

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

A. 510(k) Number:

k081857

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:

Dimension SIRO Flex® reagent cartridge

Dimension Sirolimus Calibrator

G. Regulatory Information:

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

H. Intended Use:

1. Intended use(s):

The SIRO Flex® reagent cartridge is an in vitro diagnostic test for the quantitative measurement of sirolimus in human whole blood on the Dimension® clinical chemistry system. Measurements of sirolimus are used as an aid in the management of sirolimus therapy in renal transplant patients.

The sirolimus calibrator is an in vitro diagnostic product for the calibration of sirolimus (SIRO) on the Dimension® clinical chemistry system.

2. Indication(s) for use:

See intended use

3. Special conditions for use statement(s):

See expected values section, below.
For prescription use.

4. Special instrument requirements:

For use on the DIMENSION® instrument.

I. Device Description:

The reagent cartridge consists of wells containing the following:

<u>Form</u>	<u>Ingredient</u>	<u>Concentration</u>	<u>Source</u>
Liquid	Ab-β-galactosidase		mouse monoclonal
Tablets	Sirolimus-CrO2	30 mg/tab	
Tablets	CPRG	8.4 mg/tab	
Liquid Substrate Diluent			
Liquid Pretreatment reagent			

Tablets contain excipients, buffers, and stabilizers.

The calibrator is supplied as a frozen liquid product packaged as a single vial for each of five levels. The matrix is human whole blood hemolysate with preservatives. Levels 2, 3, 4, and 5 contain sirolimus drug at target values of 5, 10, 20, and 30 ng/mL, respectively. Level 1 is a human whole blood hemolysate that does not contain 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 (not repeatedly reactive). 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 the Dimension[®] and the predicate sirolimus assay have similar intended use and both are enzyme immunoassays. They are for use on different instrument systems and reagents and antibodies differ. Sample pretreatment is automatic on the Dimension System; pretreatment is performed manually for the predicate device.

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

None noted by the sponsor.

L. Test Principle:

The automated Dimension[®] SIRO method uses an immunoassay technique in which free and sirolimus-bound antibody-enzyme conjugates are separated using magnetic particles. The assay is performed using a method specific Flex[®] reagent cartridge. The Flex[®] cartridge contains a pretreatment reagent, antibody- β -galactosidase conjugate, sirolimus immobilized on chromium dioxide particles, chlorophenol red β -d-galactopyranoside (CPRG) substrate, and diluent to hydrate the tablets. The Dimension[®] system automatically mixes and lyses the whole blood sample. The lysed sample is then mixed with the antibody enzyme conjugate. The sirolimus present in the sample is bound by the sirolimus antibody conjugate reagent. Magnetic particles coated with sirolimus are added to bind free (unbound) antibody-enzyme conjugate. The reaction mixture is then separated magnetically. Following separation, the supernatant containing the sirolimus-antibody-enzyme complex is transferred to another cuvette and mixed with the substrate. β -galactosidase catalyzes the hydrolysis of CPRG (chlorophenol red β -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 sirolimus 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:*

Precision studies were conducted internally, and at two external sites. One reagent product lot and 1 instrument was evaluated at each site. For each test level, samples were measured in duplicate twice per day, for 20 days (total n=80). Testing was conducted with one reagent lot across multiple instruments at one internal and two external locations. The repeatability and within-lab standard deviations were calculated by the analysis of variance method independently for each site. Samples included whole blood pools as well as quality control materials. Results are shown below for the study at the manufacturer's site:

<u>Sample</u>	<u>Mean (ng/mL)</u>	<u>SD(ng/mL) Within-run</u>	<u>%CV</u>	<u>SD(ng/mL) Within-lab (total)</u>	<u>%CV</u>
<u>Whole Blood Pools</u>					
<u>Pool 1</u>	4.4	0.35	8	0.38	8.6
<u>Pool 2</u>	12.6	0.72	5.7	0.72	5.8
<u>Pool 3</u>	25.4	1.69	6.7	1.79	7.1
<u>Whole Blood Quality</u>					
<u>Controls</u>					
<u>Level 1</u>	2.7	0.35	13	0.39	14.5
<u>Level 2</u>	10.6	0.58	5.5	0.61	5.8
<u>Level 3</u>	22.3	1.09	4.9	1.2	5.4

Percents CV for whole blood pools evaluated by similar methods at external sites are tabulated below:

Mean (ng/mL)	Within run		Within lab	
	%CV	SD (ng/mL)	%CV	SD (ng/mL)
3.0	6.3	0.36	12.6	0.41
3.7	9.7	0.35	12.7	0.46
11.7	2.3	0.27	4.8	0.56
24.0	2.0	0.48	4.6	1.1

A separate precision study was conducted using fresh whole blood pools from patients

taking sirolimus. This study was conducted over 5 days. Results are shown below:

Mean	within run %CV	total % CV
5.2	9.2	9.2
8.8	9.0	9.0
10.5	7.6	8.1

In addition, an analysis of replicate samples from the method comparison study was performed. The pooled replicate SD results were as follows: for samples ranging from 2.0 to 5.9 ng/mL, SD = 0.284 ng/mL; for samples ranging from 6.0 to 10.9 ng/mL, SD = 0.347; for samples greater than 11.0 ng/mL, pooled replicate SD = 0.413ng/mL.

b. Linearity/assay reportable range:

The manufacturer's claimed assay range is 2.0-30 ng/mL.

Linearity was evaluated with samples prepared using a sirolimus stock solution spiked into a whole blood transplant patient pool to produce concentrations across the assay range. Each sample was assayed five times in random order. The mean observed result (y) was plotted against the expected concentration (x). The data was analyzed by linear regression and yielded slope of (1.01) and intercept of (0.08 ng/mL). Recoveries relative to the expected concentrations determined gravimetrically, are tabulated below:

Dimension SIRO Linearity/Recovery

Expected	Measured	% Recovery
0	0.4	-
2	2.2	107.7%
5	4.8	95.5%
10	9.2	92.4%
15	16.4	109.6%
31	31.2	100.8%

The method for diluting high samples recommended in the manufacturer's package insert was validated with patient samples containing sirolimus at high concentrations. Samples were diluted 1:1 with calibrator. Observed percents recovery (averaged across replicates of each individual sample) relative to expected concentrations ranged from -4% to -7%.

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

Calibrator values are traceable to values determined by two HPLC-MS-MS methods (which were described in the 510(k)). In an analysis of a representative calibrator lot, calibrator values measured by the Dimension SIRO assay recovered within 90-99% of the HPLC-MS-MS methods.

Calibrator stability was evaluated at multiple time points under various storage conditions, including all storage conditions recommended to users in the labeling. Open-Vial stability studies were conducted under conditions of 2-8 degrees. Observed percent drift within 30 days ranged from -5 to -6%. Under conditions of -20 degrees C, calibrator recovery was within +/- 10% throughout expiration dating claim. The “control” calibrator was stored at -70 degrees C during testing. Multiple lots were included in the evaluation.

d. Detection limit:

A precision study was conducted using samples prepared by spiking sirolimus drug into EDTA whole blood at multiple concentrations near the lower assay limit. The study was conducted over 20 days, 2 runs per day with samples run in duplicate (n=80). Each of 3 lots was tested (at different times) and evaluated separately. The within-lab %CV was plotted against the sample concentration. At a concentration of 2.0 ng/mL (the claimed lower limit of the assay) the within-lab %CV was ≤ 20%. In addition, a sample at 2 ng/mL was included in the linearity study. Recovery at 2.0 ng/mL was approximately 108%. These data support the lower limit of the analytical measuring range of 2.0 ng/mL claimed by the manufacturer.

e. Analytical specificity:

Studies were conducted to evaluate the effects of potential interferents on assay performance. Potential sources of interference evaluated included endogenous compounds, co-administered immunosuppressive drugs including cyclosporine, mycophenolic acid and its metabolite MPAG, tacrolimus and common co-administered drugs with which the sample is likely to come in contact.

Endogenous compounds

Interference from endogenous compounds was evaluated by preparing test samples containing the potential interferent in the presence of 6 ng/mL and 18 ng/mL sirolimus in whole blood hemolysate. All samples were assayed in replicates of five and the mean was used in the analysis. The interference of each compound was calculated as: Interference = (Test sample – Blank)/ Blank x 100%. Recoveries observed were within +/- 10%, as shown below.

Compound	% recovery relative to control sample	
	6 ng/mL sirolimus	18 ng/mL sirolimus
Cholesterol (400mg/dL)	-2.2	-9.9
Triglyceride (1000mg/dL)	-5.2	-2.5
Albumin (6g/dL)	-7.1	-8.2
Gamma Globulin (6g/dL)	-7.1	-8.2
Ditaurobilirubin (40mg/dL)	9.5	0.2
Unconjugated Bilirubin (40mg/dL)	-8.2	0.8

Rf (500IU/mL)	-0.5	-0.5
Uric Acid (20mg/dL)	-2.7	-1.6
HAMA	2.3	-0.5
HAMA 1	9.8	0
Hematocrit 15.8-52.4% (relative to 44% hematocrit)	-2 to 6%	-0.5 to 3%

Cross-reactivity

Four major sirolimus metabolites were evaluated for cross-reactivity, at a concentration of 25 ng/mL, in the presence of 0 ng/mL [0.0 nmol/L] and 12 ng/mL [13.2 nmol/L] sirolimus. The percent cross-reactivity was calculated as follows:

$$\% \text{ cross rct} = \frac{\text{measured sirolimus} - \text{control sirolimus (ng/mL [nmol/L])}}{\text{metabolite added}} \times 100$$

Percent Cross-reactivities observed under these conditions are summarized below. (The ranges refer to cross-reactivities observed under the two sirolimus concentrations tested):

16-O-demethyl sirolimus	6-10%
12-hydroxy sirolimus	46-53%
39-O-demethyl sirolimus	52-53%
27,39-O-didesmethyl sirolimus	83- 89%

Co-administered drugs and physiological substances

Interference from commonly co-administered drugs, including immunosuppressive drugs was evaluated. A sample was prepared containing the co-administered drug in the presence of 6 ng/mL and 18 ng/mL sirolimus in whole blood. The sample containing the co-administered drug was compared to the control, which contained only 6 ng/ml or 18 ng/mL sirolimus in whole blood (no drug). All samples were assayed n=5 and the mean result were calculated. At the concentrations tested, the co-administered drugs did not cause significant interference with the assay, as defined by the manufacturer. All recoveries were within +/- 10% of the control samples.

f. Assay cut-off:

Not applicable – this is a quantitative assay.

2. Comparison studies:

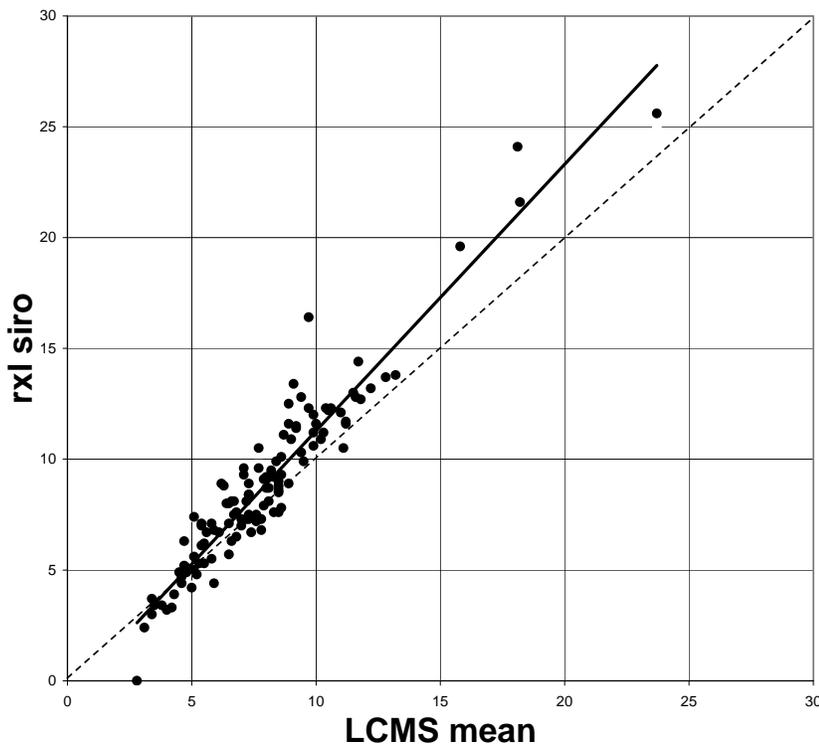
a. Method comparison with predicate device:

Method comparison studies were conducted at two external clinical sites. There were a total of 119 samples from adult kidney transplant patients (EDTA plasma)

evaluated at these sites. All 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). In addition, analysis of the data showed there was no significant effect of repeat measurements (from a single patient) on the regression statistics. The range of time post transplant was 7 weeks to 25 years. Samples were measured by the Dimension SIRO method and a well-validated HPLC-MS method.

The linear regression provided a slope of 1.2 (95% CI = 1.13 to 1.27) with an intercept of -0.7 (95% CI = -1.26 to -0.06), correlation coefficient (r) of 0.95 and Sy/x of 1.2 ng/mL. These results, and the graph below were derived from the mean of HPLC/MS results compared to the first replicate for the Dimension RXL SIRO assay. Data from both sites were poolable, and are shown combined below.

Linear regression for Method Comparison



b. Matrix comparison:

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 a, 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.