *			Sample Range (by comparator method, ng/mL)
		Slope (95% CI)	**Intercept (95% CI)**	**r value (95% CI)**	
Site 1					
FDI Tacrolimus vs. LC-MS/MS	125	1.07x (1.01 – 1.12)	+ 0.22 (0.02 – 0.48)	0.92 (0.88 – 0.94)	1.8 – 19.2
FDI Tacrolimus vs. IMx Assay	124	0.81x (0.75 – 0.88)	+ 0.37 (0 – 0.68)	0.92 (0.86 – 0.93)	2.1 – 15.9
Site 2					
FDI Tacrolimus vs. IMx Assay	133	0.92x (0.87 – 0.99)	- 0.07 (-0.47 - 0.37)	0.93 (0.91 – 0.93)	2.7 – 27.4
Site 3					
FDI Tacrolimus vs. IMx Assay	63	0.97x (0.86 – 1.12)	- 1.19 (-1.71 – 0.39)	0.84 (-2.52 – -0.2)	3.1 – 18.4
* Passing-Bablok Method					

There were no significant differences in the performance of the assay between liver and renal allograft patient samples.

   b. *Matrix comparison:*
      Not applicable; EDTA whole blood is the recommended assay matrix.

3. Clinical studies:
   a. *Clinical Sensitivity:*
      Not applicable for this assay.
   b. *Clinical specificity:*
      Not applicable for this assay.
   c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off**:**
   Not applicable for this assay.

5. Expected values/Reference range:
   The recommended range of tacrolimus concentrations in whole blood for effective postoperative management of kidney and liver allograft transplant patients is 5 ng/mL to 20 ng/mL **using LC/MS**. The optimal therapeutic range for tacrolimus in whole blood has not been established with this assay.

   The complexity of the clinical state, individual differences in sensitivity to

immunosuppressive and nephrotoxic effects of tacrolimus, co-administration 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. Therefore, individual tacrolimus values cannot be used as the sole indicator for making changes in treatment regimen and each patient should be thoroughly evaluated clinically before changes in treatment regimens are made. Each user must establish his or her own ranges based on clinical experience.

Therapeutic ranges vary according to the commercial test used, and therefore should be established for each commercial test. 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. When elimination of tacrolimus is impaired (e.g. during cholestasis), tacrolimus metabolites may accumulate. The immunoassay may overestimate the concentration of tacrolimus. In such cases, the use of a specific assay (e.g. Liquid Chromatography Mass Spectrometry/Mass Spectrometry [LC/MS/MS]) could be considered.

N. Proposed Labeling:
The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

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