

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

A. 510(k) Number:

k053061

B. Purpose for Submission:

Addition of extended range application

C. Measurand:

Cyclosporine

D. Type of Test:

Quantitative Immunoassay

E. Applicant:

Dade Behring Inc.

F. Proprietary and Established Names:

EMIT® 2000 CSAE Cyclosporine Specific Assay

G. Regulatory Information:

1. Regulation section:

21 CFR §862.1235, Cyclosporine test system

2. Classification:

Class II

3. Product code:

MKW

4. Panel:

91 Clinical Toxicology

H. Intended Use:

1. Intended use(s):

See item 2 below.

2. Indication(s) for use:

The Emit® 2000 Cyclosporine Specific Assay is for in vitro quantitative analysis of cyclosporine (CsA) in human whole blood as an aid in the management of cyclosporine therapy in kidney, heart and liver transplant patients.

3. Special conditions for use statement(s):

None

4. Special instrument requirements:

The Emit 2000 Cyclosporine Specific Assay Extended Range requires either the Viva-E™ or Viva-Twin ® analyzer.

I. Device Description:

The Emit 2000 Cyclosporine Specific assay employs a homogeneous enzyme immunoassay technique for the analysis of cyclosporine in whole blood. The assay reagents are liquid and consist of the Antibody/Substrate Reagent A and the Enzyme Reagent B. There was no change to the reagents to accommodate the extended range application.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Abbott TDx/TDxFLx Cyclosporine Monoclonal Whole Blood Assay

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

k040761

3. Comparison with predicate:

Similarities		
Item	EMIT 2000 Cyclosporine Specific Assay	Abbott TDx/TDxFLx Cyclosporine Assay
Intended Use	In vitro quantitative analysis of cyclosporine (CsA) in human whole blood as an aid in the management of cyclosporine therapy in kidney, heart, and liver transplant patients	In vitro quantitative measurement of cyclosporine (Sandimmune®, Cyclosporine A) in human whole blood as an aid in the management of cardiac, liver, and renal transplant patients
Sample type	Human whole blood	Human whole blood
Antibody	Mouse monoclonal	Mouse monoclonal
Sample pretreatment	Requires manual pretreatment	Requires manual pretreatment

Differences		
Item	Device	Predicate
Technology	EMIT (Enzyme multiplied immunoassay technique) technology	FPIA (Fluorescence Polarization Immunoassay) technology
Assay Range	40-500 ng/mL or 350-2000 ng/mL	25-1500 ng/mL

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

- Evaluation of the Linearity of Quantitative Analytical Methods (EP6-P, version August 1986)
- Interference Testing in Clinical Chemistry (EP7-P, version February, 1982)
- Evaluation of Precision Performance of Clinical Chemistry Devices (EP5-A February, 1999)
- Class II Special Controls Guidance Document: Cyclosporine and Tacrolimus Assays; Guidance for Industry and FDA (September, 2002)

L. Test Principle:

The Emit 2000 Cyclosporine Specific Assay is based on the competition immunoassay principle. Cyclosporine in the sample competes with cyclosporine in Enzyme Reagent B that is labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH). Active (unbound) enzyme converts the oxidized nicotinamide adenine dinucleotide (NAD) in Antibody Reagent A to NADH, resulting in a kinetic absorbance change that can be measured spectrophotometrically. Enzyme activity decreases upon binding the antibody, allowing the cyclosporine concentration in the sample to be measured in terms of enzyme activity.

The assay requires the use of whole blood. Samples, calibrators, and controls must first be pretreated with the Emit Cyclosporine Pretreatment Reagent. The reagent lyses the cells, extracts the cyclosporine, and precipitates most of the blood proteins. The pretreated samples are then centrifuged and an aliquot of the resulting supernatant containing the cyclosporine can be assayed using the Emit 2000 Cyclosporine Specific Assay.

The assay may either be run to measure cyclosporine concentrations from 40-500 ng/mL or as the extended range application from 350-2000 ng/mL. Separate calibrators are required for each application.

M. Performance Characteristics (if/when applicable):1. Analytical performance:*a. Precision/Reproducibility:*

Precision studies were performed in accordance with CLSI EP5-A (formerly NCCLS). Whole blood pools at three different levels were prepared by spiking cyclosporine into EDTA whole blood, resulting in samples ~400 ng/mL (Level 1), ~900 ng/mL (Level 2), and ~1400 ng/mL (Level 3). The study was conducted for 20 days, with 2 runs per day with samples run in duplicate. For the duplicate measurements, the sample was first pretreated as per the Instructions for Use, and then split for each replicate measurement.

Within run imprecision ranged from 3.3-4.8% c.v. and total imprecision ranged from 5.9-8.7%.

b. Linearity/assay reportable range:

Linearity was performed according to CLSI EP6-P. Cyclosporine-free EDTA whole blood was spiked with cyclosporine at concentrations covering the extended application range (250, 350, 500, 750, 1000, 1250, 1500, 1750, and 2000 ng/mL). Four separate runs of each level were pretreated and assayed five times (n=20 at each concentration) on the V-Twin analyzer.

The data was analyzed by linear regression and yielded a slope of 1.02, intercept 3.80 and r^2 0.996. The data supports the assay range of 350-2000 ng/mL.

The accuracy of diluting samples with high cyclosporine concentration was evaluated by spiking five patient samples with cyclosporine (2000-2400 ng/mL), and then diluting 1:2 and 1:4 with either cyclosporine-negative EDTA whole blood or Emit 2000 Cyclosporine Specific Calibrator 0. The diluted samples were pretreated and then assayed on the V-Twin analyzer. The result for each diluted sample was compared to the nominal concentration of the spiked patient samples. All diluted samples quantitated within the established acceptance criteria of 90-110% compared to the reference.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):
The shelf-life of the assay was previously established for the PMA submission P920031. No changes were made to the reagents for the extended range claim.

Calibrators were reviewed under k053108.

d. Detection limit:

In order to describe the functional sensitivity, whole blood pools and control samples with values near the lower end of the measuring range were tested over a 20 day period. The functional sensitivity of the assay is considered to be concentration at which the inter-assay coefficient of variation is $\leq 20\%$. For this assay, a whole blood pool with a mean value of ~ 360 ng/mL had a %CV of 10.2 demonstrating a functional sensitivity of < 350 ng/mL.

e. Analytical specificity:

The effects of major cyclosporine metabolites, potential endogenous interferents, and co-administered common or immunosuppressive drugs on assay performance were evaluated.

In order to assess cross-reactivity of cyclosporine metabolites, 1000 ng/mL of each metabolite (AM1, AM4n, AM9, AM19, AM1c, AM1c9, AM4c9) were spiked into whole blood samples containing 800 ng/mL of cyclosporine. AM1c9 was also tested at 800 ng/mL. The reference was the same sample without metabolite. The cross-reactivity of the metabolites ranged from -5.9% to 5.5% and met the manufacturer's specification of values within $\pm 10\%$ of the reference sample.

The effects of bilirubin (60 mg/dL), cholesterol (500 mg/dL), uric acid (20 mg/dL), albumin (12 g/dL), IgG (12 g/dL), triglycerides (1500 mg/dL), rheumatoid factor (500 IU/ml), hematocrit (66.5%), and HAMA were evaluated. A sample containing the interferent was spiked with 800 ng/mL cyclosporine and compared to the same sample with cyclosporine alone. Interference is considered to be any sample that shows greater than 10% bias. The endogenous compounds tested did not interfere with the cyclosporine assay.

Commonly co-administered drugs, including immunosuppressive drugs, were evaluated for interference. Samples were prepared by adding each drug to a whole blood sample containing 800 ng/mL cyclosporine. The control was the sample without the added drug. Sixty drugs that were tested and the list can be found in the package insert under Specificity section. Interference was defined as values greater than $\pm 10\%$ of the control sample. Each drug tested did not interfere with the cyclosporine assay at the level described for each drug.

f. Assay cut-off:
Not applicable

2. Comparison studies:

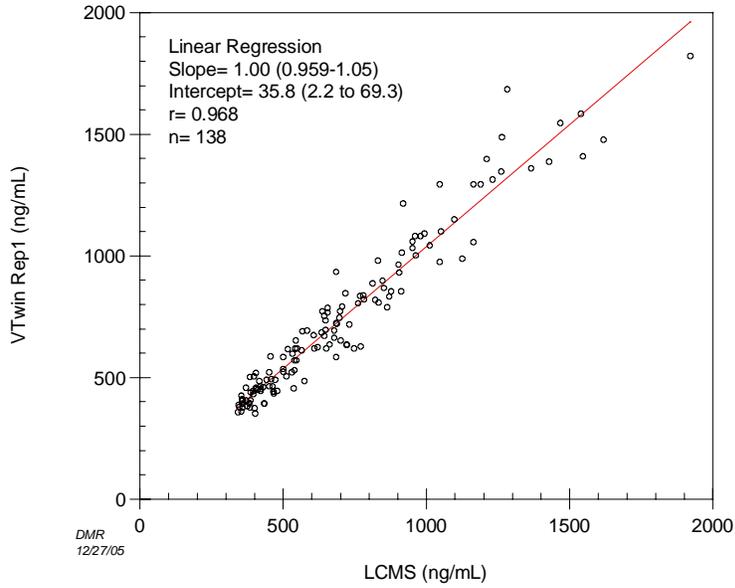
a. Method comparison with predicate device:

Studies for the extended range application were conducted at two hospitals: Methodist Medical Center of Illinois (MMCI) and University of Maryland Medical Center (UMMC). A total of 138 banked retrospective samples from heart, liver, or kidney transplant patients on cyclosporine therapy were included in the study. Samples from both male and female patients, with varying ranges of time post-transplant, and varying ranges of time of blood draw with respect to drug administration were collected. Samples were distributed across most of the assay range however supplemental samples from kidney transplant patients were included to cover the range from 1500-2000 ng/mL. Whole blood EDTA samples were kept frozen until testing. At each site samples were tested using the EMIT 2000 Cyclosporine extended range application on the V-Twin analyzer, on the Abbott TDx/TDxFLx Cyclosporine Monoclonal Whole Blood assay, and on LC/MS following established protocols.

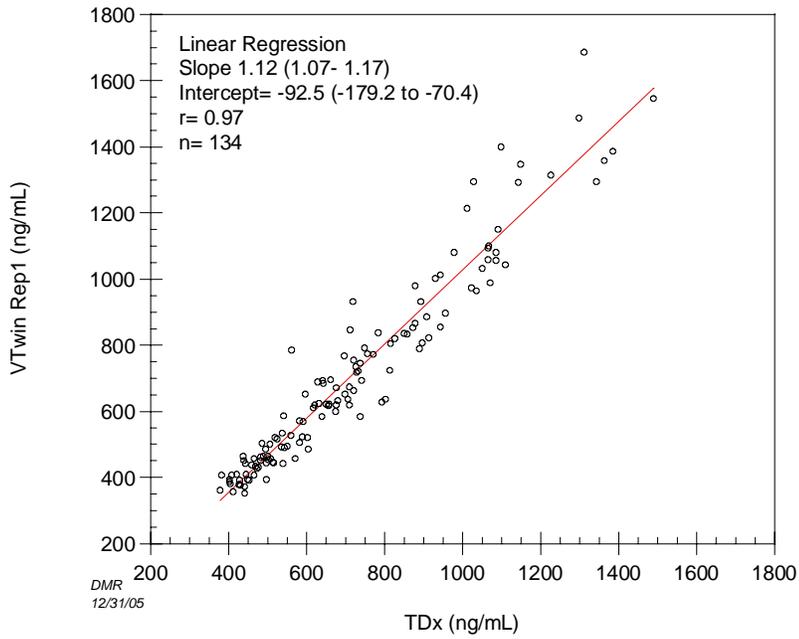
Results are presented in the following summary table and regression graphs. All three organ types and both clinical sites are included.

Method Comparison Statistics – sites combined				
Comparative Method	Slope	Intercept ng/mL	Correlation Coefficient	n
LC/MS				
All*	1.00	35.8	0.968	138
Heart	1.05	10.5	0.98	33
Liver	0.99	55.3	0.96	39
Kidney	1.12	-45.4	0.95	60
Abbott TDx®/TDx/FLx® Assay				
All*	1.12	-92.5	0.97	134
Heart	1.13	-95.3	0.97	33
Liver	1.13	-91.1	0.97	39
Kidney	1.14	-108.9	0.95	60
* Additional transplant patients were added to encompass the assay range.				

Cyclosporine Extended Range Both Sites- All Organ



Cyclosporine Extended Range Both Sites- All Organ



b. Matrix comparison:

Not applicable. Only whole blood EDTA may be used.

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 states in the labeling that no firm therapeutic range exists for cyclosporine in whole blood. .

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 and substantial equivalence decision.