

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

**A. 510(k) Number:**

k060385

**B. Purpose for Submission:**

New Device

**C. Measurand:**

Tacrolimus

**D. Type of Test:**

Quantitative

**E. Applicant:**

Dade Behring Inc.

**F. Proprietary and Established Names:**

Emit Tacrolimus Assay

**G. Regulatory Information:**

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

**H. Intended Use:**

1. Intended use(s):

The Emit® 2000 Tacrolimus Assay is for 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.

The Emit® 2000 Tacrolimus Sample Pretreatment Reagent is an accessory reagent for use with the Emit® 2000 Tacrolimus Assay.

2. Indication(s) for use:

The Emit® 2000 Tacrolimus Assay is for 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.

The Emit® 2000 Tacrolimus Sample Pretreatment Reagent is an accessory reagent for use with the Emit® 2000 Tacrolimus Assay.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

The Emit® 2000 Tacrolimus Assay is for use on a variety of chemistry analyzers from other manufacturers. Performance characterization was performed on the Vital Scientific V-Twin analyzer.

**I. Device Description:**

The Emit® 2000 Tacrolimus Assay are liquid reagents, comprised of an antibody reagent, a buffer reagent and an enzyme reagent. This assay contains mouse monoclonal antibodies with a high specificity for tacrolimus. The Emit® 2000 Tacrolimus Sample Pretreatment Reagent is a liquid reagent that is used to pretreat the whole blood samples, calibrators, and controls prior to testing with the Emit® 2000 Tacrolimus Assay.

**J. Substantial Equivalence Information:**

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

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
<b>Intended Use</b>	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.
<b>Sample type</b>	Human whole blood.	Human whole blood.
<b>Antibody</b>	Mouse monoclonal antibody.	Mouse monoclonal antibody.

<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
<b>Assay technology</b>	The Emit 2000 Tacrolimus Assay is based on EMIT technology.	The Abbott IMx Tacrolimus II Assay technology uses the MEIA technology.
<b>Assay range</b>	The Emit 2000 Tacrolimus Assay has an assay range of 2-30 ng/mL.	The Abbott IMx Tacrolimus II Assay has an assay range of 1.5-30 ng/mL.
<b>Sample pretreatment</b>	The Emit 2000 Tacrolimus Assay requires a manual pretreatment of the sample.	The Abbott IMx Tacrolimus II Assay does not have a sample pretreatment requirement.
<b>Analyzers</b>	The Emit 2000 Tacrolimus Assay is used on chemistry analyzers.	The Abbott IMx Tacrolimus II Assay predicate device is used on the IMx analyzer.

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

<b>STANDARDS</b>
<b>Title and Reference Number</b>
NCCLS Document: Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline (EP5-A)
NCCLS Document: Interference Testing in Clinical Chemistry; Approved Guideline (EP 7-A)

<b>GUIDANCE</b>			
<b>Document Title</b>	<b>Office</b>	<b>Division</b>	<b>Web Page</b>
<b>Class II Special Controls Guidance Document: Cyclosporine and Tacrolimus Assays; Guidance for Industry and FDA</b>	<b>OIVD</b>	<b>DCTD</b>	<a href="http://www.fda.gov/cdrh/ode/guidance/1380.html">http://www.fda.gov/cdrh/ode/guidance/1380.html</a>

**L. Test Principle:**

The Emit® 2000 Tacrolimus Assay utilizes competition for tacrolimus antibody binding sites. Tacrolimus in the sample competes with tacrolimus in the Enzyme Reagent that is labeled with recombinant enzyme glucose-6-phosphate dehydrogenase (rG6PDH). Active (unbound) rG6PDH enzyme converts the oxidized nicotinamide adenine dinucleotide (NAD) in the antibody reagent to NADH, resulting in a kinetic absorbance change that can be measured

spectrophotometrically. Enzyme activity decreases upon binding to the antibody, allowing tacrolimus concentrations to be measured in terms of enzyme activity.

The Emit® 2000 Tacrolimus Sample Pretreatment Reagent contains cupric sulfate in water and is used to pretreat the whole blood samples, calibrators, and controls prior to testing with the Emit® 2000 Tacrolimus Assay. The pretreatment process lyses the cells, extracts the 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 Emit® 2000 Tacrolimus Assay.

**M. Performance Characteristics (if/when applicable):**

All of the performance data for the Emit® 2000 Tacrolimus Assay was gathered on the V-Twin® Analyzer.

1. Analytical performance:

a. *Precision/Reproducibility:*

An external site precision study was conducted by assaying 4 levels of spiked whole blood hemolysate pools according to NCCLS EP5-A. The pools were prepared from EDTA whole blood with tacrolimus spiked in 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 shown in the table below.

Material	Mean (ng/mL)	Within-Run (ng/mL)		Total (ng/mL)	
		SD	%CV	SD	%CV
Whole Blood Pool, Level 1	5.10	0.40	7.8	0.83	16.4
Whole Blood Pool, Level 2	9.98	0.50	5.0	1.03	10.3
Whole Blood Pool, Level 3	15.11	0.59	3.9	1.27	8.4
Whole Blood Pool, Level 4	23.87	0.81	3.4	1.62	6.8

Tacrolimus was assessed using tacrolimus-free EDTA whole blood spiked to 5, 15 and 25 ng/mL of tacrolimus. Four separate runs were pretreated and assayed in replicates of 5 (n=20 for each level). The percent recovery was calculated using the following equation:

$$\% \text{ Recovery} = (\text{V-Twin mean result}) / (\text{Nominal spiked concentration}) \times 100$$

Recovery study results are summarized below. The percent recovery for all levels met the sponsors' acceptance criteria of 90 – 110%.

Spike	5 ng/mL	15 ng/mL	25 ng/mL
Vtwin Mean , ng/mL	5.3	16.1	27.6
SD	0.5	0.7	1.4
%CV	9.9	4.5	5.1
Nominal Recovery	106%	107%	110%

b. *Linearity/assay reportable range:*

Linearity was assessed according to NCCLS EP6-A. Standard solutions were prepared by sequential mixing a high (30 ng/mL) and a low (0 ng/mL) pool to create a set of 5 samples pools with concentrations of 30, 22.5, 15, 7.5 and 0 ng/mL. Each pool was pretreated and assayed in replicates of 5. The mean result (y) was compared to the spiked concentration (x) and plotted. The linear regression equation was  $y=1.0235x + 0.244$  with a correlation coefficient of 0.9984.

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 Emit® 2000 Tacrolimus Calibrator (0 ng/mL). Ten patient negative samples were spiked with either 35 or 80 ng/mL tacrolimus and assayed twice in replicates of 5 in either 1:2 or 1:3 dilutions with either negative whole blood or the Emit® 2000 Tacrolimus zero calibrator. The percent recovery was calculated using the following equation:

$$\% \text{ Recovery} = (\text{V-Twin mean result}) / (\text{Nominal spiked concentration}) \times 100.$$

The % recoveries for the negative whole blood or calibrator diluted 1:2 ranged from 97 to 101.4%. The % recoveries for the negative whole blood or calibrator diluted 1:3 ranged from 90.0 to 101.2%. The results are summarized below.

<b>Spiked Sample Concentration at 35 ng/mL</b>				
		<b>V-Twin mean ng/mL</b>	<b>x dilution factor ng/mL</b>	<b>% Recovery</b>
Negative Whole Blood 1:2	Sample 1	17.2	34.5	98.5
	Sample 2	17.8	35.5	101.4
	Sample 3	17.7	35.4	101.2
	Sample 4	17.0	34.0	97.0
	Sample 5	17.6	35.2	100.5
<b>Spiked Sample Concentration at 35 ng/mL</b>				
		<b>V-Twin mean ng/mL</b>	<b>x dilution factor ng/mL</b>	<b>% Recovery</b>
Negative Whole Blood 1:3	Sample 1	11.3	33.8	96.5
	Sample 2	10.6	31.7	90.5
	Sample 3	11.7	35.2	100.6
	Sample 4	10.9	32.8	93.8
	Sample 5	11.8	35.4	101.2
<b>Spiked Sample Concentration at 35 ng/mL</b>				
		<b>V-Twin mean ng/mL</b>	<b>x dilution factor ng/mL</b>	<b>% Recovery</b>
Cal zero 1:3	Sample 1	17.4	34.9	99.7
	Sample 2	17.6	35.3	100.7
	Sample 3	17.4	34.8	99.4
	Sample 4	17.6	35.2	100.5
	Sample 5	17.1	34.1	97.5
Cal zero 1:3	Sample 1	11.8	35.3	100.8
	Sample 2	11.9	35.7	102.0
	Sample 3	11.8	35.3	100.8

		<i>V-Twin mean ng/mL</i>	<i>x dilution factor ng/mL</i>	<i>% Recovery</i>
	Sample 4	11.7	35.0	100.0
	Sample 5	11.7	35.1	100.3
<b>Spiked Sample Concentration at 80 ng/mL</b>				
		<b>V-Twin mean ng/mL</b>	<b>x dilution factor ng/mL</b>	<b>% Recovery</b>
Negative Whole Blood 1:3	Sample 1	24.0	72.0	90.0
	Sample 2	24.2	72.6	90.8
	Sample 3	24.8	74.5	93.2
	Sample 4	24.4	73.2	91.5
	Sample 5	24.5	73.6	92.0
Cal zero 1:3	Sample 1	24.3	73.0	91.2
	Sample 2	25.0	75.0	93.8
	Sample 3	25.4	76.1	95.1
	Sample 4	26.4	79.1	98.9
	Sample 5	26.4	79.1	98.9

The percent recovery met the sponsors' acceptance criteria of 90 – 110% and support their reportable range claim of 2.0 to 30 ng/mL.

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

Real-time stability studies were conducted on the Emit® Tacrolimus Assay reagents. The studies met the sponsors' acceptance criteria and supported the 18 month shelf life and 12 week open bottle stability when stored at 2-8°. The sponsor also notifies the user to look for reagent deterioration by the following statement "If the antibody reagent turns yellow, it has deteriorated and, therefore, must be discarded along with its accompanying buffer and enzyme reagents."

The sponsor conducted a freeze-thaw study using five whole blood samples from transplant patients of varying levels of tacrolimus. The frozen samples were thawed and assayed 5 times to determine their mean tacrolimus

concentration. The five runs for the five samples are shown in the chart below.

	Sample #1 Mean (ng/mL)	Sample #2 Mean (ng/mL)	Sample #3 Mean (ng/mL)	Sample #4 Mean (ng/mL)	Sample #5 Mean (ng/mL)
Initial Result	4.6				
After freeze-thaw	4.4	9.1	29.2	13.9	14.8
% Difference	-5	5	2	10	2

*d. Detection limit:*

An analytical sensitivity (limit of the blank) study was conducted by assaying the 0 ng/mL tacrolimus calibrator in replicates of 20. The sponsor defined analytical sensitivity as the mean of the 0 ng/mL samples plus 2 SD. This level is the lowest concentration of tacrolimus that can be distinguished from zero with a 95% confidence. The mean was 0.455 ng/mL and the SD was 0.528. The mean + 2SD was calculated to be 1.511 ng/mL. The sensitivity results obtained supports the sponsors' analytical sensitivity claim of 2 ng/mL.

A functional sensitivity study was conducted with 4 patient sample pools (values ranging from 0.00 to 3.0 ng/mL). The sponsor defined functional sensitivity as the lowest drug concentration for which acceptable assay precision is noted. The four patient samples were measured once a week for six weeks with two different lots of reagents and calibrators. The mean, standard deviation and %CV (y) were calculated and plotted against the mean of the test sample (x). The regression line of  $y = -0.3727x + 1.2413$  was obtained. The analyte concentration corresponding to a 20% CV is 2.8 ng/mL. Based on the studies conducted, the analytical sensitivity is 2 ng/mL and the functional sensitivity is 2.8 ng/mL.

The sponsor stated that the EMIT technology is not based on the "sandwich" principle and is not susceptible to hook effect.

*e. Analytical specificity:*

Potential interferences were evaluated by adding known amounts of exogenous and endogenous substances in the presence of 15 ng/mL tacrolimus in negative whole blood samples. The sample containing the interferent was compared to the control, which contained only 15 ng/mL tacrolimus in the whole blood. All samples were pretreated and assayed in duplicate. The results are summarized in the chart below.

Interferant	Tacrolimus (ng/mL)		Recovery (%)
	Test Sample	Control Sample	
IgG (12 g/dL)	16.10	17.65	91.2
Triglycerides (Intralipid) 1500 mg/dL	16.10	17.65	91.2
Uric Acid (26 mg/dL)	14.25	13.75	103.6
Cholesterol (500 mg/dL)	17.55	17.65	99.4
Rheumatoid Factor (500 IU/mL)	17.40	17.65	98.6
Albumin (12 g/dL)	16.20	17.65	91.8
Bilirubin (60 mg/dL)	16.90	17.65	95.8
HAMA	16.2	17.6	91.9
Hematocrit (15%)	14.40	14.20	101.4
Hematocrit (30%)	15.00	14.20	105.6
Hematocrit (45%)	14.95	14.20	105.3
Hematocrit (60%)	13.30	14.20	93.7

Potential cross-reactivity with tacrolimus metabolites was evaluated by adding different concentrations of the major metabolites of tacrolimus to negative whole blood samples. Five replicates of each sample were assayed and the cross-reactivity was calculated. The results are shown in the chart below.

	Mean of Metabolite (ng/mL)	Mean of Blank (ng/mL)	Percent (%) Cross-Reactivity
13-O-demethyl tacrolimus	11.04	0.6	10.4%
31-O-demethyl tacrolimus	2.22	0.6	1.6%
15-O-demethyl tacrolimus	2.84	0.6	2.2%
12-hydroxy tacrolimus	21.66	0.6	21.1%
15,31-O didemethyl tacrolimus	3.1	0.6	2.5%
13,31-O didemethyl tacrolimus	1.18	0.6	0.6%
13,15-O didemethyl tacrolimus	1.54	0.6	0.9%
M-8	2.8	0.6	2.2%

The sponsor conducted an interference study on forty-five commonly co-

administered drugs, including four immunosuppressive drugs. A sample was prepared containing the co-administrated drug in the presence of 15 ng/mL tacrolimus in negative whole blood. All samples were pretreated and assayed five times. No cross-reactivity was observed and a complete list of the compounds can be found in the assay package insert.

The sponsor recommends that the blood should be drawn using EDTA as the anticoagulant. Heparinized samples are not recommended because they may form clots during storage. The sponsor notifies users of this in the storage and collection section of the package insert.

*f. Assay cut-off:*

Not applicable.

2. Comparison studies:

*a. Method comparison with predicate device:*

The Emit® Tacrolimus Assay 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. The samples were pretreated with the sample pretreatment reagent.

Study	Transplant Type	N	Slope (ng/mL)	Intercept (ng/mL)	Correlation (r)
<b>Site 1</b>					
Emit® Tacrolimus Assay vs. LC-MS/MS	Kidney and Liver	71	0.93 +/- 0.05	0.15 +/- 0.37	0.908
Emit® Tacrolimus Assay vs. IMx Assay	Kidney and Liver	67	1.14 +/- 0.08	-1.28 +/- 0.54	0.881
<b>Site 2</b>					
Emit® Tacrolimus Assay vs. LC-MS/MS	Kidney and Liver	84	1.09 +/- 0.04	0.591 +/- 0.33	0.947
Emit® Tacrolimus Assay vs. IMx Assay	Kidney and Liver	83	0.76 +/- 0.06	1.08 +/- 0.64	0.822
<b>Combined sites 1 and 2</b>					
Emit® Tacrolimus Assay vs. LC-MS/MS	Kidney and Liver	155	1.06 +/- 0.04	0.09 +/- 0.29	0.916
Emit® Tacrolimus Assay vs. IMx Assay	Kidney and Liver	150	0.80 +/- 0.04	0.84 +/- 0.39	0.844

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 known 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 interchangeable 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.

Jusko WJ, Thomson AW, Fung J, et.al. Consensus document: therapeutic monitoring of tacrolimus (FK-506). *Ther. Drug Monit.* 1995;17:606-614.

Physician's Desk Reference, 57<sup>th</sup> ed. Montvale, NJ: Medical Economics Co. Inc; 2003:1371-1376.

**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.