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

A. 510(k) Number:

k080751

B. Purpose for Submission:

New Device

C. Measurand:

Cyclosporine

D. Type of Test:

Quantitative chemiluminescent microparticle immunoassay

E. Applicant:

Fujirebio Diagnostics, Inc.

F. Proprietary and Established Names:

Architect Cyclosporine Assay

Architect Cyclosporine Calibrators

Whole Blood Precipitation Reagent kit

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
MKW	II	21 CFR 862.1235	91 (Toxicology)
DLJ	II	21 CFR 862.3200	91 (Toxicology)

H. Intended Use:

1. Intended use(s):

   See indications for use statement below.

2. Indication(s) for use:

   The ARCHITECT Cyclosporine assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of cyclosporine in human whole blood on the ARCHITECT *i* System. The ARCHITECT Cyclosporine assay is used an aid in the management of heart, liver and kidney transplant patients receiving cyclosporine therapy.

3. Special conditions for use statement(s):

   For prescription use only.

   See Expected Range, below.

4. Special instrument requirements:

   Architect *i* System

I. Device Description:

The ARCHITECT Cyclosporine assay is comprised of the ARCHITECT Cyclosporine Reagent Kit that contains microparticles bottles, conjugate, and assay diluent. The microparticle bottles contain anti-cyclosporine (mouse, monoclonal) coated microparticles in MOPS buffer with protein (bovine) stabilizer and sodium azide and ProClin 950 as preservatives. The conjugate is cyclosporine acridinium-labeled conjugate in citrate buffer. The assay diluent contains MES buffer and sodium chloride. Also included in the ARCHITECT cyclosporine assay are the Whole Blood Precipitation and Whole Blood Solubilization reagents. The Whole Blood Precipitation reagent contains zinc sulfate in methanol and ethylene glycol. The Whole Blood Solubilization reagent contains surfactants in water.

The ARCHITECT Cyclosporine Calibrator Kit contains 6 bottles of cyclosporine calibrators (calibrator A is 9.0 mL and calibrators B-F are 4.5 mL each). Calibrator A is used as a diluent for out of range specimens. Calibrators A through F are prepared with processed human whole blood. Calibrators B through F contain cyclosporine at the following levels: 40,150,400,800 and 1500.

Components contain human sourced materials. Donor blood has been tested and found to be no-reactive to HBsAg, HIV-1 RNA, or HIV-1 Ag, anti-HCV, and anti HIV-1/HIV-2.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   TDx/TDxFLx Cyclosporine Monoclonal Whole Blood Assay

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

   P890025 (subsequently down-classified into Class II)

3. Comparison with predicate:

   Similarities include the following: The intended use and indications for use are the same as the predicate device. Both devices utilize mouse monoclonal anti-cyclosporine antibodies. Both devices include 6 levels of calibrators, with concentrations 0-1500 ng/mL.

   The differences between the devices include the following:

Differences are shown in the table below:

Differences		
	ARCHITECT Cyclosporine (Proposed Device)	**TDx/TDxFLx Cyclosporine Monoclonal Whole Blood (Predicate Device) P890025 and supplement 7**
Instrument System	ARCHITECT System	TDx/TDxFLx System
Principle of Operation	Chemiluminscent Microparticle Immunoassay (CMIA)	Fluorescence Polarization Immunoassay (FPIA)
Detection	Cyclosporine acridinium-labeled conjugate in citrate buffer with detergent	<0.01% fluorescein tracer in buffer containing surfactant and protein stabilizer.
Capture	Anti-cyclosporine (mouse, monoclonal) coated paramagnetic microparticles in MOPS buffer with protein stabilizers.	Antibody (mouse monoclonal) in buffer with protein stabilizer.
Specimen Collection Method	EDTA Whole Blood Collection Tubes	EDTA and Heparin Whole Blood Collection Tubes

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

STANDARDS
Title and Reference Number
Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline (EP09-A2)
Interference Testing in Clinical Chemistry; Approved Guideline (EP 7-A)
Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline (EP5-A)
Stability Testing of In Vitro Diagnostic Reagents (13640)
Medical Devices - Symbols to be used with medical device labels, labeling and information to be supplied (15223)
Class II Special Controls Guidance Document: Cyclosporine and Tacrolimus Assays; Guidance for Industry and FDA

L. Test Principle:

The ARCHITECT Cyclosporine assay is a two-step immunoassay for the quantitative determination of cyclosporine 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 lysed and extracted. The supernatant is placed onto the ARCHITECT *i* System.

In the first step, sample, assay diluent, and anti-cyclosporine coated paramagnetic microparticles are combined. Cyclosporine present in sample binds to the anti-cyclosporine coated microparticles. Cyclosporine acridinium-labeled conjugate is added in the second step. Following another wash cycle, pre-trigger and trigger solutions 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 cyclosporine 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 studies were preformed in accordance with CLSI guideline EP5-A2 "Evaluation of Precision Performance of Quantitative Measurement Methods (2004)". Three studies (one at the manufacturer's site and two at external sites) were performed to assess the precision including the effect of the manual drug extraction procedure. Prior to running each replicate the manual pretreatment step was performed using the ARCHITECT Cyclosporine Whole

Blood Precipitation reagent kit.

Site 1 (internal Fujirebio location) evaluated eight samples. The samples included pooled specimens from patients undergoing cyclosporine therapy, control materials and human whole blood samples spiked with cyclosporine (~90, 250 and 900 ng/ml).

Results are shown below:

Sample	Instrument	Reagent Lot	n	Mean Conc. (ng/mL)	Within-run %CV	Between-run %CV	Between-day %CV	Total %CV
Control 1	1	1	80	92.6	7.3	6.8	6.9	12.2
	2	2	80	92.1	7.5	2.3	3.8	8.7
Control 3	1	1	80	463.9	7.2	2.2	5.9	9.6
	2	2	80	407.5	6.0	0.7	1.4	6.2
Control 4	1	1	80	975.4	7.1	0.0	4.3	8.3
	2	2	80	925.7	8.9	0.0	0.9	8.9
Panel 1 (pooled specimens)	1	1	80	161.2	8.9	3.7	4.0	10.5
	2	2	80	153.4	6.3	4.4	2.0	7.9
Panel 2 (pooled specimens)	1	1	80	686.0	7.1	0.0	5.0	8.7
	2	2	80	607.0	5.4	2.4	1.1	6.0
Low spiked sample	1	1	80	88.6	12.1	0.0	4.0	12.8
	2	2	80	87.1	8.7	7.3	0.0	11.4
Med spiked sample	1	1	80	261.4	9.1	0.0	4.0	10.0
	2	2	80	231.7	5.4	2.1	4.4	7.3
High spiked sample	1	1	80	949.7	7.3	0.0	2.7	7.7
	2	2	80	877.0	6.4	2.0	2.5	7.1

The two external sites evaluated pooled patient samples from patients undergoing cyclosporine therapy as well as, human whole blood samples (in house quality control material) spiked with cyclosporine (~900 ng/ml). Data was reviewed from the three sites and were shown to be similar. Results from a representative site are summarized below:

Precision evaluation at external site:

Sample	Instrument	Reagent Lot	n	Mean Conc (ng/mL)	Within-run %CV	Between-run % CV	Between day % CV	Total % CV
Panel 1	1	1	80	151.9	8.5	0.0	5.2	10.0
	2	2	80	159.0	9.0	1.2	8.0	12.0
Panel 2	1	1	80	612.6	6.2	4.8	0.0	7.9
	2	2	80	658.9	7.0	3.5	5.9	9.8
High Control	1	1	80	914.7	6.9	5.0	0.7	8.5
	2	2	80	970.6	7.4	0.8	7.0	10.2

Results for between-site precision are as follows:
For patient samples with mean concentration 156 ng/mL, between-site CV ranged from 0-4%. For patient samples with mean concentration 645 ng/mL, between-site CV ranged from 0-6.5%.

An additional 5 day study was performed to evaluate precision at the high end of the assay range. One sample was tested in replicates of eight (8) using two (2) lots of reagents, at two (2) separate times per day, for five (5) days on two (2) separate instruments. The extraction procedure was performed for each individual replicate to best reflect total assay precision.

Cyclosporine Precision Summary High Panel				
	Instrument 1 Lot 1 n = 80		Instrument 2 Lot 2 n = 80	
Mean (ng/mL)	1313.9		1195.8	
	SD	CV	SD	CV
Total	102.8	7.8%	77.9	6.5%
Within run	99.5	7.6%	76.9	6.4%
Between run	25.9	2.0%	4.9	0.4%
Between day	0.0	0.0%	11.5	1.0%

b. Linearity/assay reportable range:

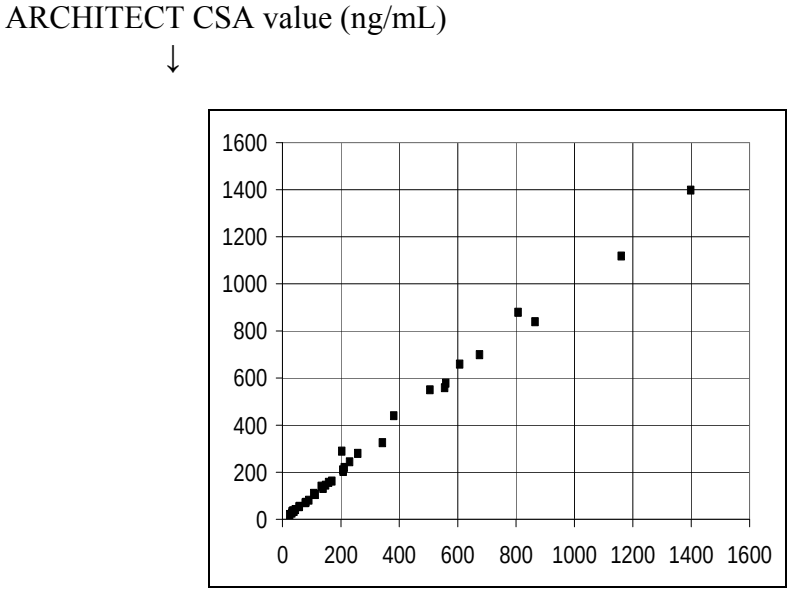
The following evaluations were performed to determine the linearity across the reportable range of 30-1500 ng/mL.

Recovery:
EDTA whole blood samples were spiked to a cyclosporine concentration of 1500 ng/mL (determined by gravimetric methods). A Cyclosporine-free EDTA whole blood sample was used as the diluent for the spiked samples. Eleven dilutions

ranging from 38-1500 ng/mL were evaluated. A parallel set of dilutions was prepared using ARCHITECT Cyclosporine Calibrator A as the diluent. Following the manual pretreatment step which was performed separately for each dilution, samples were evaluated on the ARCHITECT in replicates of five, and mean percent recovery (of the 5 replicates) was calculated:
Averaged recoveries relative to gravimetrically-determined values were within 10% (99-109%) without observed trends (for both whole blood and Calibrator A diluent).

Linearity:
Whole blood specimens were spiked to achieve cyclosporine values ranging from approximately 30 ng/mL 1400 ng/mL, and EDTA whole blood samples were used as the diluent for the respective spiked samples. The manual pretreatment step was performed after the spiked whole blood specimens were diluted with the respective EDTA whole blood. Samples were tested in replicates of five (5) from one (1) transplant pretreatment tube using the ARCHITECT Cyclosporine assay.

Results supporting linearity to the assay lower limit of 30 ng/mL are shown in the graph below. The resulting regression equation is y=1.0084x + 4.563 with a correlation coefficient of 0.997.



High concentration samples were diluted to assess the recommended dilution procedure of 1:2 for “out of range” samples. Recovery observed for this procedure was comparable to recovery shown above.

The claimed assay range based on results of the detection limit (see below) study and linearity studies is 30-1500 ng/mL.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

The ARCHITECT Cyclosporine Calibrators kit is comprised of 6 levels (A-F) of cyclosporine (0, 40, 150, 400, 800 and 1500 ng/ml).
Open vial stability was assessed via real time studies Three lots of the calibrators stored at 2-8 C were used to evaluate two samples and three controls (MCC) at monthly intervals. Closed vial stability was assessed by stress testing 2 lots of calibrators stored at 2-8 ˚C, 15 ˚C and 25 ˚C. Each lot and storage condition was assayed at day 0, 3, 5, 7, 10, 13, 17, 26, 28, 35, 42, 56, 65, 75, 85, 95, 105 and 120. Recovery of the test samples were consistent (no apparent trends) within the time range tested.

An on-board sample stability study was conducted for 3 hours, as claimed by the manufacturer. Samples with concentrations spanning much of the assay range were spilt (one was stored at 2-8 C) and run at time 0 and after 3 hours. . Percent recovery was within 10% of the mean value obtained from time 0.

The calibrators are traceable to a USP reference material. Cyclosporine reference calibrators B-F are tested and value assigned by HPLC with MS/MS detection.

d. *Detection limit:*

The Limit of Detection (LOD) of the ARCHITECT Cyclosporine assay supports the manufacturer's claim of a low reportable limit of $\leq$ 30.0 ng/mL.

The sponsor conducted a study to determine the lowest concentration of cyclosporine at which the sample CV is equal to 20% for the ARCHITECT Cyclosporine assay in accordance with CLSI EP5-A2. Seven samples were prepared by spiking EDTA whole blood with cyclosporine stock solution to obtain concentrations of 5, 10, 15, 20, 30, 40 and 50 ng/ml. Ten replicates for each of the samples were run twice per day for five days. Results support $\leq$20% CV at the lower limit of the reportable range of 30.0 ng/mL.

e. *Analytical specificity:*

Cross reactivity:

The effects of major cyclosporine metabolites, potential endogenous interferent and co-administered common or immunosuppressive drugs on assay performance were evaluated based on CLSI EP7-A. In order to assess cross-reactivity of cyclosporine metabolites, 1000 ng/ml of each metabolite (AM19, AM1c, AM9, AM4N and AM1) were spiked into whole blood samples containing a cyclosporine concentration ranging from 70.0 ng/ml to 1357 ng/ml. Percent cross-reactivity was calculated using the equation:

$$\% \text{ Cross-Reactivity} = 100*(\text{Measured Value}_{\text{With Cross-Reactant}} - \text{Measured Value}_{\text{Control}} / \text{Amount of Cross-Reactant Added}$$

Results are shown in the table below:

Metabolite	Amount Added (ng/mL)	Mean Excess Detected (ng/mL, n=5)	% Cross Reactivity
AM1	1000	0.7	-0.7 to 2.9 %
AM1c	1000	10.2	-0.8 to 3.3 %
AM4N	1000	-5.9	-2.3 to 3.1 %
AM9	1000	-1.6	-3.8 to 1.9 %
AM19	1000	-4.5	-2.9 to 2.1 %

The effects of bilirubin (40 mg/dL), Cholesterol (500 mg/dl), uric acid (20 mg/dl), triglycerides (1500 mg/dl), hematocrit (25% and 55%), total protein (3 and 12 g/dl), HAMA and RF were evaluated through a recovery study. Whole blood samples were spiked with various levels of cyclosporine ranging from 87.1 to 693.3 ng/mL. The recovery observed during the study ranged from 97 % to 108%.

Commonly co-administered drugs, including immunosuppressant drugs, were evaluated for interference. Whole blood was spiked with cyclosporine to achieve a low and high level cyclosporine concentrations ranging from 80 to 800 ng/mL. The control was the sample without the added drug. A list of the 66 drugs tested can be found in the package insert under the potentially interfering pharmaceutical substances section. Cyclosporine recoveries in the presence of most drugs tested were within 90-110% of the control sample. Observed recoveries for substances that fell outside of the +/-10% were observed for the compounds. Recoveries ranged from 84% to 118%.

	Spike Level 1 of Cyclosporine (ng/mL)			Spike Level 2 of Cyclosporine (ng/mL)		
Substance Tested	**Observed (control)**	**Observed (test substance)**	**Percent Recovery**	**Observed (control)**	**Observed (test substance)**	**Percent Recovery**
Heparin (High MW)	79.8	67.1	84%	637.0	649.9	102%
Kanamycin B Sulfate	77.0	68.7	89%	671.8	669.9	100%
Lidocaine	81.1	69.3	85%	564.6	636.3	113%
Mycophenolic Acid Glucuronide	70.9	61.6	87%	588.4	589.2	100%
Phenobarbital	72.3	80.6	111%	598.8	528.4	88%
Spectinomycin	67.9	76.4	113%	684.7	667.4	97%
Vancomycin	72.7	72.0	99%	778.8	919.2	118%

Potentially Interfering Endogenous Substances:

Whole blood specimens spiked with cyclosporine targeting concentrations between 70 and 900 ng/mL were supplemented with the following potentially interfering endogenous substances. The mean recoveries for the following substances tested ranged from 92% to 110%.

Potential Interfering Substance	Concentration	Mean Percent Recovery
Hematocrit	25%, 55%	106 and 106% respectively
Bilirubin	40 mg/dL	102%
Total Protein	3 and 12 g/dL	106 and 102% respectively
Uric Acid	20 mg/dL	97%

Observed averaged (n=5) percent recoveries for the following substances tested during the study ranged from 101% to 113%.

Potential Interfering Substance	Interferent Concentration	Cyclosporine concentration Range ng/mL	Percent Recovery Ranges
Triglycerides	1500 mg/dL	87-693	102 to 108%
Total Protein	3 g/dL	83-716	103 to 113 %
Cholesterol	500 mg/dL	77-687	101 to 112%

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

Method comparison studies were performed at three sites including the manufacturer's site and two external sites. The ARCHITECT Cyclosporine Assay was compared to LCMS at one of the sites. The sponsor also compared the ARCHITECT results to those of the TDx/TDxFLx Cyclosporine Monoclonal Whole Blood.

Samples tested were from unaltered whole blood specimens from patients undergoing cyclosporine therapy following heart, liver, or kidney transplants. Studies were performed using routine methods for quality control and calibration as described in the package insert. The patient samples included 73 from cardiac transplant patients; 77 from kidney transplant patients; and 77 from liver transplant patients. Patient samples included 191 samples from 169 individuals. The mean patient age was 54 (ranging from 22-79). Samples

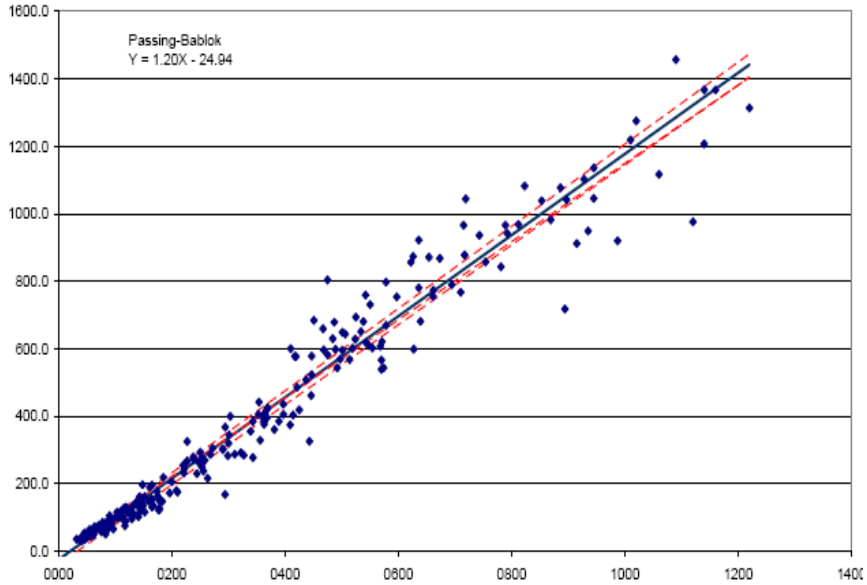
were from men (125) and women (66). Samples included C2 time points as well as pre-dose (Co) time points and were from patients representing both acute (short time post-transplant) and chronic (longer time post-transplant).

The sponsor reported results of Passing-Bablok and linear regression analyses for both the overall results and by transplant type. The data below and correlation coefficients are based on single measurements of each sample. Samples spanned the range from 31 to 1460 ng/mL.

Comparison to LC/MS reference method:
The table below summarizes the regression/correlation results for the cardiac, liver, and renal samples against the predicate device and LC/MS.

	Method Comparison against LC/MS results		
	Passing-Bablok Values and 95% CI (ng/mL)		
	Cardiac Transplant	Liver Transplant	Renal Transplant
Slope	1.21	1.22	1.18
	(1.15 to 1.25)	(1.17 to 1.27)	(1.14 to 1.25)
Intercept	-15.77	-40.57	-21.54
	-27.2 to -8.45	-57.18 to -26.71	-34.56 to -14.66
Correlation Coefficient	0.99	0.98	0.99
	(0.98 to 0.99)	(0.96 to 0.99)	(0.98 to 0.99)
	Linear Regression Values and 95% CI (ng/mL)		
Slope	1.22	1.16	1.13
	(1.17 to 1.26)	(1.1 to 1.23)	(1.07 to 1.2)
Intercept	-17.93	-25.84	-8.06
	-43.25 to 7.39	-54.9 to 3.21	-33.05 to 16.94
correlation coefficient	0.99	0.98	0.99
CI	(0.98 to 0.99)	(0.97 to 0.99)	(0.98 to 0.99)
Sy/x	72 ng/mL	70 ng/mL	67 ng/mL

There were no significant differences observed among results of different transplant types. The method comparison for all samples is shown below.



CsA concentration (ng/mL) by LCMS

Y-axis= CsA concentration
by ARCHITECT (ng/mL)

In this study, trough specimens (which had cyclosporine values typically < 400) exhibited less variability and less bias than C2 specimens. Similarly, "chronic" (greater time post-transplant) condition specimens exhibited less variability and less bias than "acute" (shorter time post-transplant) condition specimens. This may also be related to the fact that trough specimens and chronic condition specimens had a greater proportion of low cyclosporine values (which had lower bias and less variability) relative to C2 and acute condition specimens (which had relatively more bias and variability). Bias and variability relative to LCMS are presented in the package insert.

Comparison to ARCHITECT:
Results of the ARCHITECT assay were also compared to those of the predicate device. The graph and table below summarizes the overall regression/correlation results compared to the predicate device:

ARCHITECT Cyclosporine vs. TDx/TDxFLx Cyclosporine Monoclonal Whole Blood				
Number of Observations	Intercept (95% CI[a])	Slope (95% CI)	Correlation Coefficient	Square Root of MSE ($s_{y\|x}$)[b]
227	-24.65 (-32.54 to -19.99)	0.93 (0.91 to 0.95)	0.99	53.46 ng/mL

Results obtained at the two external sites using the ARCHITECT assay were compared. Each site obtained measurements for 192 samples that spanned the assay range. Results of linear regression analysis, comparing results from the 2 sites are shown below:

Number of Observations	Intercept (95% CI)	Slope (95% CI)	Correlation Method	Correlation Coefficient (95% CI)
192	15.03 (4.01 to 26.04)	0.96 (0.93 to 0.98)	Pearson	0.98 (0.98 to 0.99)

b. Matrix comparison:

Not applicable. The device is intended for use with EDTA whole blood only.

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 sponsor provides the following statement in the labeling regarding expected values:

No firm therapeutic range exists for cyclosporine in whole blood. The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic effects of cyclosporine, 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 cyclosporine. Therefore, individual cyclosporine 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.

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.