

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

A. 510(k) Number:

k083487

B. Purpose for Submission:

New assay

C. Measurand:

Sirolimus

D. Type of Test:

Quantitative enzyme immunoassay

E. Applicant:

Siemens Healthcare Diagnostics, Inc.

F. Proprietary and Established Names:

EMIT 2000 Sirolimus Assay

EMIT 2000 Sirolimus Calibrators

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
<u>NRP</u>	<u>Sirolimus Test System</u>	<u>862.3840</u>	<u>Toxicology</u>
<u>DLJ</u>	<u>Clinical Toxicology Calibrator</u>	<u>862.3200</u>	<u>Toxicology</u>

H. Intended Use:

1. Intended use(s):

The Emit® 2000 Sirolimus Assay is for the in vitro quantitative analysis of sirolimus in human whole blood as an aid in the management of sirolimus therapy in kidney transplant patients.

The Emit® 2000 Siro/Tacro Sample Pretreatment Reagent is an accessory reagent for use with the Emit 2000 Sirolimus Assay and/or the Emit 2000 Tacrolimus Assay.

The Emit® 2000 Sirolimus Calibrators are intended for use in the calibration of the Emit 2000 Sirolimus Assay.

2. Indication(s) for use:

See intended use above.

3. Special conditions for use statement(s):

For prescription use.

See expected values section, below regarding interpretation of results.

4. Special instrument requirements:

Performance was evaluated on the Vital Scientific V-Twin Analyzer.

I. Device Description:

The Emit® 2000 Sirolimus Assay contains liquid reagents, including (mouse monoclonal) antibody, buffer, a pretreatment and enzyme reagents.

The Emit® 2000 Sirolimus Calibrators are frozen material containing sirolimus in a preserved human whole blood hemolysate. There are six calibrator levels with nominal concentrations of 0, 3, 6, 12, 24 and 36 ng/mL sirolimus.

The calibrators contain human source material. Each donor unit used in the preparation of this product was tested by FDA-approved methods for the presence of antibodies to human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2), as well as for hepatitis B surface antigen and antibody to hepatitis C virus (HCV), and found to be negative. Because no testing can offer complete assurance that these or other infectious agents are absent, this material should be handled using good laboratory practice to avoid skin contact and ingestion.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Abbott IMx Sirolimus Microparticle Enzyme Immunoassay

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

k042411

3. Comparison with predicate:

Both devices are immunoassays intended for use in the quantitative measurement of sirolimus in human whole blood. Both devices require a manual pretreatment. The predicate device uses microparticle enzyme immunoassay technology; this device uses a homogeneous enzyme immunoassay technology.

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

Conformance to standards was not noted by the sponsor.

L. Test Principle:

The Emit® 2000 Sirolimus Assay employs a homogeneous enzyme immunoassay technique used for analysis of sirolimus in whole blood. This assay contains mouse monoclonal antibodies reactive to sirolimus. The assay is based on competition for sirolimus antibody binding sites. Sirolimus in the sample competes with sirolimus 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 Sirolimus concentrations to be measured in terms of enzyme activity. Endogenous serum G6PDH does not interfere with the assay because coenzyme NAD functions only with bacterial (*Leuconostoc mesenteroides*) enzyme employed in this assay.

The pretreatment process lyses the cells, extracts the analyte and precipitates most of the blood proteins. The pretreated samples are centrifuged, the supernatant containing the analyte is assayed.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision studies were conducted at the manufacturer's site and at two external sites using whole blood pools at concentrations which represent sub-therapeutic, mid-therapeutic and upper levels. The pools were prepared from sirolimus spiked

into EDTA whole blood. A sample pool from patients treated with sirolimus was also evaluated. The study was conducted with one reagent lot. Multiple calibrations were performed during the study, as needed. Specimens at each level were analyzed in duplicate twice per day for 20 days (n=80). A separate (pre-analytical) extraction was performed for each sample. Results are summarized below.

Site 1: External		Repeatability		Within Lab	
Material	Mean, ng/mL	SD (ng/mL)	% CV	SD	%CV
Whole Blood Pool, Level 1	5.0	0.40	8.0	0.67	13.4
Whole Blood Pool, Level 2	10.7	0.38	3.6	0.88	8.3
Whole Blood Pool, Level 3	25.3	1.05	4.2	2.10	8.3

Site 2: External		Repeatability		Within Lab	
Material	Mean, ng/mL	SD	% CV	SD	%CV
Whole Blood Pool, Level 1	4.9	0.27	5.4	0.50	10.1
Whole Blood Pool, Level 2	10.3	0.50	4.9	0.77	7.4
Whole Blood Pool, Level 3	23.6	1.00	4.2	1.93	8.2

Manufacturer's site		Repeatability		Within Lab	
Material	Mean, ng/mL	SD	% CV	SD	%CV
Whole Blood Pool, Level 1	5.6	0.33	5.9	0.62	11.2
Whole Blood Pool, Level 2	10.8	0.29	2.7	0.86	8.0
Whole Blood Pool, Level 3	23.8	0.93	3.9	1.93	8.1
Patient Sample Pool	11.6	0.46	4.0	0.72	6.2

b. Linearity/assay reportable range:

Linearity/recovery by dilution:

A linearity study was performed using a patient sample spiked with a sirolimus stock solution (prepared from purified sirolimus) to a concentration of 30 ng/mL determined gravimetrically (independently of the EMIT assay). Intermediate concentrations were prepared by sequentially mixing the high sample with the Emit® 2000 Sirolimus Calibrator (0 ng/mL) to produce concentrations equally

spaced across the assay range. Each sample was pretreated first, and then and assayed n=5 times. Linear regression analysis showed slope = 1.02, intercept = -0.466ng/mL. Observed recoveries are shown in the table below.

	#1	#2	#3	#4	#5
Predicted Value (gravimetrically determined) ng/mL	0	7.2	15.1	23.0	31.0
V-Twin Mean, ng/mL	0	6.6	14.7	22.5	31.7

Spike recovery:

A recovery study was performed with a sirolimus stock solution (prepared from purified sirolimus) spiked into EDTA whole blood. The predicted concentration was determined gravimetrically (independently of the EMIT assay). Four separate runs were pretreated and assayed five times (total n=20 at each level). The percents recovery observed are shown below.

Nominal value ng/mL	V-Twin Mean ng/mL	% recovery
2.5	2.7	106.6
5	5.3	106.9
10	10.2	102.3
15	15.3	102.0
20	20.5	102.4
25	25.6	102.5
30	32.3	107.7

These studies support linearity and recovery/accuracy to 30 ng/mL. For lower limits of the assay, see *Detection Limit*, below.

High sample dilution:

The method for diluting high samples recommended in the manufacturer’s package insert was validated with patient samples, including samples containing sirolimus concentrations near the upper end of the assay range. Samples were diluted 1:1 with calibrator or negative whole blood. Observed percents recovery (averaged across replicates of each individual sample) were within +/-10%.

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

Calibrators are traceable to master pools formulated with sirolimus reference material of high purity. The master pool levels are confirmed by chromatographic methods from three reference labs. Estimated uncertainties

in calibrator measurements provided in the 510(k) were shown to be within acceptable limits.

The shelf-life of the Emit® 2000 Sirolimus Calibrators is established using real-time data collected from multiple lots. Recoveries, tested at 5 equal intervals throughout the expiration dating period are determined using a standard curve based on “control” calibrators stored at -70°C. Similar testing was performed for opened calibrator vials. At testing time points, calibrators were removed from storage at 2-8° C, sampled and returned to storage at that temperature. Recovery of the test calibrators (measured in replicates) are monitored over time. The sponsor indicated that real-time testing showed no significant drift during testing (< 0.02% change for shelf life studies) and that stability was within the manufacturer’s criteria.

d. Detection limit:

The limit of blank, limit of detection, and limit of quantitation were determined. The blank samples were sirolimus-free patient samples. The low-analyte samples were prepared from individual sirolimus patient samples that were diluted with sirolimus-free patient samples. The study utilized one reagent lot, two instruments, multiple (28) calibration curves, five blank samples and five low-analyte samples, and was conducted over 3 days. Blank and low analyte samples were assayed in duplicate on each instrument. Analyses showed LoB = 2.3 ng/mL; LoD=3.5 ng/mL.

To determine limit of quantitation, seven patient samples ranging from 1.6 – 6.7 ng/mL were measured on five consecutive days (over a period of 48 days), 1 run per day. Four replicate measurements were measured for each run. The study was performed with one instrument and one reagent lot. Recovery studies, as well as limit of detection and limit of quantitation, support the sponsor’s claimed limit of quantitation of 3.5 ng/mL.

e. Analytical specificity:

The effects of potential interferents on assay performance were evaluated. Potential sources of interference tested were hematocrit, endogenous compounds, other co-administered or OTC drugs.

a) A hematocrit range of 12.5% to 60% was evaluated. Sirolimus recovery relative to the control calibrator ranged from 90-102%. No trends were apparent.

b) Endogenous compounds:

Sirolimus was spiked into samples containing the interferents at concentrations listed in the table below. Sirolimus concentrations were 5

ng/mL and 20 ng/mL. Samples were pretreated and measure in replicates, n=5. Percents bias were all within +/-10%. Results are listed below.

Endogenous Compound (with 5 ng/mL Sirolimus)	% Bias observed with 5ng/mL sirolimus	% Bias observed with 20 ng/mL sirolimus
Albumin (12 g/dL)	0.1	-0.6
Bilirubin (60 mg/dL)	2.2	2.8
Cholesterol (500 mg/dL)	3.7	-0.7
Creatinine (30 mg/mL)	3.2	-3.7
HAMA A	5.3	1.7
HAMA B	7.2	-9.7
IgG (12 g/dL)	1.6	7.7
Rheumatoid Factor (500 IU/mL)	-4.5	1.1
Triglycerides (Intralipid®) (1500 mg/dL)	-3.1	3.2
Uric acid (20 mg/dL)	-1.5	-1.0

c) Co-administered drugs and common OTC drugs

Samples were prepared to contain potential interferences and 6 or 20 ng/mL sirolimus. All samples were pretreated and assayed n=5 times. The percent interference of each drug was calculated relative to control samples containing only sirolimus. The immunosuppressive drugs that were tested included: cyclosporine, mycophenolic acid and its metabolite MPAG, and tacrolimus. A list of available drugs (with concentrations) tested is included in the package insert. At the concentrations tested, the drugs did not cause significant interference with the assay. Recoveries were within +/-10% of the control sample.

d) Cross-reactivity

Major sirolimus metabolites were evaluated for cross-reactivity, at a concentration of 25 ng/mL, in the presence of 0 ng/mL and 10 ng/mL sirolimus. The samples were pretreated, and assayed (n=5 replicates). Results, listed in the table below, were compared against a control sample. Cross reactivity was calculated as:

$$\frac{\text{Mean of metabolite} - \text{Mean of Control}}{\text{Metabolite concentration}} \times 100$$

Metabolite Name (Alternate Name) 0 ng/mL sirolimus	Percent (%) Cross- Reactivity in presence of 0ng/mL sirolimus	Percent (%) Cross- Reactivity in presence of 10 ng/mL sirolimus
7-O-demethyl sirolimus (16-O-demethyl sirolimus)	15%	21%

12-hydroxy sirolimus (11-hydroxy sirolimus)	4%	6%
41-O-demethyl sirolimus (39-O-demethyl sirolimus)	38%	42%
27, 39-O-didemethyl sirolimus (41, 32-O-didemethyl sirolimus)	52%	61%

f. *Assay cut-off:*

Not applicable – this is a quantitative assay.

2. Comparison studies:

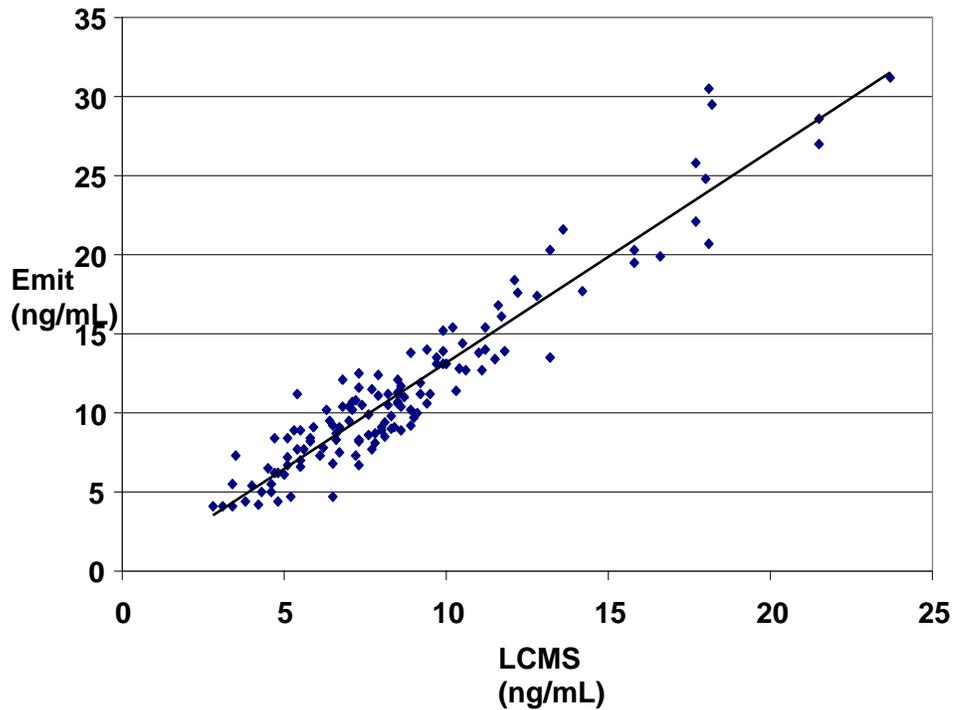
a. *Method comparison with predicate device:*

Measurements of patient samples with the Emit® 2000 Sirolimus Assay were compared to LC/MS/MS. (Information on the comparator method was included in the 510(k)). The studies were conducted externally at two sites. Samples (n=123) were whole blood EDTA collected from kidney transplant patients treated with sirolimus. The mean time since transplant was 6.9 years, and all but six samples were from patients > 6 months post-transplant. Samples evaluated were trough (C0) samples. The majority of the samples were from one draw per patient. In cases where patient samples were from two draws per patient, these were separated in time (2 weeks to 4 months). Analysis of the data showed there was no significant effect of repeat measurements (from a single patient) on the regression statistics.

Samples were pretreated with the Emit® 2000 Siro/Tacro Sample Pretreatment Reagent and run in random order on the V-Twin Analyzer. Calibrations occurred as needed during the course of testing at each site (4 calibrations at site 1, two calibrations at site 2). The data was analyzed by least squares linear regression. The first replicate of the Emit Assay result and LC/MS/MS result was used in the data analysis. The values obtained on the V-Twin Analyzer spanned the assay range from 4.1 – 29.5 ng/mL. Results from each site separately were included in the 510(k), and are pooled below.

# of samples	Combined sites
Slope (95% CI)	1.300 +/- 0.040
Intercept (ng/mL) (95% CI)	0.054 +/- 0.370
Correlation Coeff., r	0.946
Sy,x (ng/mL)	1.66

The graph below represents the data pooled from both sites. Each data point represents a single measurement:



b. *Matrix comparison:*

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

3. Clinical studies:

a. *Clinical Sensitivity:*

Not applicable; Clinical sensitivity and specificity is not typically provided in 510(k)s for this type of assay.

b. *Clinical specificity:*

See 3a, above.

c. Other clinical supportive data (when a. and b. are not applicable):

Data regarding patient demographics and selection criteria were provided in the method comparison evaluation in the 510(k).

4. Clinical cut-off:

Not applicable; this is a quantitative assay.

5. Expected values/Reference range:

The following is stated in the package insert:

The optimal concentration range for sirolimus in whole blood using this assay has not been established. Optimal concentration ranges vary according to the specific assay used, and therefore should be established for each specific assay. Values obtained with different assay methods should not be used interchangeably due to differences in cross-reactivity with metabolites, nor should correction factors be applied. Laboratories should include identification of the assay used in order to aid in interpretation of results. Each institution should establish the optimal ranges based on the specific assay used and other factors relevant to their patient population. Optimal ranges depend upon the patient's clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic effects of sirolimus, co-administration of other immunosuppressants, time post transplant and a number of other factors. Therefore, individual sirolimus 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.

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.