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

A. 510(k) Number:

k063868

B. Purpose for Submission:

New device

C. Measurand:

Tacrolimus

D. Type of Test:

Quantitative LC/MS/MS

E. Applicant:

Waters Corporation

F. Proprietary and Established Names:

Proprietary: MassTrak Immunosuppressants Kit
Established: LC/MS/MS Analysis for Tacrolimus in Whole Blood

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
MLM	II	21 CFR 862.1678	75 Chemistry
JIT	II	21 CFR 862.1150	75 Chemistry

H. Intended Use:

1. Intended use(s):

   Refer to Indications for Use

2. Indication(s) for use:

The Waters MassTrak Immunosuppressants Kit is indicated for the quantification of the immunosuppressive drug Tacrolimus (FK506; Prograf) in liver and kidney transplant patient whole blood samples as an aid in the management of tacrolimus therapy.

3. Special conditions for use statement(s):

   Prescription Use Only

4. Special instrument requirements:

   These reagents, calibrators, and controls are designed to be used with a Liquid Chromatography / Tandem Mass Spectrometry (LC/MS/MS) system only.

I. Device Description:

The device consists of six levels of calibrator, three levels of controls, neat solution, internal standard, and an extraction column. Other reagents required but not included are HPLC grade water, zinc sulfate heptahydrate, HPLC grade methanol, HPLC grade acetonitrile, ammonium acetate, and formic acid.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   EMIT 2000 Tacrolimus Assay
   CEDIA Tacrolimus Assay

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

   k060385
   k050206

3. Comparison with predicate:

Similarities			
Item	Device	Predicate 1	Predicate 2
Intended Use	The Waters MassTrak Immunosuppressants Kit is indicated for the quantification of the immunosuppressive drug Tacrolimus (FK506; Prograf) in liver and kidney transplant patient whole blood samples as an aid in the management of tacrolimus therapy.	Intended 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.	Intended for the quantitative determination of Tacrolimus in human whole blood using automated clinical chemistry analyzers as an aid in the management of kidney and liver transplant recipients receiving Tacrolimus therapy.
Matrix	Whole Blood	Whole Blood	Whole Blood
Assay Technology	LC/MS/MS	Immunoassay	Immunoassay
Assay Range	0.5 – 31.7 ng/mL	2 – 30 ng/mL	2 – 30 ng/mL
Sample Pretreatment	Whole blood samples treated with organic solvent to precipitate protein and extract Tacrolimus; assay performed on supernatant	Whole blood samples treated with cupric sulfate in water; assay performed on supernatant	Whole blood samples treated with Zinc sulfate; assay performed on supernatant
Differences			
Item	Device	Predicate 1	Predicate 2
Instrumentation	Liquid chromatography / tandem mass spectrometry (LC/MS/MS)	Clinical Chemistry Analyzers	Clinical Chemistry Analyzers
Calibrators	Six (6) levels 0,3,6,12 20, and 30 ng/mL of Tacrolimus	Six (6) levels 0,2.5,5,10, 20, and 30 ng/mL of Tacrolimus	Two (2) levels 0 and 30ng/mL
Kit/Reagent Storage	-20°C	2-8°C	2-8°C
Antibody	None	mouse monoclonal anti-Tacrolimus antibodies	mouse monoclonal anti-Tacrolimus antibodies

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

Class II Special Controls Guidance Document: Cyclosporine and Tacrolimus Assays; Guidance for Industry and FDA

CLSI EP-5A2: Evaluation of Precision Performance of Quantitative Measurement Methods

CLSI EP-6A: Evaluation of the Linearity of Quantitative Measurement

CLSI EP-7A: Interference Testing in Clinical Chemistry

CLSI EP-9A2: Method Comparison and Bias Estimation

CLSI EP-17A: Protocols for Determination of Limits of Detection

L. Test Principle:

LC/MS/MS utilizes three dimensions of separation/selection for the target analyte prior to the detection step.

1. A chromatographic dimension in which the selection is based on the ability to separate the target analyte from interferences under the conditions of the separation;

2. A primary mass separation, MS 1, in which the selection is based on the molecular mass of the target analyte. The sample for the mass-based separation is a defined retention window from the chromatography selected to specifically to eliminate interferences; and

3. A secondary mass separation, MS 2, which occurs after the target mass window from the primary mass separation is subjected to conditions to fragment the target analyte. The secondary mass separation is then performed on the fragments from the primary mass separation target mass window. The signal for measurement is then obtained from the mass window known to contain the analyte fragment target mass again to specifically eliminate interferences.

In a tandem quadrupole mass spectrometer, (MS/MS) the mass windows described above are typically 1.0 – 1.5 Da (atomic mass units) in width and therefore provide highly selective filters for the analytes of interest.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

The sponsor evaluated precision at three external sites by assaying three levels of tacrolimus according to CLSI EP5-A2. Samples were prepared by spiking tacrolimus into pooled patient whole blood. Concentrations were chosen to represent the middle of the reportable range and the low and high medical decision points. Each level was analyzed in duplicate, twice per day over 20 days (n = 80). Results were as follows:

Site 1

Material	Mean (ng/mL)	Total		Within-run	
		SD	CV%	SD	CV%
Low Pool	2.807	0.14	4.7	0.10	3.4
Medium Pool	8.996	0.31	3.4	0.24	2.7
High Pool	20.025	0.73	3.6	0.38	1.9

Site 2

Material	Mean (ng/mL)	Total		Within-run	
		SD	CV%	SD	CV%
Low Pool	2.594	0.21	7.9	0.15	5.7
Medium Pool	11.335	0.57	5.1	0.39	3.5
High Pool	27.784	0.76	2.7	0.66	2.4

Site 3

Material	Mean (ng/mL)	Total		Within-run	
		SD	CV%	SD	CV%
Low Pool	2.041	0.16	7.6	0.11	5.6
Medium Pool	10.913	0.40	3.7	0.35	3.2
High Pool	29.898	1.12	3.7	0.72	2.4

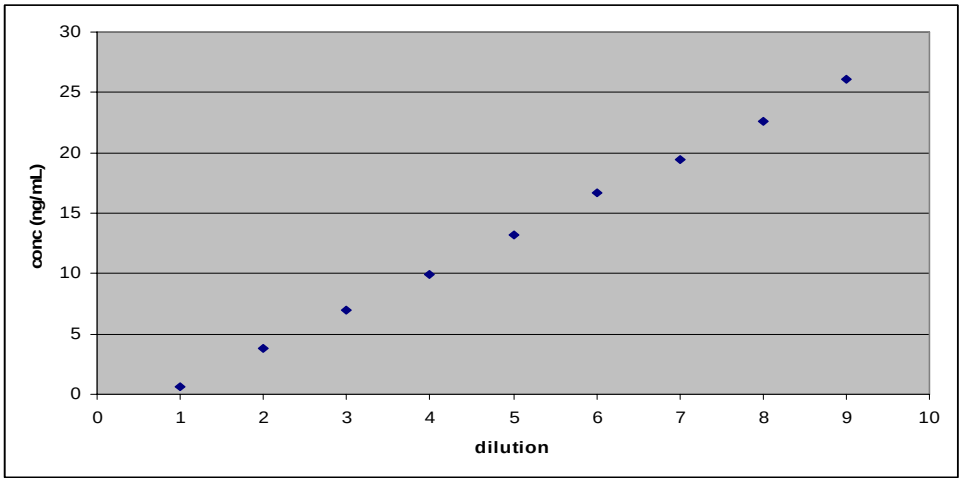
b. Linearity/assay reportable range:

Linearity was assessed at two sites according to both CLSI EP-6 and EP-6A. The latter document utilizes polynomial regression analysis to assess whether the data set is statistically non-linear. Using this method, none of the second or third order coefficients were significantly different from zero at the 95% confidence level, indicating that the data are linear. EP-6 assesses linearity using lack-of-fit modeling. According to this assessment the data were also

found to be linear. The dilution series was made starting with one high and one low sample and making evenly spaced intermediate dilutions according to the CLSI guideline for a total of nine concentrations. Below is a summary of the raw data collected and the appearance of a simple XY plot.

Site 1

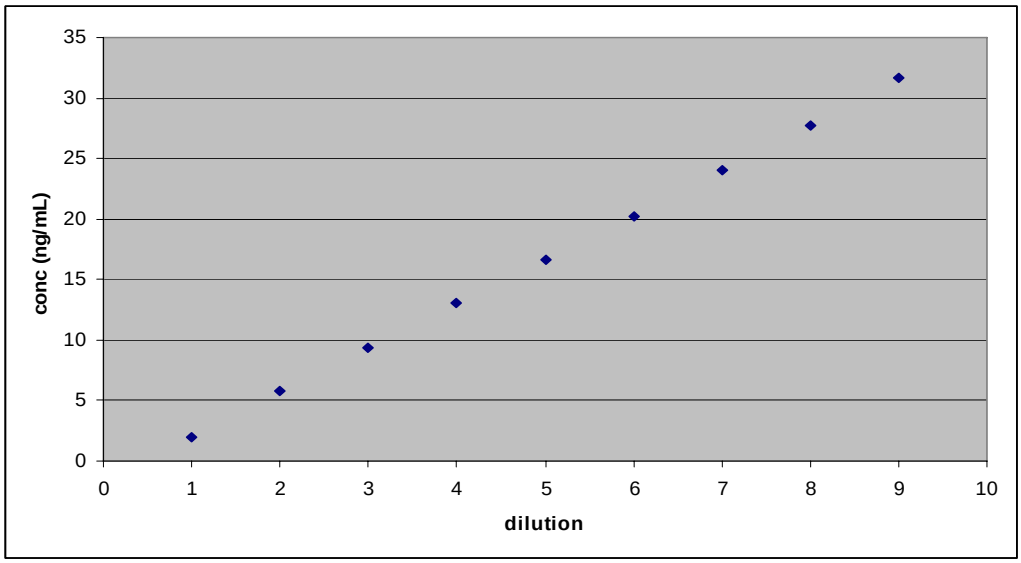
Dilution	Mean conc (ng/mL) n = 4
1	0.68
2	3.84
3	6.92
4	9.88
5	13.25
6	16.71
7	19.47
8	22.63
9	26.10



Site 2

Dilution	Mean conc (ng/mL) n = 4
1	1.98
2	5.85
3	9.38
4	13.08
5	16.68
6	20.18
7	24.05

Dilution	Mean conc (ng/mL) n = 4
8	27.75
9	31.70



The sponsor also assessed recovery of the assay at one site by spiking tacrolimus into six whole blood samples. Each sample had an initial tacrolimus concentration of 5 – 10 ng/mL which was measured prior to spiking. Aliquots of each of the six samples were then spiked with an additional 5, 10, and 20 ng/mL Tacrolimus (n = 18). Target values were calculated for each aliquot as (initial concentration + amount of spike). Each of the 18 samples were analyzed in triplicate. Recovery study results are summarized in the following table:

Sample	Mean % Recovery		
	5 ng/mL spike (measured in triplicate)	10 ng/mL spike (measured in triplicate)	20 ng/mL spike (measured in triplicate)
1	96.27	108.83	101.88
2	117.13	109.30	108.82
3	86.27	92.73	103.82
4	99.60	102.67	107.97
5	100.67	115.10	110.17
6	92.87	86.03	98.40
Grand Mean	98.80	102.44	105.18

The reportable range of the assay is 0.5 – 31.7 ng/mL

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

Although the use of fresh samples is recommended, the sponsor has made the following claims for sample stability in the labeling:

- Room temperature (22° C) for 24 hours
- Refrigerator temperature (4° C) for 7 days
- Frozen (-20° C) for 60 days and stable through three freeze/thaw cycles

These claims were validated by the sponsor's sample stability testing protocol

Calibrators, controls, and internal standard are prepared by spiking tacrolimus into tacrolimus-free whole blood. Samples are then freeze-dried, reconstituted and assayed as unknowns by seven different laboratories using validated Tacrolimus methods to assign values to a master lot. Production lots are traceable to the master lot. The sponsor states that there are currently no recognized reference standards for tacrolimus.

Calibrator and control stability dating is established by assessing kit recovery at various time points. Frozen proficiency testing samples are thawed, analyzed, and compared to the peer group mean. The sponsor's results were within ± 10% of the peer group mean.

d. *Detection limit:*

The lower limit of quantification (LLoQ) was determined using CLSI EP-17A as a guide. Twelve clinical samples were selected for analysis ranging in concentration from 0.125 to 0.6 ng/mL. Each of the samples was analyzed on four different days with four replicate injections of each sample (n=16). The LLoQ was defined as the lowest concentration where the CV<20% with <20% deviation from the expected concentration. Samples at a concentration of 0.5 ng/mL (the sponsor's claimed LOQ) met these criteria.

e. *Analytical specificity:*

Potential interferences due to the following substances or conditions were evaluated using CLSI EP7-A as a guide:

Potential Interferent	**Maximum Concentration**
Anticoagulants	
K_2EDTA	9.0 mg/mL (5 X normal)
Endogenous Substances	
Hematocrit	15 – 60%
Bilirubin	60 mg/dL
Albumin	12 g/dL

Potential Interferent	Maximum Concentration
Cholesterol	500 mg/dL
Triglycerides	1500 mg/dL
Uric Acid	20 mg/dL
Vitamin B12	1000 pg/mL
Exogenous Substances	
Amphotericin B	100 µg/mL
Cyclosporine	5000 ng/mL
Digoxin	25 nmol/L
Rifampin	390 µmol/L
Sirolimus	200 ng/mL
Vancomycin	270 µmol/L

A base pool of ≈20 ng/mL tacrolimus was first analyzed for tacrolimus as the control concentration. Each substance at the concentration in the list above was then added to an aliquot of the base pool and reanalyzed for tacrolimus. All of the compounds tested caused a change in tacrolimus concentration of ≤ ±10% from the control concentration.

Potential interferents with a molecular mass of < 750 Daltons were excluded from testing because they would not reasonably be expected to cause interference with this LC-MS-MS method due to the difference in mass between the potential interferent and the target analyte. .

The identities of three known metabolites are 12-hydroxy-Tacrolimus, demethyl-Tacrolimus (13-0, 15-0 and 31-0) and 13, 31-0-didemethyl-Tacrolimus. Five clinical samples with Tacrolimus levels > 20 ng/mL were reanalyzed according to a standard curve with quality control materials. An instrument method was created to record transitions from masses of the precursor ammonium adducts to the respective product daughter ions for the individual metabolites at *m/z* 793.5>740.4 (didemethyl), *m/z* 807.5>754.4 (demethyl), *m/z* 809.5>756.4 (ascomycin - internal standard), *m/z* 821.5>768.4 (Tacrolimus) and m/z 837.5>786.4 (hydroxy), as per tuning with Tacrolimus and ascomycin.

Comparison of the traces of the different transitions showed that there was no evidence of any *m/z* 793.5>740.4, *m/z* 807.5>754.4 or *m/z* 837.5>786.4 products in the standard curve or quality control materials. However, all five clinical samples exhibited one or more peaks in the m/z 807.5>754.4 channel at 0.80, and 0.80 and 1.10 minutes in the m/z 809.5>756.4 channel (Tacrolimus and ascomycin elute at 0.90 minutes). The maximum intensity of the demethyl-metabolite peaks was about one factor of ten lower than the Tacrolimus peak. There was no evidence of the 12-hydroxy- or the 13, 31-O-didemethylmetabolites.

Metabolite	Parent (Precursor) Ion (m/z)	Product (Daughter) Ion (m/z)	Peak Detected? Retention Time
13, 31-0-didemethyl Tacrolimus	793.5	740.4	No
13-0-demethyl-, 15-0-demethyl- & 31-0-demethyl Tacrolimus	807.5	754.4	Yes, at 0.8 minutes
12-hydroxy Tacrolimus	837.5	786.4	No
Ascomycin (internal standard)	809.5	756.4	Yes, at 0.80 and 1.10 minutes

f. Assay cutoff:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

The Waters Tacrolimus method was compared with an LC/MS method and an LC/MS/MS method in three separate studies. Patients included both males and females, ranged in age from 2 – 73 years, and had received either a kidney or liver transplant. A summary of the studies follows:

Method Comparison Study 1 - LC/MS		
n		
Liver (58)	Slope (95% CI)	1.103 (1.089 – 1.118)
	Intercept (95% CI)	-0.192 (-0.313 - -0.071)
	r	0.9975
Kidney (51)	Slope (95% CI)	1.078 (1.058 – 1.098)
	Intercept (95% CI)	-0.128 (-0.290 – 0.033)
	r	0.9956

Method Comparison Study 2 - LC/MS/MS		
n		
Liver (50)	Slope (95% CI)	1.048 (1.006 – 1.091)
	Intercept (95% CI)	0.124 (-0.153 – 0.401)
	r	0.9797
Kidney (50)	Slope (95% CI)	1.112 (1.075 – 1.149)
	Intercept (95% CI)	-0.100 (-0.446 – 0.246)
	r	0.9864

Method Comparison Study 3 - LC/MS/MS		
n		
Liver (50)	Slope (95% CI)	1.006 (0.973 – 1.038)
	Intercept (95% CI)	-0.11 (-0.34 – 0.12)
	r	0.9872
Kidney (50)	Slope (95% CI)	0.985 (0.962 – 1.008)
	Intercept (95% CI)	-0.02 (-0.22 – 0.17)
	r	0.9933

b. Matrix *comparison:*

Not applicable.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable.

b. Clinical specificity:

Not applicable.

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

No firm therapeutic range exists for tacrolimus in whole blood. The optimum therapeutic range of tacrolimus used in each institution relies on factors that pertain to the needs of its patient population and the specific assay used. 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 several other factors also contribute to different requirements for optimal blood levels of tacrolimus. Furthermore, the wide variety of parameters leading to optimal tacrolimus therapy on an individual basis means that tacrolimus measurements alone cannot be used as an indication for changing treatment regimens. Each patient should be thoroughly evaluated clinically before changes in treatment regimens are made.

It should be noted that LC/MS/MS target ranges may be lower than immunoassay ranges, owing to the lack of metabolite cross-reactivity.

Recommended therapeutic ranges for Tacrolimus

Method	Kidney (ng/mL)	Liver (ng/mL)
Initial MEIA	10 – 15	10 – 15
Maintenance MEIA	5 – 10	5 – 10

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR 809.10.

O. Conclusion:

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