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

A. 510(k) Number:

k100144

B. Purpose for Submission:

New device

C. Measurand:

Everolimus

D. Type of Test:

Quantitative immunoassay

E. Applicant:

Microgenics Corporation, Thermo Fisher Scientific, Clinical Diagnostics Division

F. Proprietary and Established Names:

Thermo Scientific QMS® Everolimus Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR§862.3840, Sirolimus Test System

   21 CFR§862.3200, Clinical Toxicology Calibrator

   21 CFR§862.3280, Clinical Toxicology Control Material

2. Classification:

   Class II

3. Product code:

   OUF, DLJ, LAS

4. Panel:

   Toxicology (75)

H. Intended Use:

1. Intended use(s):
   See indications for use below.

2. Indication(s) for use:
   The QMS® Everolimus assay is intended for the quantitative determination of everolimus, the active ingredient of Zortress® in human whole blood on automated clinical chemistry analyzers. The results obtained are used as an aid in the management of kidney transplant patients receiving Everolimus therapy. This in vitro diagnostic device is intended for clinical laboratory use only.

   The QMS® Everolimus Calibrator set is intended for use in calibration of the QMS® Everolimus Assay.

   The QMS® Everolimus Control set is intended for use in quality control of the QMS® Everolimus Assay.

3. Special conditions for use statement(s):

   For prescription use.

   See expected values section, below regarding interpretation of results.

   The manufacturer includes the following in the package insert:
   On average, the assay is designed so that bias for patient samples relative to LC-MS/MS systems is within 0 to ±10%. However, as with other immunosuppressant immunoassays, caution should be exercised because results for individual patient samples may vary (in a positive or negative direction) due to the difference in metabolite accumulation or other errors.

   The QMS Everolimus Assay is calibrated based on a training set of trough samples from adult renal transplant patients co-administered with cyclosporine, basiliximab induction therapy and corticosteroids. Relative to LC-MS/MS, comparison for patients under different conditions has not been evaluated and may be different. Comparability to LC-MS/MS has not been evaluated in pediatric patients and may be different due to differences in metabolism.
   The assay is indicated ONLY for renal transplant patients, and performance has not been determined for any other type of patients.

   The assay should not be used on patients who have recently been administered

sirolimus (until sirolimus parent compound and metabolites are fully cleared) since the assay cross-reacts with sirolimus and its metabolites.

Certain conditions, such as hepatic impairment, that can affect the parent compound to metabolite ratio in patient samples may affect performance (e.g. bias relative to an LC-MS/MS assay). For such patients, consider confirming results with an LC-MS/MS method specific for the parent compound.

Test findings should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

4. Special instrument requirements:

Performance was evaluated on the Roche Hitachi 917.

I. Device Description:

Device Description
The QMS® Everolimus Assay consists of separately packaged reagents (R1, R2 and Precipitation Reagent).
R1: Everolimus Antibody Reagent: <1.0% Anti-Everolimus polyclonal antibody (rabbit) in a buffer as stabilizer and <0.09% sodium azide as preservative.
R2: <0.5% Everolimus-coated microparticles in buffer containing <0.09% sodium azide as preservative.

Calibrators and controls are prepared in a human whole blood hemolysate matrix with preservatives.

The QMS® Everolimus Calibrators are supplied in a set of six with assigned values including an everolimus-free calibrator and everolimus calibrators ranging from approximately 2 ng/mL to 20 ng/mL. (See Traceability in section C below for information on how values are assigned; values do not correspond to a spiked concentration). Calibrators contain everolimus in hemolysate with < 1% sodium azide.

The QMS® Everolimus Controls are supplied in a set of three with target assigned values of 4 ng/mL, 8 ng/mL, and 15 ng/mL.

The package insert contains the following caution: CAUTION: This product contains human sourced and/or potentially infectious components. Components sourced from human blood have been tested and found to be nonreactive for HBsAg, anti-HIV 1/2, and anti-HCV. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, it is recommended that all human sourced materials be considered potentially infectious and handled with appropriate biosafety practices.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   CEDIA® Sirolimus Assay

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

   k034069

3. Comparison with predicate:

   The predicate and candidate device are immunoassays intended for use in the quantitative measurement of the respective m-TOR inhibitors, sirolimus and everolimus, in human whole blood. Both devices are homogeneous immunoassays. The predicate device uses enzyme immunoassay technology; this device uses particle agglutination and immunoassay technology. The predicate device is intended to measure sirolimus; this device is intended to measure everolimus. Everolimus is derived from sirolimus by addition of a 2-hydroxyethyl group at position 40. Additional comparisons are shown below.

Device	Predicate: CEDIA sirolimus assay	New device: QMS everolimus assay
Indication(s) for use	Intended for the quantitative determination of everolimus, the active ingredient of Zortress® in human whole blood on automated clinical chemistry analyzers. The results obtained are used as an aid in the management of kidney transplant patients receiving Everolimus therapy.	Intended for the quantitative determination of sirolimus in human whole blood, using automated clinical chemistry analyzers, as an aid in the management of sirolimus therapy in renal transplant patients taking sirolimus.
Calibrators	Lyophilized (0 and 30 ng/mL)	Liquid ready-to-use (0, 1.5, 3.0, 6.0,12.0 and 20.0 ng/mL)
Controls	Lyophilized (Low, Medium, High)	Liquid ready-to-use (Low, Medium, High)
Traceability of Calibrators	Traceable to purified sirolimus	Ultimately traceable to calibration based on minimization of bias between the QMS assay and an LCMSMS assay for a specified adult renal transplant sample set.

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

CLSI EP-5, Evaluation of Precision Performance of Quantitative Measurement Methods.

CLSI EP-9, Method Comparison and Bias Estimation using Patient Samples.

L. Test Principle:

The QMS Everolimus Assay is a homogeneous particle-enhanced turbidimetric immunoassay based on competition between drug in the sample and drug coated onto a microparticle for antibody binding sites of the everolimus antibody reagent. The principle is based on everolimus-coated microparticle reagent agglutinating in the presence of the anti-everolimus antibody reagent and in the absence of any competing drug in the sample. The rate of absorbance change is measured photometrically at a wavelength of 700 nm. When a sample containing everolimus is added, the agglutination reaction is partially inhibited, slowing down the rate of absorbance change. A concentration-dependent classic agglutination inhibition curve can be obtained with maximum rate of agglutination at the lowest everolimus concentration and the lowest agglutination rate at the highest everolimus concentration.

The approach taken by the manufacturer was that QMS Everolimus calibrators and controls were value-assigned by using a representative set of clinical trough samples from renal transplant patients with traceability to LC-MS/MS values. Limitations associated with this approach are described in the package insert.

M. Performance Characteristics (if/when applicable):

Performance was validated on the Hitachi 917.

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

   a. *Precision/Reproducibility:*
   Everolimus samples were used for precision testing, following a CLSI protocol. A tri-level human hemolysate-based control containing Everolimus, and 3 renal transplant patient whole blood sample pools were assayed on the Hitachi 917 for this study at the manufacturer's site. For 20 non-consecutive days, each sample was run in duplicate. After a minimum of two hours within the same day, the samples were run again in duplicate, resulting in a total of 80 replicates. Each replicate was separately extracted. Calibration was performed initially and at intervals (n=4) throughout the precision evaluation. Results are tabulated below.

Lot 1

Controls	N	Mean (ng/mL)	Within-run CV(%)	Between Run CV (%)	Between day CV(%)	Total (within lab) CV(%)
1	80	4.1	3.9	2.8	2.7	5.6
2	80	8.1	3.8	2.6	3.4	5.7
3	80	16.3	3.6	3.4	3.7	6.2
Patient Pools						
1	80	2.8	5.6	4.8	4.5	8.6
2	80	5.8	2.3	3.6	1.9	4.7
3	80	11.72	2.4	2.1	2.8	4.2

Lot 2

Controls	N	Mean (ng/mL)	Within-run CV(%)	Between Run CV (%)	Between day CV(%)	Total (within lab) CV(%)
1	80	4.3	3.4	2.2	5.2	6.6
2	80	8.3	3.1	2.0	5.1	6.3
3	80	15.9	2.3	3.3	4.4	6.0
Patient Pools						
1	80	2.9	4.8	4.1	6.4	9.0
2	80	6.1	2.6	3.5	4.8	6.5
3	80	10.5	2.1	2.6	4.8	5.9

Evaluations were also conducted to demonstrate overall precision for 3 lots at the manufacturer's site. Results are shown below:

		Mean	Within Run		Between Day		Total	
Controls	N	(ng/mL)	SD	CV(%)	SD	CV(%)	SD	CV(%)
1	240	3.87	0.23	5.86	0.21	5.4	0.34	8.83
2	240	8.03	0.31	3.84	0.34	4.18	0.52	6.51
3	240	14.48	0.71	4.88	0.68	4.7	1.07	7.4
Transplant Patient Pools								

1	240	3.93	0.46	11.68	0.02	0.57	0.5	12.8
2	240	8.29	0.21	2.57	0.52	6.29	0.68	8.24
3	240	11.64	0.42	3.63	0.66	5.68	0.95	8.16

Results of precision evaluations at external sites are shown below. Replicates were individually extracted to reflect actual procedures with patient samples.

External Site 1

This evaluation was performed over 5 days (with 2 runs per day, 2 samples per run) at an external site.

Controls	N	Mean (ng/mL)	Within Run CV(%)	Between Day CV(%)	Between run CV(%)	Total CV(%)
1	20	4.3	2.8	0.7	4.27	5.1
2	20	7.9	5.0	0.0	5.45	7.4
3	20	15.4	5.6	0.2	1.43	5.8

External Site 2; Controls and patient pools
Patient pools were run once a day. Patient pool 1 was run for 16 days and patient pools 2 and 3 for 20 days. Controls were run twice a day, with two replicates per run for 20 days.

			Within Run		Between Day		Total	
Controls	N	Mean (ng/mL)	SD	CV(%)	SD	CV(%)	SD	CV(%)
1	80	3.96	0.16	3.95	0.18	4.59	0.31	7.84
2	80	8.51	0.28	3.27	0.39	4.63	0.59	6.91
3	80	15.30	0.66	4.33	0.72	4.71	1.16	7.58

Transplant Patient Pools	N	Mean	Total SD	Total percent CV
1	32	3.86	0.24	6.28
2	40	9.12	0.60	6.55
3	40	12.46	0.53	4.24

Reproducibility between sites was also evaluated by having two sites each run 121 patient samples. Results are shown below:

Site 1 vs manufacturer site	N	Slope	Intercept	R
	121	0.97 (0.94 to 1.00)	0.16 (0.01 to 0.31)	0.99

Site 2 vs manufacturer site	N	Slope	Intercept	R
	121	1.01 (0.96 to 1.06)	0.11 (-0.22 to 0.41)	0.97

b. Linearity/assay reportable range:

Linearity/recovery by dilution:

Linearity was determined by diluting renal transplant patient samples (containing near 20 ng/mL as determined by an LCMSMS method) with hemolysate negative for everolimus, to yield 10 concentrations spanning the assay range from < 2 to near 20 ng/mL. Each dilution was tested in triplicate using the QMS® Everolimus Assay on two instruments using two calibration curves per instrument (for a total n=12). Sample extractions were performed after preparation of each dilution. The averaged percent recovery, standard deviation, and %CV are shown below.

Within the concentration ranges of 2.36 ng/mL to 20 ng/mL, recoveries relative to expected concentrations (based on the high sample concentration and the dilution factor) were within +/- 10%, and no trends in recovery were observed. At lower measured concentrations (<2 ng/mL) recoveries were near 115%.

LCMSMS Concentration (ng/mL)	**Avg. of 12 Reps (ng/mL)**	**% CV**	**% Recovery**
0.00	0.33	-	0.00
1.12	1.29	17.28	114.70
2.23	2.36	8.36	105.17
4.46	4.34	5.25	96.53
6.70	6.24	3.03	92.51
8.93	8.60	2.55	95.64
11.16	11.14	3.72	99.11

LCMSMS Concentration (ng/mL)	Avg. of 12 Reps (ng/mL)	% CV	% Recovery
13.39	13.21	2.63	97.94
15.63	15.85	3.17	100.72
17.86	17.88	6.73	99.42
20.09	19.88	3.83	98.24
22.32	22.32	7.82	99.30

Results shown above are based on dilution of patient samples. The manufacturer will note in the labeling that recovery studies with gravimetrically prepared spiked everolimus samples will under-recover significantly.

The LoQ study described below (see Detection Limit) further demonstrated that recovery at 2.0 ng/mL was observed to be within +/-15% of the theoretical value. The claimed measuring range is 2 to 20 ng/mL.

High sample dilution:

A manual dilution study was conducted to verify the dilution procedure recommended in the package insert. Five patient samples containing concentrations near the high end of the assay were diluted 1:1 using the QMS® Everolimus Calibrator A (0.0 ng/mL Everolimus) and were then extracted. The samples were assayed undiluted and diluted using the QMS® Everolimus Assay. Each was run in triplicate. The recovery was calculated as follows:
(Recovered value) x (dilution factor) = Calculated value
The % difference and % Recovery were then calculated.
The data is shown in the table below.

Sample	**neat**	**1:1**	**neat**	**1:1**	**neat**	**1:1**	**neat**	**1:1**	**neat**	**1:1**
Mean of triplicate (ng/mL)	18.78	18.75	16.36	14.73	18.12	17.03	13.5	11.99	15.91	15
% Recovery	-	99.8	-	90		94		88.8		94.3

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

Calibrator and control composition:
The QMS calibrator set consists of six levels of human whole blood hemolysate with everolimus using purified material from the drug manufacturer. Controls are prepared from the same types of materials. Also see device description above.

Calibrator and control value assignment and traceability:
Initially, calibrator values were assigned by comparison of results of a patient sample set to an LCMSMS method, and adjustment of the assigned calibrator assigned values to minimize average bias relative to LCMSMS results. The sample set used for calibration consisted of trough samples from adult kidney transplant patients included in the initial Everolimus drug trials. Master calibrators values are traceable to these initially assigned values. Subsequent calibrator preparations are value assigned on the Hitachi 917 by ratio testing to the Master Calibrators. The ratio for the subsequent calibrator value must be 1.00+/-0.05 ng/mL for Cal B relative to the master calibrator and 1.00 +/-0.02 ng/mL for all other levels.

The sponsor's estimated uncertainty of kit calibrators values used throughout the studies in the 510(k) is 0.2 ng/mL (for lower concentration calibrators) to < 0.7 ng/mL (for higher concentration calibrators).

The manufacturer notes in the package insert that third party control material containing spiked values of everolimus will not give the appropriate results when used in conjunction with the QMS Everolimus Assay, unless the values have been defined for the QMS Everolimus Assay. In addition, using the QMS Everolimus Assay calibrators and controls in analytical methods other than the QMS Everolimus Assay is not appropriate, as this would result in incorrect values being obtained.

Control materials are assigned by assaying each level on a Hitachi 917 clinical chemistry analyzer with five replicates per run, two runs per day for four days within a two week period. At least two operators, two lots and two instruments are used. Manufacturer's ranges included in the package inserts are determined based on analyses of these results. The package insert states that each laboratory should determine its ranges for each new lot.

Calibrator and control stability:
Results supporting the current claimed calibrator storage stability of 12 months (closed vial) at -20 degrees C were included in the 510(k). At each time point, test calibrators stored at -20 degrees C (the test temperature) were used to calibrate the assay and control material stored at a lower known stable temperature (-80 degrees C) was measured. Control recovery at each time point was compared to the zero time point. No trends in changes in recovery were observed over this time period.

Results supporting the current claimed calibrator storage stability of six weeks at 2-8 degrees C (opened vial) were included in the 510(k). At each time point, test calibrators stored at 2-8 degrees (the test temperature) were used to calibrate the assay and control material stored at a lower temperature (-20 degrees C) was measured. Control recovery at each time point was compared to the zero time point. No trends in changes in recovery were observed.

d. Detection limit:

The limit of quantitation was evaluated using patient samples with everolimus values known based on the LCMSMS method used in the drug clinical trials. Three kit lots were used in this evaluation. Samples with six levels of everolimus surrounding the estimated LoQ were measured with the QMS assay over five days with two runs per day. Recoveries for the sample closest to 2 ng/mL (1.8 ng/mL as determined by the LCMSMS method used during the everolimus drug clinical trials) ranged from 89% to 109% for the three lots measured.

Results are shown in the table below:

	Mean observed	Observed percent CV	Fitted percent CV (95% CI)	Percent recovery (relative to LCMSMS)
Lot 1	2.0	7.6	5.1 (0.8 to 9.4)	109
Lot 2	1.6	11.7	12.6 (9.4 to 15.7)	89
Lot 3	1.7	9.1	12.9 (5.9 to 19.9)	95

e. Analytical specificity:

Interference studies were conducted using CLSI protocol EP7-A2 as a guideline. Cross-reactivity was tested for major metabolites of everolimus. Other medications routinely administered with everolimus and endogenous substances were also tested to determine whether these compounds affect the quantitation of everolimus concentrations using the QMS Everolimus assay. The sponsor defined interference or cross-reactivity as ND (not detectable) if the difference between test and control samples was smaller than the SD of repeat control samples.

<u>Cross-reactivity:</u>

The cross-reactivity of the QMS Everolimus assay to everolimus metabolites was evaluated. The compounds tested were added in two concentrations (5 ng/mL and

20 ng/mL metabolite) to human blood hemolysate, which contained either 5 ng/mL of everolimus or no everolimus. Samples were tested with the QMS everolimus assay and results were compared to control samples (without metabolites).

Percent cross-reactivity was calculated as shown in the equation below; observed concentrations were based on average of 5 replicates. The results are shown below in the table below:

Percent Cross-Reactivity = ((DA - DT) / C) X 100)
Where:
DT =average observed concentration of the control solution
DA =average of the observed concentration of the cross-reactant test solution
C =concentration at which the cross-reactant is tested

Tabulated data is shown in Table 18.14.

Metabolite tested	Concentration of metabolite tested (ng/ml)	Observed % Cross-reactivity in presence of 5 ng/mL of everolimus	Observed % Cross-reactivity in absence of everolimus
RADSA	5	7	1.8
RADSA	20	2	1.7
RAD PSA	5	12	21.4
RAD PSA	20	16	16.9
RAD PC	5	63	76.36
RADPC	20	59	80.89
45/46 OH RAD	5	ND	
45/46 OH RAD	20	2	
24 OH RAD	5	9	
24 OH RAD	20	5	
25 OH RAD	5	15	
25 OH RAD	20	22	

Cross-reactivity with sirolimus and its metabolites was also tested. Observed cross-reactivity ranged from 39 – 46% for parent sirolimus, 36-35% for a mixture of 41-O-desmethyl sirolimus and 32-O-desmethyl sirolimus, 11-12% for 11-hydroxy sirolimus and < 10% for other sirolimus metabolites. The manufacturer notes in the labeling that the test should not be used on samples from patients who have recently been administered sirolimus, until sirolimus and its metabolites have been completely cleared.

Other exogenous compounds tested:
Other compounds tested for interference were spiked into normal human hemolysate containing everolimus (at concentrations of 5 ng/mL, and in some cases 0, and 2.6 ng/mL). Samples were assayed by QMS in triplicate and mean results compared to control samples without the test compounds.

Results are shown below. The manufacturer includes a separate table in the package insert for the drugs that were observed to affect recovery by greater than 10%.

Compound	Concentration Tested ug/mL	Percent Cross Reactivity	% Recovery
Acetaminophen	200	ND	103.16%
N-Acetylprocainamide	120	ND	105.67%
Acyclovir	1000	ND	104.78%
Albuterol	0.18	ND	101.32%
Allopurinol	60	ND	103.85%
Amikacin	150	ND	112.85%
Amphotericin B	100	ND	102.73%
Ascorbic Acid	30	ND	100.57%
Atenolol	40	ND	103.36%
Azathioprine	10	ND	99.27%
Caffeine	100	ND	99.50%
Captopril	50	ND	105.05%
Carbamazepine	120	ND	109.04%
Cefaclor	230	ND	102.02%
Chloramphenicol	250	ND	105.62%
Cimetidine	100	ND	104.39%
Ciprofloxacin	250	ND	122.52%
Cyclosporin A	1	ND	102.81%
Digoxin	0.01	ND	96.53%
Disopyramide	30	ND	96.04%
Erythromycin	200	ND	96.40%
Ethanol	3500	ND	104.91%
Fluconazole	75	ND	97.78%
Flucytosine	300	ND	102.16%
Folic Acid	0.01	ND	101.44%
Furosemide	100	ND	100.68%
Ganciclovir	1000	ND	101.28%
Gentamicin	20	ND	105.86%
Glipizide	60	ND	103.30%
Glyburide	40	ND	99.49%
Heparin	16	ND	108.13%
Hydralazine	32	ND	103.40%
Hydrochlorothiazide	40	ND	101.71%
Ibuprofen	400	ND	100.00%
Insulin	0.0167	1.18%	103.78%
Intralipid	15000	ND	104.54%
Isoniazid	70	ND	102.46%
Isoproterenol	0.06	ND	102.08%

Itraconazole	17	ND	97.89%
Kanamycin A	100	ND	100.57%
Kanamycin B	100	ND	100.95%
Ketoconazole	10	ND	106.33%
Labetalol	200	ND	102.99%
Lidocaine	100	ND	100.53%
Lovastatin	4	ND	93.51%
Metformin HCl	5100	ND	105.67%
Metoclopramide	4	ND	103.59%
Misoprostol	0.015	ND	100.19%
Morphine Sulfate	6	ND	98.38%
Mycophenolic Acid	250	ND	98.20%
Nadolol	333	ND	98.20%
Naproxen	1000	ND	93.15%
Niacin	800	ND	100.68%
Nifedipine	120	ND	103.58%
Omeprazole	14	ND	104.57%
Penicillin G	100	ND	104.78%
Phenobarbital	150	ND	101.54%
Phenytoin	100	ND	103.41%
Piperacillin	8	ND	103.40%
Prazosin	25	ND	99.66%
Prednisone	12	ND	100.18%
Prednisolone	12	ND	100.88%
Primidone	100	ND	102.05%
Procainamide	25	ND	106.05%
Propanolol	0.5	ND	101.51%
Quinidine	100	ND	98.46%
Ranitidine	200	ND	101.32%
Rifampin	50	ND	106.83%
Salicylic Acid	500	ND	99.65%
Sotrastaurin	40	ND	106.83%
Spectinomycin	100	ND	101.32%
Sulfamethoxazole	400	ND	103.07%
Tacrolimus	0.04	1.0	107%
Theophylline	250	ND	100.92%
Tobramycin	20	ND	98.87%
Triamterene	600	ND	73.64%
Trimethoprim	20	ND	99.66%
Valproic Acid	1000	ND	96.22%
Vancomycin	630	ND	102.46%
Verapamil	10	ND	98.11%

In addition the manufacturer tested the following: gemfibrozil (75 ug/mL), lithium (22 ug/mL), methicillin (240 ug/mL), MTX (910 ug/mL). No interference was observed with these additional drugs.

Endogenous compounds

Results of testing the QMS assay for interference by endogenous compounds are shown in the table below:

Interfering Substance Tested	Concentration Tested	N	Measured Test Sample (ng/mL)	% Recovery
Bilirubin	60 mg/dL	10	4.45	95.86
Cholesterol	500 mg/dL	3	5.61	96.9
Creatinine	5 mg/dL	3	5.40	99.6
Gamma Globulin	12 g/dL	3	4.06	92.86
HAMA type 1*	Normal Human Level	3	4.22	102.92
HAMA type 2*	Normal Human Level	3	4.22	95.02
Hematocrit	60%	10	4.18	101.89
Rheumatoid Factor	1350 IU	3	4.22	101.42
Total Protein	12 g/dL	3	4.06	105.17
Triglyceride	1500 mg/dL	3	4.22	100.6

f. Assay cut-off:

Not applicable – this is a quantitative assay.

2. Comparison studies:

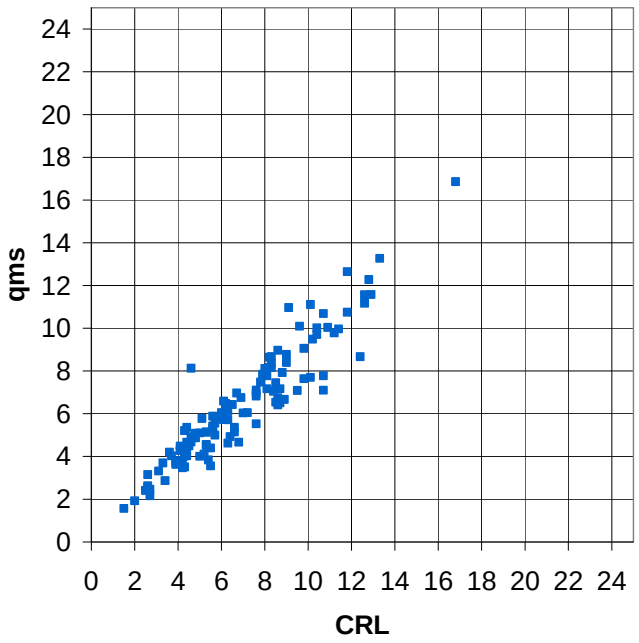
a. Method comparison with predicate device:

Clinical samples were evaluated by the QMS method and the LCMSMS method (CRL method) used in the everolimus drug trials to support approval

for everolimus use in kidney transplant patients. Samples were trough samples from 121 adult renal transplant patients. Results below are based on analysis of single measurements of each sample by each method. (Analyses showed there was no significant affect of within-patient correlation on results). Also see the site to site comparison in the Precision/Reproducibility section above.

Table 5: Summary of Method Comparison Regression Analysis

		Deming Regression		Passing-Bablok regression		
Comparator Method used	**N**	**Slope (95% CI)**	**Intercept (95% CI)**	**Slope (95% CI)**	**Intercept (95% CI)**	**R**
LCMSMS 1 (drug trial assay)	124	0.93 (0.87 to 0.98)	0.03 (-0.41 to 0.46)	0.92 (0.87 to 0.98)	0.17 (-0.15 to 0.54)	0.94
LCMSMS 2 (used for initial QMS calibration)	124	1.00 (0.95 to 1.06)	-0.08 (-0.48 to 0.33)	1.01 (0.95 to 1.08)	-0.15 (-0.50 to 0.17)	0.95



In other studies additional high concentration samples were tested by both the LCMSMS1 method and the QMS method. Results are shown below:

Sample	LCMS	QMS	QMS/LCMS
1	22.48	22.32	99.%
2	17.18	18.8	106%
3	18.6	16.4	88%

The manufacturer includes additional information in the package insert to further represent the bias and variability observed for samples in this specific study.

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:

Not applicable.

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 recommended everolimus therapeutic range specified in the Zortress® package insert is 3 to 8 ng/mL for prophylaxis of organ rejection in adult patients at low to moderate immunologic risk receiving a kidney transplant. This range is recommended when Zortress® is administered in combination with basiliximab induction and concurrently with reduced doses of cyclosporine and corticosteroids. Kidney transplant patients achieving everolimus whole blood trough concentrations less than 3.0 ng/mL (as determined by an LC-MS/MS

assay) are at increased risk for rejection.

The QMS Everolimus assay has been calibrated using a set of trough samples from adult renal transplant patients administered everolimus in combination with basiliximab and concurrently with reduced doses of cyclosporine and corticosteroids, so that on average results for these specimens will be within +/- 10% of an LC-MS/MS system.
Test findings should always be assessed in conjunction with the patient's medical history, clinical signs and symptoms, and other laboratory parameters. Everolimus values cannot be used as the sole indicator for making changes in treatment regimen.

Values obtained with different methods cannot be used interchangeably due to differences in methods and cross-reactivity with metabolites, nor should correction factors be applied. Consistent use of the same assay for individual patients is strongly recommended.

Optimally, dose adjustments of everolimus should be based on trough concentrations obtained 4 or 5 days after a previous dosing change. See the drug package insert for further information, including potential drug interactions.

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.