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

A. 510(k) Number:

k060502

B. Purpose for Submission:

New Device

C. Measurand:

Tacrolimus

D. Type of Test:

Quantitative

E. Applicant:

Dade Behring Inc.

F. Proprietary and Established Names:

Dimension TACR Flex reagent cartridge

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
MLM	II	21 CFR 862.1678	75 Chemistry

H. Intended Use:

1. Intended use(s):

See the Indications for use below.

2. Indication(s) for use:

The Dimension TACR Flex reagent cartridge is an in vitro diagnostic test intended to quantitatively measure tacrolimus in human whole blood on the Dimension Clinical Chemistry system as an aid in the management of tacrolimus therapy in liver and kidney transplant patients.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Dade Behring Dimension Clinical Chemistry system

I. Device Description:

The Dimension TACR Flex reagent consists of prepackaged reagents in a plastic eight well cartridge for use on the Dimension Clinical Chemistry system. The Flex cartridge contains a pretreatment reagent, antibody-ß-galactosidase conjugate, tacrolimus

immobilized on chromium dioxide particles, chlorophenol red ß-d-galactopyranoside (CPRG) substrate, and diluent to hydrate the tablets.

J. Substantial Equivalence Information:

Predicate Device Name and Number: Abbott IMx Tacrolimus II Assay, P970007

Similarities		
Item	**Device**	**Predicate**
Intended Use	For the in vitro quantitative analysis of tacrolimus and metabolite in human whole blood as an aid in the management of tacrolimus therapy in liver and kidney transplant patients	For the in vitro quantitative analysis of tacrolimus and metabolite in human whole blood as an aid in the management of tacrolimus therapy in liver allograft patients.
Sample type	Human whole blood.	Human whole blood.
Antibody	Mouse monoclonal antibody.	Mouse monoclonal antibody.

Differences		
Item	**Device**	**Predicate**
Assay technology	Tacrolimus Flex reagent cartridge uses an immunoassay technique.	Abbott IMx Tacrolimus II Assay uses the MEIA technology.
Assay range	1.2-30 ng/mL.	1.5-30 ng/mL.
Sample pretreatment	Automated pretreatment	No sample pretreatment required.
Analyzers	Dimension Clinical Chemistry system	Abbott IMx analyzer

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

STANDARDS			
Title and Reference Number			
CLSI Document: Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline (EP5-A)			
CLSI Document: Interference Testing in Clinical Chemistry; Approved Guideline (EP 7-A)			
GUIDANCE			
Document Title	Office	Division	**Web Page**
Class II Special Controls Guidance Document: Cyclosporine and Tacrolimus Assays; Guidance for Industry and FDA	OIVD	DCTD	**http://www.fda.gov/cdrh/ode/guidance/1380.html**

L. Test Principle:

The automated Dimension TACR method uses an immunoassay technique in which free and tacrolimus-bound antibody-enzyme conjugates are separated using magnetic particles. The assay is performed using a method specific Flex reagent cartridge.

To perform the TACR assay, a sample cup containing the whole blood sample to be analyzed and a TACR Flex reagent cartridge are placed on the Dimension system. The Dimension system mixes and lyses the whole blood sample. The lysed sample is then mixed with the antibody enzyme conjugate. The tacrolimus present in the sample is bound by the tacrolimus antibody conjugate reagent. Magnetic particles coated with tacrolimus are added to bind free (unbound) antibody-enzyme conjugate. The reaction mixture is then separated magnetically. Following separation, the supernatant containing the tacrolimus-antibody-enzyme complex is transferred to another cuvette and mixed with the substrate. ß-galactosidase catalyzes the hydrolysis of CPRG (chlorophenol red ß-d-galactopyranoside) to produce CPR (chlorophenol red) that absorbs light maximally at 577 nm. The change in absorbance at 577 nm due to the formation of CPR is directly proportional to the amount of tacrolimus in the patient's sample and is measured using a bichromatic (577, 700 nm) rate technique.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. Precision/Reproducibility:

   An internal site precision study was conducted using whole blood samples from patients taking tacrolimus. Three patient samples were chosen having low, mid and high levels. Twenty replicates of each patient sample were analyzed in one run

over one day. The with-in run results are presented in the table below:

Replicate Number	**Low level, ng/mL**	**Mid level, ng/mL**	**High level, ng/mL**
1	6.2	13.6	24.4
2	6.1	13.0	23.3
3	6.5	12.3	22.4
4	6.5	12.5	21.7
5	6.3	12.3	21.8
6	6.3	11.7	22.8
7	6.2	12.1	21.7
8	6.5	12.7	22.4
9	6.0	12.2	22.9
10	6.3	11/9	22.4
11	7.1	13.8	22.6
12	6.8	13.2	23.5
13	7.2	12.6	22.8
14	7.1	12.0	22.2
15	6.8	12.7	22.3
16	6.6	12.2	21.7
17	6.6	12.3	21.3
18	7.0	12.4	21.2
19	6.5	12.0	21.5
20	6.9	12.1	22.2
Mean	**6.6**	**12.5**	**22.4**
SD	**0.36**	**0.56**	**0.79**
%CV	**5.52**	**4.52**	**3.55**

The results demonstrated that the within-run precision met the sponsor's acceptance criteria for SD≤0.3 at 3 ng/mL, %CV≤7% at 10 ng/mL and %CV≤4% at 20 ng/mL.

An external site precision study was conducted by assaying 3 levels of spiked whole blood pools according to CLSI EP5-A. The whole blood pools were prepared from EDTA whole blood spiked with tacrolimus at low, moderate and high levels. Specimens at each level were analyzed in duplicate twice per day for 20 days (n=80). The results for within-run and total precision are presented in the table below:

Material	Mean, ng/mL	Within-Run, ng/mL		Total, ng/mL	
		SD	%CV	SD	%CV
Whole Blood Pool, Level 1	3.4	0.19	5.6	0.33	9.7

Material	Mean, ng/mL	Within-Run, ng/mL		Total, ng/mL	
		SD	%CV	SD	%CV
Whole Blood Pool, Level 2	11.5	0.28	2.5	0.38	3.3
Whole Blood Pool, Level 3	20.3	0.30	1.5	0.42	2.1

A second internal precision study was conducted by assaying 3 levels of commercially available whole blood quality control (QC) materials according to CLSI EP-5A. Specimens were analyzed in duplicate once per day for twenty days (n=80). The results for within-run and total precision are presented in the table below:

Material	Mean ng/mL	Within-Run ng/mL		Total, ng/mL	
		SD	%CV	SD	%CV
QC Level 1	4.4	0.47	10.7	0.51	11.6
QC Level 2	13.9	0.60	4.3	0.63	4.5
QC Level 3	25.6	0.67	2.6	0.74	2.9

b. Linearity/assay reportable range

Linearity was assessed with samples prepared using the tacrolimus calibrator level 5 (35.8 ng/mL) and calibrator level 1 (0.0 ng/mL) to produce concentrations evenly distributed across the assay range. Standard solutions were prepared by sequentially mixing a high calibrator (35.8 ng/mL) and a low (0 ng/mL) calibrator to create a set of samples pools with concentrations of 35.8, 28.7, 21.5, 14.3, 7.2 and 0 ng/mL. The mean observed result (y) was compared to the expected concentration (x) and plotted. The linear regression equation was y=1.0277x + 0.43 with a correlation coefficient of 0.9909. The sponsor demonstrated that the assay is linear across the measuring range of 1.2 – 30.0 ng/mL.

A high sample dilution study was conducted to evaluate the accuracy of results when a high sample is diluted with tacrolimus-negative EDTA whole blood or the Dimension Tacrolimus Calibrator (0 ng/mL). Five transplant patient negative samples were spiked with a tacrolimus methanol stock solution to a concentration of 60 ng/mL. Each spiked sample was assayed twice in replicates of 5 in either 1:2 or 1:4 dilutions with either negative whole blood or the Dimension Tacrolimus zero calibrator. The diluted samples were assayed five times.

The % recoveries for the negative whole blood or calibrator diluted 1:2 ranged from 99.6 to 107.6. The % recoveries for the negative whole blood or calibrator diluted 1:4 ranged from 99.5 to 109.2. The results are summarized below:

High Sample Dilution Study using EDTA whole blood

Sample	Spike Concentration, ng/mL	Dilution Factor		TACR mean result, ng/mL		X Dilution		% Recovery	
1	60	1:2	1:4	30.1	15.8	60.2	63.0	100.3	105.0
2	60	1:2	1:4	32.0	15.7	64.1	63.0	106.8	104.9
3	60	1:2	1:4	31.1	16.4	62.1	65.5	103.6	109.2
4	60	1:2	1:4	31.4	16.4	62.8	65.5	104.6	109.2
5	60	1:2	1:4	31.1	15.5	62.1	62.0	103.5	103.3

High Sample Dilution Study using TACR Calibrator Level 1

Sample	Spike Concentration, ng/mL	Dilution Factor		TACR mean result, ng/mL		X Dilution		% Recovery	
1	60	1:2	1:4	30.6	15.9	61.1	63.6	101.8	106.0
2	60	1:2	1:4	31.0	15.2	62.0	60.8	103.4	101.4
3	60	1:2	1:4	32.3	15.7	64.5	62.8	107.6	104.7
4	60	1:2	1:4	29.9	14.9	59.8	59.7	99.6	99.5
5	60	1:2	1:4	31.9	15.3	63.7	61.3	106.2	102.2

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

Real- time stability studies were conducted on the Dimension TACR Flex reagent cartridge. The studies met the sponsor's acceptance criteria and supported the:

- 12 month shelf life for the reagent when stored at 2-8˚C
- the 30 days stability for sealed or unhydrated wells on the instrument
- the 48 hours stability once wells 1-6 are entered by the instrument and
- the 6 days stability once wells 7 and 8 are opened.

The sponsor conducted a freeze-thaw study using four (4) whole blood samples of varying levels of tacrolimus from transplant patients. The frozen samples were thawed and assayed in triplicate to determine their mean tacrolimus concentration. The samples were re-frozen at -20°C, thawed, assayed in triplicate and the mean tacrolimus concentration calculated. The mean tacrolimus concentrations were compared. The percent difference between the mean concentrations was

calculated from the following equation:

% difference = Result after freeze-thaw – Initial result/ Initial assay result x100

The percent difference for each patient sample was ± 10% compared to the initial result and met the sponsor's acceptance criteria. The results are presented in a table below.

	Sample 1 Mean, ng/mL	Sample 2 Mean, ng/mL	Sample 3 Mean, ng/mL	Sample 4 Mean, ng/mL
Initial Result	7.9	11.0	13.1	12.5
After freeze-thaw	8.5	10.4	12.9	13.2
% Difference	6.7	-4.9	-1.5	5.2

d. Detection limit:

The analytical sensitivity (Limit of the Blank) of the TACR method was determined by assaying 20 replicates of the tacrolimus negative calibrator. The sponsor defined analytical sensitivity as the concentration at two standard deviations above the 0.0 ng/mL Level 1 Calibrator and represented the lowest concentration of tacrolimus that can be distinguished from 0 with a confidence of 95%. Based on the studies that were conducted the analytical sensitivity is 1.2 ng/mL.
A functional sensitivity study was conducted using 9 whole blood spiked pools (values ranging from 0.3 to 25.5 ng/mL). The sponsor defined functional sensitivity as the lowest drug concentration for which acceptable assay precision is noted. The study was conducted over 20 days, 2 runs per day with samples run in duplicate (n=80). The mean, standard deviation and %CV (y) were calculated and plotted against the mean of the test sample (y). The regression line of y=-0.4385x – 0.8766 was obtained. The analyte concentration corresponding to a 20% CV is 2.4 ng/mL. Based on the studies conducted, the functional sensitivity is 2.4 ng/mL.

The potential for a high dose hook effect was evaluated by preparing serial dilutions of concentrated tacrolimus stock solution in methanol. The final dilutions (35.8, 60.0, 80.0, 90.0 and 100 ng/mL) were prepared using EDTA whole blood hemolysate.and assayed in duplicate. The mean observed results were compared against the instrument response for the level 5 calibrator. Each mean observed result was higher than the level 5 calibrator and the instrument produced error messages for the high concentration samples indicating that there is no high dose hook effect or this assay up to 100 ng/mL.

e. Analytical specificity:

The sponsor evaluated the effects of potential interferents on assay performance. The sponsor conducted an interference study on endogenous compounds, commonly co-administered drugs, including four immunosuppressive drugs and anticoagulants.

A sample was prepared containing the endogenous compound in the presence of 10 ng/mL tacrolimus in negative whole blood hemolysate. All samples were assayed five times and the mean was calculated. At the concentration tested, these compounds did not cause significant interference (defined by the sponsor as ±10%). The results are presented in the table below:

Endogenous Compound	**Tacrolimus ng/mL**		**% Interference**
	Test Sample	**Control**	
Ditaurobilirubin (60 mg/dL)	9.7	10.2	-4.9
Triglycerides (1000 mg/dL)	8.6	9.6	-9.9
Cholesterol (400 mg/dL)	8.9	9.6	-7.0
Uric Acid (20 mg/dL)	8.9	9.1	-1.3
Rheumatoid Factor (500 IU/mL)	8.8	9.6	-8.7
Albumin (6 g/dL)	10.0	10.4	-3.7
Gamma globulin – IgG (6 g/dL)	10.0	10.4	-3.7
HAMA	9.0	9,7	-7.6
Hematocrit (19.5%)	9.9	-0.3	0.6
Hematocrit (31.3%)	10.1	-0.0	0.0
Hematocrit (46.4%)	9.5	0.3	-8.9
Hematocrit (52.7%)	9.8	-0.4	0.5

The sponsor evaluated potential cross-reactivity of eight major tacrolimus metabolites by using whole blood with tacrolimus spiked in at 10 ng/mL. For each metabolite, two aliquots were prepared with 50 ng/mL of the metabolite

spiked into one of the aliquots; the second aliquot contained no metabolite and served as the control. Five replicates of each sample were assayed and the cross-reactivity was calculated. The results of the study are presented in the table below.

Metabolite	**Mean metabolite, ng/mL**	**Mean control, ng/mL**	**Cross-reactivity (%)**
13-O-desmethyl tacrolimus	17.5	10.1	14.8
31-O-desmethyl tacrolimus	11.5	10.1	2.7
15-O-desmethyl tacrolimus	10.6	10.1	1.0
12-OH tacrolimus	19.4	10.1	18.0
15-31-O-didesmethyl tacrolimus	10.1	10.1	0.5
13-31-O-didesmethyl tacrolimus	10.1	10.1	-0.1
13-15-O-didesmethyl tacrolimus	11.0	10.1	1.9
M-VIII tacrolimus	10.3	10.1	0.4

The sponsor conducted a study to examine the interference from commonly co-administered drugs including 4 immunosupressive drugs - cyclosporine, mycophenolic acid and its metabolite MPAG, and rapamycin. A sample was prepared containing the co-administered drug in the presence of 10 ng/mL tacrolimus in negative whole blood. This sample was compared to a control which contained only 10 ng/mL tacrolimus in whole blood. All samples were assayed five times. At the concentrations tested, the co-administered drugs did not cause significant interference (defined by the sponsor as ±10% of the control). A complete list of the compounds is contained in the package insert.

The sponsor recommends that a trough sample should be drawn using EDTA as the anticoagulant. Samples collected in heparin are not recommended because they may form clots during storage. This recommendation is referenced in the

Specimen Collection section of the package insert.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

The Dimension TACR method was compared against two methods: liquid chromatography/tandem mass spectrometry (LC/MS/MS) and the Abbott IMx Tacrolimus II Assay at two external sites. Trough samples from patients were obtained from multiple geographic sites and encompassed liver and kidney transplant organ types.

Study – Site #1	**Transplant Type**	**N**	**Slope (ng/mL)**	**Intercept (ng/mL)**	**Correlation**
Dimension TACR Flex vs. LC-MS/MS	Kidney and Liver	97	1.00	0.0 (-0.68 to 0.62)	0.88
Dimension TACR Flex vs. Abbott IMX Assay	Kidney and Liver Transplant	90	1.14	-1.14	0.87

Study – Site #2	**Transplant Type**	**N**	**Slope (ng/mL)**	**Intercept (ng/mL)**	**Correlation**
Dimension TACR Flex vs. LC-MS/MS	Kidney and Liver	87	1.11	0.87	0.91
Dimension TACR Flex vs. Abbott IMX Assay	Kidney and Liver Transplant	85	0.82	0.73	0.81

Study – Combined Sites #1 & #2	**Transplant Type**	**N**	**Slope (ng/mL)**	**Intercept (ng/mL)**	**Correlation**
Dimension TACR Flex vs. LC-MS/MS	Kidney and Liver	184	1.13	-0.27	0.88

Study – Combined Sites #1 & #2	Transplant Type	N	Slope (ng/mL)	Intercept (ng/mL)	Correlation
Dimension TACR Flex vs. IMx Assay	Kidney and Liver	175	0.92	0.1	0.85

b. Matrix comparison:

Not applicable

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 references the Consensus Document (published in 1995) that describes targets for 12 –hour trough whole-blood concentrations and references the Fujisama Pharmaceutical Co. Ltd.'s interpretation of the PDR which describes co-medication interference. The described tacrolimus trough range (from 5 to 20 ng/mL) depends on the transplant type, stage after transplantation, and the medical practice. Higher or lower concentrations may be associated with an increase in the incidence of adverse effects. Blood levels can be affected by co-medications. Patients treated with viral protease inhibitors for HIV infection may have dramatically altered metabolism of tacrolimus, which may cause elevation of tacrolimus to at least 100 ng/mL, and would require novel dosing. Tacrolimus is extensively metabolized by the liver. Therefore, circulating tacrolimus levels may be influenced by drugs that affect hepatic microsomal enzymes, particularly the cytochrome P450 system. Substances know to inhibit these enzymes will decrease hepatic metabolism and increase tacrolimus levels.

Therapeutic ranges vary according to the commercial test used, and the sponsor recommends that ranges 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, the sponsor recommends consistent use of one assay for individual patients.

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.