

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

**A. 510(k) Number:** k042411

**B. Purpose for Submission:** New assay and calibrators.

**C. Measurand:** Sirolimus

**D. Type of Test:** Quantitative microparticle enzyme immunoassay

**E. Applicant:** Axis-Shield Diagnostics Ltd

**F. Proprietary and Established Names:** Abbott IMx® Sirolimus Microparticle Enzyme Immunoassay, IMx® Sirolimus Calibrators, IMx® Sirolimus Controls

**G. Regulatory Information:**

1. Regulation section:  
21CFR862.3840, 862.3280, 862.3200
2. Classification:  
Class II
3. Product Code:  
NRP, LAS, DLJ
4. Panel:  
91, Toxicology

**H. Intended Use:**

1. Intended use(s): See indications for use
2. Indication(s) for use

The IMx® Sirolimus assay is an *in vitro* reagent system for the quantitative determination of sirolimus in human whole blood, as an aid in the management of renal transplant patients receiving therapy with sirolimus.

The Imx® Sirolimus Calibrators are for the calibration of the IMx® Analyzer when used for the quantitative determination of sirolimus in human whole blood.

The Imx® Sirolimus Mode I Calibrator is for the adjustment of the stored calibration of the IMx® Analyzer when used for the quantitative determination of sirolimus in human whole blood.

The Imx® Sirolimus Controls are for verification of calibration of the IMx® Analyzer when used for the quantitative determination of sirolimus in human whole blood.

The Imx® Sirolimus Whole Blood Precipitation Reagent is for the extraction of sirolimus from samples (whole blood patient specimens, Imx® Sirolimus calibrators and controls).

3. Special condition for use statement(s):

The device is for prescription use.

The concentration of sirolimus in a given specimen determined with assays from different manufacturers can vary due to differences in antibody specificity.

Cross-reactivity can also lead to a positive bias with respect to HPLC. Patients with impaired clearance or metabolism or those taking drugs affecting sirolimus metabolism may show the most variation between assays. Especially for such patients, use of this assay may be supported with a chromatographic method more specific for the parent compound.

Clinical studies have shown large intra-patient variability, indicating that optimal dose adjustment should be based on more than just a single trough sample.

4. Special instrument Requirements: The assay system is for use on the Abbott IMx® System.**I. Device Description:**

The device consists of (1) mouse monoclonal anti-sirolimus-coated microparticles in buffer with preservatives and stabilizers (2) sirolimus-alkaline phosphatase conjugate in buffer with stabilizers and preservatives (3) 4-Methylumbelliferylphosphate in buffer with preservative. The device also includes precipitation reagent, calibrators and controls.

**J. Substantial Equivalence Information:**

4. Predicate device name(s): Microgenics CEDIA® Sirolimus

5. Predicate K number(s): k034069

6. Comparison with predicate:

Parameter	Abbott IMx® Sirolimus	Microgenics CEDIA® Sirolimus
<b>Intended use</b>	In vitro reagent system for the quantitative determination of sirolimus in human whole blood as an aid in the management of renal transplant patients receiving sirolimus therapy.	In vitro diagnostic medical device intended for the quantitative determination of sirolimus in human whole blood using automated clinical chemistry analysers as an aid in the management of sirolimus therapy in kidney transplants.
<b>Technology Format</b>	Automated. Competition format. Microparticle Enzyme Immunoassay (MEIA).	Automated. Competition format. EIA utilising recombinant DNA technology.
<b>Capture Antibody</b>	Anti-sirolimus (mouse, monoclonal) antibody coated microparticles.	Anti-sirolimus antibody (mouse, monoclonal) in enzyme acceptor solution.
<b>Competition Antigen</b>	Sirolimus-Alkaline Phosphatase conjugate.	Sirolimus bound to inactive fragments of $\beta$ -galactosidase.
<b>Substrate</b>	4-Methylumbelliferyl phosphate.	Not applicable.
<b>Assay End-Point</b>	Fluorescence	Colourimetry

**K. Standard/Guidance Document Referenced (if applicable):** FDA Guidance: Guidance for Industry and FDA Staff; Class II Special Controls Guidance Document: Sirolimus Test Systems.

**L. Test Principle:**

The assay is based on MEIA (microparticle enzyme immunoassay technology). Whole blood samples are first extracted with precipitation reagent. The supernatant is decanted into the sample well and reagents and sample are added (automated) to the reaction cell in sequence. Sample, antibody-coated microparticles, and sirolimus conjugate are added to the incubation well of the reaction cell. Sirolimus and conjugate competitively bind to microparticles and form antibody-antigen and antibody-antigen- alkaline phosphatase complexes. The reaction mixture bound to microparticles is transferred to a glass fiber matrix to which microparticles bind irreversibly. The matrix is washed, substrate is added and fluorescent product is measured by the MEIA optical assembly.

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

Precision and method comparison were evaluated at the manufacturer's site, as well as external sites. Other evaluations were conducted at the manufacturer's site.

1. Analytical performance:

*a. Precision/Reproducibility:*

Precision of the method, including extraction steps, was evaluated. Within-run and total precision was evaluated at the manufacturer's site (using 2 instruments) and at 2 external sites. The first study used the manufacturer's control material. Performance was similar for both external and internal sites. At each site, the study was run over 20 days, with 2 duplicates per run, 2 runs per day, n=80, sample extractions = 40. A calibration curve was run on day 1. Mode 1 assays were run for all subsequent runs. At each site, one operator performed the study. Different lots were evaluated at each site. Precision was calculated as described in NCCLS EP-5A. Acceptance criteria are total % CV < 15% for the low control and < 12% for medium and high controls. A summary of results is shown below. The ranges shown are the ranges of results across the multiple sites.

Concentration of control material	N	Within-run		Total	
		SD range observed (ng/mL)	%CV range observed	SD range observed (ng/mL)	%CV range observed
5.0-5.3	80	0.25-0.55	5.0-11.0	0.35-0.65	6.9-13.0
10.4-11.5	80	0.43-0.76	3.9-6.9	0.53-1.21	4.8-11.0
19.8-22.2	80	0.89-1.25	4.0-5.7	1.20-2.38	5.5-10.8

In a second study with a similar protocol, precision was evaluated at the manufacturer's site, using pooled patient samples. Two IMx instruments were included in the evaluation and results were similar for the 2 instruments. Results for one of the instruments is shown below.

Sample	n	Mean	Within Run		Total	
		(ng/mL)	SD	%CV	SD	%CV
1	80	5.14	0.50	9.8	0.86	16.8
2	80	8.54	0.57	6.7	0.83	9.7
3	80	13.33	0.80	6.0	1.30	9.8
4	80	19.16	1.51	7.9	2.00	10.4

For both studies shown above, sample extractions were performed for each duplicate measurement (i.e. n=40 extractions). Results were statistically evaluated to determine whether the extraction events affect results shown above. No effect of extraction on the results above was observed based on these analyses.

*b. Linearity/assay reportable range:*

Linearity by dilution:

Samples from sirolimus patients were diluted with calibrator diluent and used to produce 5 pools ranging in concentration from below 2.5 ng/mL to 22 ng/mL. Sample preparation followed NCCLS EP6-A. Dilutions were assayed in replicates of 4 on the IMx®. Samples were extracted after preparation of the various concentration levels, so that any potential effect of extraction on linearity could be evaluated.

The percent observed/expected concentrations at each level for samples > 4 ng/mL were within 91-111%. Expected concentrations were based on the observed IMx reading for the neat sample, and the dilution factor. For sirolimus values < 4 ng/mL percents observed/expected were within 91-125%.

Linearity by spiking:

Sirolimus stock solutions were prepared gravimetrically and analyzed on the IMx. These stock solutions were used to spike sirolimus into samples from patients taking sirolimus. Expected concentrations were determined based on the sum of the patient sample concentration measured on the IMx and the stock solution concentration. Percents recovery for samples in the range of 14-28 ng/mL ranged from 93-121% (mean of 108%). No trends (e.g., in terms of percent recovery versus concentrations) were observed.

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

Calibrator value assignment:

Calibrators are traceable to sirolimus powder, purified by crystallization, and assayed for purity by the drug manufacturer, using HPLC methods. Analytical error (uncertainty) of kit calibrator values was calculated by following a traceability chain to the original

Wyeth powder and analyzing each manufacturing step and the associated specifications for that step. A standard calculation of summing the relative errors was used to calculate the 95% confidence interval as recommended in the ISO document “Guide to the expression of uncertainty in measurement”, 1st edit., ISO, Genève, 1993. The estimates of the uncertainties of the manufacturing process for the kit calibrators, shown below included the uncertainty of the kit calibrator manufacturing specifications (specification for matching each kit calibrator to the corresponding primary calibrator, assuming a Gaussian distribution), and the uncertainty of the testing (considering the imprecision at each calibrator level and the number of replicates during the testing). A summation of the relative errors for both the primary calibrators and kit calibrator manufacturing was used to calculate the 95% confidence interval. The total uncertainty for the kit calibrators is summarized in the following table.

Kit calibrator	95% Confidence
A Cal (0 ng/mL)	Taken as 0
B Cal (3 ng/mL)	± 0.17 ng/mL
C Cal (6 ng/mL)	± 0.27 ng/mL
D Cal (12 ng/mL)	± 0.60 ng/mL
E Cal (20 ng/mL)	± 1.10 ng/mL
F Cal (30 ng/mL)	± 1.65 ng/mL

Control range assignment:

Ranges shown in labeling are determined based on evaluations from multiple lots, operators and instruments.

Control and calibrator stability:

Primary calibrators and controls stored at -20 degrees C, serve as reference material in stability evaluations. Calibrators/controls are stored under conditions recommended to users, including the “opened vial” conditions. Evaluations are performed using fresh reagents at time 0 and for 3 month intervals to the expiration date. The material under test is assayed directly from primary calibrators, run on each of 3 instruments. At each time point, values must deviate no more than 10% from the time 0 value.

*d. Detection limit:*

Analytical sensitivity (LOD):

LOD evaluations were performed over 2 days and included 1 operator, 2 lots and 2 instruments (i.e. 8 runs total). Cal A and Cal B are evaluated.

10 Cal A and 4 Cal B observations were made for each run. LLOD was defined as:

$$2(\text{cal A rate SD}) \times \frac{(\text{Cal B concentration})}{(\text{Cal A rate mean-cal B rate mean})}$$

LOD's for lot 1 ranged from 0.5-0.8. (avg 0.75). LOD's for lot 2 ranged from 0.71-1.85 (average 1.3).

Functional Sensitivity:

Study1

A panel of 5 spiked samples ranging in concentration from 1.5-5 ng/mL, was evaluated on the Imx<sup>®</sup> by running 3 runs/day (on 3 instruments), over 2 days, with 10 replicates per run, for a total of 60 replicates, and 30 extraction events, for each concentration evaluated. The evaluation included 1 operator and 1 lot. The mean concentrations and percents CV, calculated for each day separately, and for both days combined support the claim that at the limit of quantitation, % CV is < 20%.

#### Study 2

A panel of 4 pooled sirolimus patient samples ranging in concentration from approximately 1.5 – 5 ng/mL was tested on the Imx<sup>®</sup> in replicates of 7 for each of 8 runs (n=56, with 28 extraction events). Two lots and 2 instruments were tested on each of 2 non-consecutive days, for each level. CV's were determined based on the mean and SD of all replicates from a single lot in one day, as well as on the mean and SD of all replicates from a single lot across both days. Percents CV for samples near the LOQ concentration of 2.5 ng/mL, were less than 20%. ANOVA analyses indicated that pretreatment steps do not affect the stated LOQ.

Inter-instrument and inter-lot CV is reported as 22% and 16%, respectively.

Data from the linearity study for concentrations near the lower limit of the assay are shown below.

Observed (ng/mL)	Observed/Expected (%)
3.5	125
3.1	115
2.5	119
2.3	92
2.1	110

#### *e. Analytical specificity:*

Prescription and over-the counter drugs were tested for interference by spiking the drugs into the manufacturer's 0 calibrator and "medium" control material. The drugs, and concentrations at which they were tested, are listed in the product package insert. Control samples, comprised of diluent spiked into the 0 calibrator and the medium control, were run with the test samples. Each sample and control was run in duplicate. Percent cross-reactivity was defined as:

$$\frac{[(\text{Mean of the drug spiked Cal A rate}) - (\text{Mean of diluent Control Cal A rate}) \times (100)]}{[\text{Mean of diluent Control Cal A rate}]}$$

Percent interference is defined as:

$$\frac{[(\text{Mean concentration of the drug spiked (medium) control material}) - (\text{Mean concentration of the diluent spiked (medium) control material}) \times (100)]}{[\text{Mean concentration of diluent spiked control material}]}$$

Acceptance criteria are that percent cross-reactivity and interference are less than 10%. All drugs except Gemfibrozil, Itraconazole, MPAG, OKT3, and Trimethoprim met these criteria. Percents interference ranging from -12.9% to 13.6% were observed for these latter drugs. These latter differences were not reported to be statistically significant.

The effect of endogenous compounds was evaluated by spiking the compounds into pooled whole blood containing approximately 0, 5, 10, and 15 ng/mL sirolimus. Control samples contained diluent spiked into whole blood at the same sirolimus concentrations. Means of replicates (n=4) were determined. Percent interference was defined as  $\frac{([\text{Observed mean sirolimus concentration in sample with spiked endogenous compound}] - [\text{Observed sirolimus concentrations in control samples}]) \times 100}{\text{observed sirolimus concentrations in control samples}}$ .

For the following compounds, < 10% interference was observed.

Bilirubin , 40 mg/dL.
Triglycerides, 1000 mg/dL
Uric Acid, 20 mg/dL
HAMA, 60 ng/mL
RF, 500 IU/mL
Cholesterol, 500 mg/dL
Total protein, 3-12 g/dL

The effect of hematocrit was evaluated by preparing samples ranging from 15-60% hematocrit, and spiked with sirolimus to 5, 11 and 22 ng/mL. Samples with 45% hematocrit are used as the control in calculations. Less than 25% interference was observed for all samples tested. Less than 10% interference was observed for samples with 35-45% hematocrit (5 ng/mL sirolimus). Up to 21% interference was observed for samples with 25-55% hematocrit (11-22 ng/mL sirolimus).

HAMA levels up to 75 ng/mL were evaluated. On average (with exception of 1 sample) interference was < 10% (within acceptance criteria), no trends were observed.

Metabolite fractions were isolated by HPLC separation of a synthetic mixture of metabolites and spiked to final concentrations of 10 ng/mL, into the manufacturer's "medium control" material and assayed in triplicate. Final concentrations were 10 ng/mL, based on the gravimetric preparations of fractions of known concentration and purity. These samples were evaluated and compared to diluent-spiked medium controls. Results are shown below:

Fraction	sirolimus metabolites (10 ng/mL, total)	Concentration change/ amount of spiked metabolite
2	41-O-demethyl- hydroxy-sirolimus	6%
3	41-O-demethyl- hydroxy-sirolimus; 7- O-demethyl-sirolimus (70:30 ratio)	77%
4	11-hydroxy sirolimus	37%
5	41-O-demethyl- sirolimus	58%

*f. Assay cut-off:* N/A. See detection limit

2. Comparison studies:

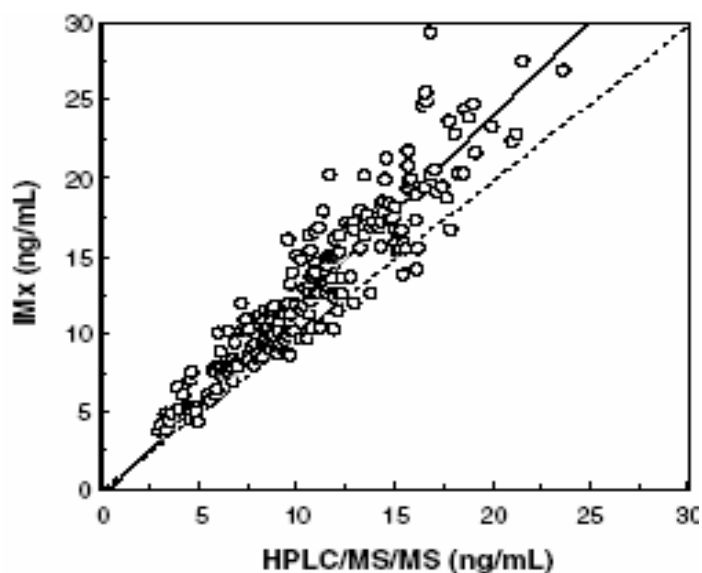
*a. Method comparison with predicate device:*

Two hundred and twenty one patient samples were tested among 3 external sites, using the Abbott IMx® Sirolimus Microparticle Enzyme Immunoassay. Results were compared to an LC/MS/MS method, conducted in accordance with a validated procedure (specified within the 510(K)). Patient samples were obtained from 2 sources. One set included 173 trough samples obtained from a drug study representing 76 “high risk” renal transplant patients. (e.g., African American, high PRA value, or second transplant). The second set included 48 trough samples from 39 transplant patients and are believed to represent “archetypal” renal transplant patients. Time-post transplant ranged from 3 days to 6 months. Sample selection criteria were samples from renal transplant patients that had been stored  $\leq 3$  months.

For both samples sets: Samples were stored frozen, then aliquoted, and refrozen for shipping to 3 testing sites.

Samples were measured in singlicate. Testing was performed using the assay protocol described in the package insert. Data was evaluated using Passing-Bablok analyses. Results obtained at the various sites, and presented in the 510(k) show data at various sites are comparable. Therefore, the graph below illustrates combined data.





Regression parameters from combined sites are shown in the table below:

		All sites and samples combined
Number of samples		215
Concentration range (ng/mL)		3.8-29.4
Slope (95% CI)		1.23(1.17 to 1.30)
Intercept (95% CI)		-0.28(-0.87 to 0.382)
Dispersion of residuals	md (68)	1.05
	md (95)	2.57
Sy/x (based on linear regression)		1.57

*b. Matrix comparison:*

N/A. The assay is for use with EDTA whole blood only. All samples tested were EDTA whole blood.

3. Clinical studies:

*a. Clinical Sensitivity:*

N/A. (Not typically reviewed for this type of test)

*b. Clinical specificity:*

N/A. (Not typically reviewed for this type of test)

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

4. Clinical cut-off:

N/A. See expected values

5. Expected values/Reference range:

Optimal sirolimus concentration ranges vary according to the commercial test used, and therefore should be established for each commercial test. Values obtained with different assay methods cannot 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.

Optimal ranges depend upon the patient's clinical state, individual differences in sensitivity to immunosuppressive and adverse 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. Each institution should establish the optimal ranges based on the specific assay used and other factors relevant to their patient population

**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.