QMS® Everolimus (EVER)

Thermo SCIENTIFIC

IVD **For In Vitro Diagnostic Use Only**

Rx Only

REF **0380000**

This Quantitative Microsphere System (QMS) package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Intended Use

The QMS® Everolimus assay is intended for the quantitative determination of everolimus in human whole blood on automated clinical chemistry analyzers.

The results obtained are used as an aid in the management of kidney and liver transplant patients receiving everolimus therapy. This in vitro diagnostic device is intended for clinical laboratory use only.

Summary and Explanation of the Test

Everolimus is a macrolide immunosuppressant derived by chemical modification of the natural product rapamycin. Rapamycin is produced by certain strains of Streptomyces hygroscopicus.[1,2]

Immunosupressive treatment strategies are aimed at prevention of T cell activation and/or proliferation. Everolimus acts as a proliferation inhibitor. On a cellular level everolimus inhibits, in general, growth factor-stimulated cell proliferation irrespective of the cell lineage or growth factor involved. Inhibition is reversible since everolimus is not a cytotoxic compound. Everolimus inhibits the T cell response to growth factors arresting clonal expansion of activated T cells by inhibiting G1 to S phase.[3] Calcineurin inhibitors, such as cyclosporine (CsA), prevent the activation of T cells by inhibiting G0 to G1 phase transition. The different modes of action for everolimus and calcineurin inhibitors such as cyclosporine provide adequate rationale for the pharmacodynamic synergy.[2-5]

Monitoring blood level everolimus concentrations is recommended as an aid in patient management with the clinical use of everolimus.[6,7] The preferred matrix is whole blood because, at therapeutic concentrations the compound is predominately partitioned into erythrocytes. Liquid chromatography coupled to mass spectrometry (LC-MS/MS) has been used to measure the concentration of everolimus in blood.[8-10]

Principles of the Procedure

Overview of Technology

The QMS Everolimus assay is a homogeneous particle-enhanced turbidimetric immunoassay. The assay is based on competition between drug in the sample and drug coated onto a microparticle for antibody binding sites of the everolimus antibody reagent. The everolimus-coated microparticle reagent is rapidly agglutinated 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. 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 the maximum rate of agglutination at the lowest everolimus concentration and the lowest agglutination rate at the highest everolimus concentration.

Value Assignment and Traceability

One of the tools that transplant physicians use to monitor patients is measurement of the drug concentrations in blood.[2,4] One factor that can impact the drug concentration measured in a patient blood sample is the analytical method used to measure the drug. As a result, the target concentration range may differ depending on the assay used. See method comparison section.

LC-MS/MS method has become the acknowledged reference method (gold standard) for immunosuppressive drug (ISD) monitoring. With the rise in LC-MS/MS centers and the general acceptance of results based on LC-MS/MS method, QMS Everolimus assay was designed to provide clinicians with an assay that would produce patient results that are similar on average to values obtained by LC-MS/MS method. Bias for individual patient samples may vary in either direction depending on cross-reactivities with metabolites and other potential errors.

The QMS Everolimus Calibrators and Controls are prepared gravimetrically by spiking everolimus in human whole blood hemolysate. In order to minimize the bias between the QMS Everolimus assay and LC-MS/MS method, the QMS Everolimus Calibrators and Controls were initially value-assigned by using a representative set of clinical trough samples from renal transplant patients with traceability to LC-MS/MS values. The value-assigned concentrations of calibrators and controls are approximately 70% of their gravimetric concentrations. Because the QMS Everolimus Calibrators and Controls have been value assigned specifically for the QMS Everolimus assay, 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 Calibrators and Controls in analytical methods other than the QMS Everolimus assay is not appropriate, as this would result in incorrect values being obtained.

Reagents

QMS Everolimus is supplied as a liquid, ready-to-use, three-reagent kit that contains:

REF	0380000	
R1	Reagent 1	1 x 22 mL
R2	Reagent 2	1 x 8 mL
PRE	Precipitation Reagent	1 x 8 mL

Materials Required but not Provided

REF	**Kit Description**
0380005	QMS Everolimus Calibrators CAL A-F: 1 x 3.0 mL each
0373878	QMS Everolimus Controls Levels 1-3: 1 x 3.0 mL each
	Methanol (HPLC grade)

Reactive Ingredients

	Ingredient	Concentration
R1	IgM Antisera (Goat)	≤3.5%
	Human Serum Albumin (HSA)	≤1.0%
	Anti-Everolimus Polyclonal Antibody (Rabbit)	<1.0%
	Sodium Azide	0.09%
R2	Everolimus-coated Microparticles	<0.6%
	Sodium Azide	0.05%
PRE	Copper (II) Sulfate	≤6.4%
	Sodium Azide	0.09%

Reagent Handling and Storage

- **R1**, **R2**, and **PRE** Ready for Use
- Before use, invert several times, avoiding the formation of bubbles.
- Remove air bubbles, if present in the reagent cartridge, with a new applicator stick. Alternatively, allow the reagent to sit at the appropriate storage temperature to allow the bubbles to dissipate. To minimize volume depletion, do not use a transfer pipette to remove the bubbles.
- When either the **R1** or **R2** cartridge becomes empty, replace both cartridges and verify calibration with at least two levels of controls according to the established Quality Control requirements for your laboratory. If control results fall outside acceptable ranges, recalibration may be necessary.
- Refer to the analyzer specific Assay System Parameter sheet for system specific information.
- In the case of accidental spill, clean and dispose of material according to your laboratory's SOP, local, and state regulations.
- In the case of damaged packaging on arrival, contact your technical support representative (refer to back page of this PI).

⚠ **CAUTION:** Reagent bubbles may interfere with proper detection of reagent level in the cartridge, causing insufficient reagent aspiration that could impact results.

2°C 8°C The unopened reagents are stable until the expiration date when stored at 2 to 8°C. **Do not freeze reagents or expose them to temperatures above 32°C.**

Light may affect **R2** stability. Keep stored reagents out of light.

⚠ Warnings and Precautions

For in vitro diagnostic use. Do not mix materials from different kit lot numbers. Avoid the use of short drawn samples. Increased amounts of anticoagulant may produce erroneous results.

☣ **CAUTION:** This product contains human sourced and/or potentially infectious components. Components sourced from human blood have been tested by FDA-approved methods 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.

DANGER: QMS Everolimus **R1** contains ≤3.5% IgM Antisera (Goat) serum.
H317 - May cause allergic skin reaction.
H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Avoid breathing mist or vapor. Contaminated work clothing should not be allowed out of the workplace. Wear protective gloves/eye protection/face protection. In case of inadequate ventilation wear respiratory protection. If on skin: Wash with plenty of soap and water. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. If skin irritation or rash occurs: Get medical advice/attention. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. Wash contaminated clothing before reuse. Dispose of contents/container to location in accordance with local/regional/national/international regulations.

WARNING: QMS Everolimus **PRE** contains ≤6.4% Copper (II) sulfate.
H400 - Very toxic to aquatic life.
H410 - Very toxic to aquatic life with long-lasting effects.

Avoid release to the environment. Collect spillage. Dispose of contents/container to location in accordance with local/regional/national/international regulations.

Reagents used in the assay components contain ≤0.09% sodium azide. Avoid contact with skin and mucous membranes. Refer to SDS for additional precautions, handling instructions, and accidental exposure treatment.

Specimen Collection and Handling

The following specimen collection tubes may be used for the QMS Everolimus assay:

	Plastic
Whole Blood	EDTA (K_2)

- Other specimen collection tubes have not been validated for use with the QMS Everolimus assay. Follow the manufacturer's processing instructions for all collection tubes.
- The use of short drawn samples may yield erroneous results.
- Specimens may be stored up to 3 days at 2 to 8°C. If testing will be delayed more than 3 days, specimens should be stored frozen (-20 ± 5°C) up to 14 days prior to being tested.
- Light may affect sample stability. Keep stored samples out of light.
- Samples for the QMS Everolimus assay should be drawn just prior to a dose (trough level), to confirm that an adequate dose has been prescribed. The trough concentration is most indicative of the therapeutic level of everolimus.[2,4]

Procedure

Extraction Procedure for Samples, Calibrators, and Controls

Extracts must be run immediately after extraction.

1. Refer to the supplementary training materials in the start-up materials for more details.
2. Laboratories should verify their entire procedure with patient samples before reporting out results.
3. Prepare micro-centrifuge tubes for extraction of samples, calibrators and controls.
4. Samples, calibrators and controls should be thawed completely and brought to room temperature before extraction. Mix samples, calibrators and controls well by inversion (use of a rocker is preferred). Avoid the formation of bubbles.
5. Accurately pipette 300 µL of each calibrator, control, or sample to be assayed into the appropriate micro-centrifuge tube. Avoid bubbles.
6. Accurately dispense 350 µL of methanol into each microcentrifuge tube. Avoid bubbles.
7. Accurately pipette 50 µL of QMS Everolimus Precipitation Reagent into each micro-centrifuge tube. Avoid bubbles.
8. Cap each micro-centrifuge tube immediately to prevent evaporation, then vigorously mix/vortex at the highest speed for at least 35 seconds. Note: it may be necessary to invert the tube and remix to ensure complete mixing. After mixing, the sample color should change from red to brown. Check to ensure the mixture is homogenous.
9. Place the tubes in a micro-centrifuge and centrifuge for at least 8 minutes at 13,400 x g.
10. After centrifuging, decant the supernatant into appropriate sample cups. Avoid bubbles. Load cups on to the instrument.
11. Begin the analyzer calibration or assay process immediately to minimize sample evaporation.
12. Dispose of extracts after analysis. Retesting of samples requires fresh extractions.

Barcode Usage

Reagent labels have a dedicated system barcode that most analyzers will ignore if unrecognized. If the analyzer returns an error code, overlay the barcode with solid-colored tape. Contact Technical Services for assistance if needed.

Assay Procedure

The assay is performed at a wavelength of 700 nm. For a detailed description of how to run and calibrate an assay, refer to the instrument specific operations manual.

Specimen Dilution Procedure

Use QMS Everolimus CAL A (0.0 ng/mL) to manually dilute samples outside the linearity of the assay.

Manual Dilution Protocol

A manual dilution can be performed on patient samples with everolimus concentrations reported as greater than 20.0 ng/mL by making a 1:1 dilution of the specimen with QMS Everolimus CAL A (0.0 ng/mL) before extracting the sample. The dilution must be performed so the diluted test result reads greater than the assay sensitivity of 2.0 ng/mL. The concentration reported must be multiplied by the manual dilution factor to obtain the final sample concentration.

$$\text{Final Sample Concentration} = \text{Reported Concentration} \times \text{Manual Dilution Factor}$$

$$\text{Manual Dilution Factor} = \frac{\text{(Volume of Sample + Volume of CAL A)}}{\text{Volume of Sample}}$$

Calibration

The QMS Everolimus assay must be calibrated using a full calibration (6-point) procedure. To perform a full calibration, test the QMS Everolimus Calibrators A, B, C, D, E, and F in duplicate.

Calibration is required with each new lot number. Verify the calibration curve with at least two levels of controls according to the established Quality Control requirements for your laboratory. If control results fall outside acceptable ranges, corrective action should be taken.

Note: Everolimus CAL A is the calibration blank for this assay.

Quality Control

As appropriate, refer to your laboratory Standard Operating Procedure(s) and/or Quality Assurance Plan for additional quality control requirements and potential corrective actions. All quality control requirements should be performed in conformance with local, state, and/or federal regulations or accreditation requirements. Each laboratory should establish its own control ranges and calibration frequency.

Recommended control requirements for the QMS Everolimus assay:

- The manufacturer will provide validated samples to all laboratories implementing the assay. These materials are for initial laboratory verification of assay performance only. Ongoing enrollment in additional external proficiency testing schemes is needed.
- A minimum of two levels of controls spanning the medical decision range should be run as frequently as is needed to control for extraction batches.
- If more frequent control monitoring is required, follow the established Quality Control procedures for your laboratory.
- If quality control results do not fall within an acceptable range defined by your laboratory, patient values may be suspect and should not be reported. Corrective action should be taken.

Results

The result units for the QMS Everolimus assay are reported as ng/mL.

As with all analyte determinations, the everolimus value should be used in conjunction with information available from clinical evaluations and other diagnostic procedures.

Reporting Results

Laboratories must report the following key information to physicians together with the actual results:

- The assay is designed to produce similar results to LC-MS/MS, on average, for trough samples from adult renal transplant patients co-administered with basiliximab, reduced concentrations of cyclosporine and corticosteroids and adult hepatic transplant recipients co-administered with or without reduced concentrations of tacrolimus and with or without corticosteroids. Comparability to LC-MS/MS has not been evaluated for other patient groups, and may be different for other groups including pediatric patients due to differences in metabolite profiles.

Result Error Codes

Some results may contain Result Error Codes. Refer to the instrument specific operations manual for a description of the error codes.

Limitations Of The Procedure

Procedural limitations:

The QMS Everolimus assay has been designed to only recover clinical patient samples accurately and not artificially spiked samples.

Only QMS Everolimus Calibrators and Controls should be used with the QMS Everolimus assay. Accurate quantitative determination of everolimus cannot be obtained if the QMS Everolimus Calibrators set, **REF** 0380005, is not used in calibration of the QMS Everolimus assay.

Limitations regarding test interpretation:

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 Immunoassay is calibrated based on a training set of trough samples from adult renal transplant patients co-administered with cyclosporine, basiliximab induction therapy and corticosteroids. In addition, the assay has been validated with samples from adult liver transplant patients co-administered with or without reduced concentrations of tacrolimus and with or without 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 measuring adult renal and hepatic transplant patient blood samples and performance has not been determined for any other type of patients.

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

Interfering heterophile antibodies occur at a low frequency in the general population. These antibodies can cause autoagglutination of the microparticle reagent leading to undetected erroneously low results.

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

See the Specimen Collection and Handling and Specific Performance Characteristics sections of this package insert.

Expected Values

The recommended everolimus therapuetic range using an LC-MS/MS method is 3 to 8 ng/mL for pre-dose samples. See therapeutic drug monitoring information in the drug package insert.[2,4]

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 the *average bias* for this population across the assay range should be within ±10% of the LC-MS/MS system used. **However this may vary depending on the nature of the samples, individual metabolite concentrations and the specific assay used. Test findings should always be assessed in conjunction with the patient's medical history, clinical signs and symptoms and other laboratory parameters. Assay 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.

Specific Performance Characteristics

Representative performance results obtained on a commercially available automated clinical chemistry analyzer that employs turbidimetric quantitative analysis are shown below.

Sensitivity

Limit of Quantitation (LOQ)

The LOQ of the QMS Everolimus assay is defined as the lowest concentration for which acceptable inter-assay precision and recovery is observed. The LOQ was determined to be 2.0 ng/mL. At the LOQ level, the precision with kidney transplant patient samples was observed to be 9.3% (with a range of 7.6% - 11.8% across lots) and recovery observed to be 98% (89% - 109%). For liver transplant patient samples, the precision near the LOQ level was 11.8% (range of 9.8% - 13.7% across lots) and the recovery was observed to be 101% (96% - 105%).

Assay Range

The range of the assay is 2.0 to 20 ng/mL.

Accuracy

Linearity studies were performed by diluting a high patient sample or patient sample pool to concentrations across the assay range. The dilutions were made with whole blood hemolysate. The theoretical concentrations are based on the high sample concentration determined by LC-MS/MS multiplied by the dilution factor. Linearity at specific dilutions was considered acceptable if the percent recovery was 100 ± 10%.

Spiked sample recovery is not appropriate for laboratory validation of this assay because spiked samples may under-recover by as low as 59 to 68% of the gravimetric value.

Recovery Across the Assay Range for Linearity Study with Kidney Transplant Patient Sample

Theoretical Concentration (ng/mL)	Avg. of 12 Reps (ng/mL)	% CV	% Recovery
0.00	0.33	-	-
1.12	1.29	17	115
2.23	2.36	8	105
4.46	4.34	5	97
6.70	6.24	3	93
8.93	8.60	3	96
11.16	11.14	4	99
13.39	13.21	3	98
15.63	15.85	3	101
17.86	17.88	7	99
20.09	19.88	4	98
22.48	22.32	8	99

Recovery Across the Assay Range for Linearity Study with Liver Transplant Patient Sample

Theoretical Concentration (ng/mL)	Avg. of 5 Reps (ng/mL)	% CV	% Recovery
0.00	0.00	-	-
0.55	0.53	7	95
1.10	1.10	8	100
1.66	1.49	6	90
2.21	1.98	6	90
4.41	4.11	3	93
6.62	6.34	2	96
8.83	9.00	2	102
11.04	11.90	1	108
13.24	14.43	2	109
15.45	16.73	1	108
17.66	19.22	1	109
19.86	20.69	2	104
22.07	22.06	3	100

Method Comparison

A correlation study was performed using 124 trough samples from adult kidney transplant patients in the everolimus drug trial. Results from the QMS Everolimus assay were compared with results from two LC-MS/MS methods (System 1 and System 2). The LC-MS/MS System 1 was used in the everolimus drug trial. LC-MS/MS System 2 is used in QMS Everolimus calibrator value assignment. Results of the Passing-Bablok[11] and Deming regression analyses for the study are shown below.

Summary of Method Comparison Regression Analysis with Kidney Transplant Patient Samples

Methods	N	Deming		Passing-Bablok		R	$S_{y/x}$ (ng/mL)
		Slope (95% CI)	Intercept (95% CI)	Slope (95% CI)	Intercept (95% CI)		
QMS vs. System 1*	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	0.95
QMS vs. System 2*	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	0.88

*System 1 and System 2 refer to two LC-MS/MS Systems

Bias Analysis (Kidney Transplant Sample Data)

	QMS vs. System 1 (N = 124)	QMS vs. System 2 (N = 124)
Average Bias (ng/mL)	-0.5	0.0
Bias SD (ng/mL)	1.00	0.87
Average % Bias	-8.4%	-2.0%

*System 1 and System 2 refer to two LC-MS/MS Systems

A correlation study was performed using 178 samples from adult liver transplant recipients, where the majority of patients were co-administered tacrolimus. The samples were collected from this population pre-dose (except for some of the samples at the assay upper limit), at a time post-transplant mostly ranging from 9 to 26 months. Results from the QMS Everolimus assay were compared with results from two LC-MS/MS methods; both methods were used in the everolimus drug trial for liver transplantation. Two lots of reagents were used to measure liver transplant patient samples by QMS Everolimus assay. Results of the Passing-Bablok[11] and Deming regression analyses for the study are shown below.

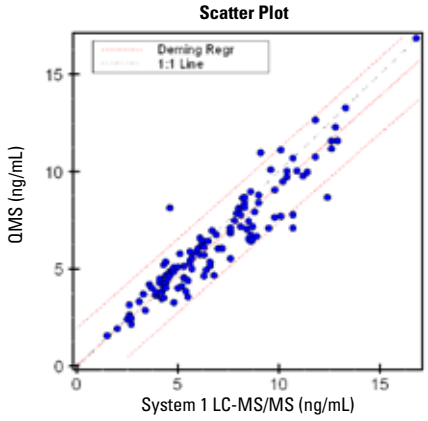
Summary Method Comparison Regression Analysis with Liver Transplant Patient Samples

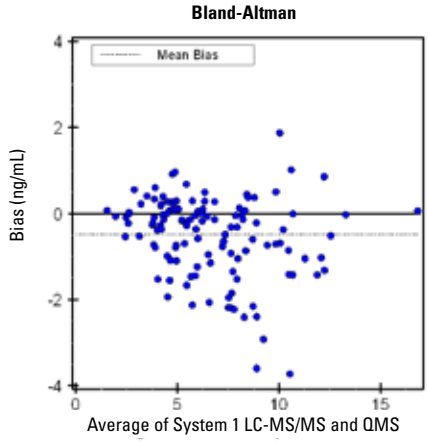
Methods	N	Deming		Passing-Bablok		R	$S_{y/x}$
		Slope (95% CI)	Intercept (95% CI)	Slope (95% CI)	Intercept (95% CI)		
System 1 LC-MS/MS vs. QMS (Rgt Lot 1)	178	1.070 (1.028 to 1.112)	0.099 (-0.214 to 0.412)	1.070 (1.026 to 1.117)	0.072 (-0.221 to 0.329)	0.9649	0.916
System 1 LC-MS/MS vs. QMS (Rgt Lot 2)	178	1.051 (1.016 to 1.087)	-0.142 (-0.406 to 0.121)	1.044 (1.008 to 1.086)	-0.220 (-0.409 to -0.014)	0.9742	0.772
System 3 LC-MS/MS vs. QMS (Rgt Lot 1)	178	1.041 (1.001 to 1.080)	0.394 (0.106 to 0.682)	1.073 (1.024 to 1.126)	0.185 (-0.141 to 0.432)	0.9681	0.873
System 3 LC-MS/MS vs. QMS (Rgt Lot 2)	178	1.023 (0.988 to 1.057)	0.145 (-0.108 to 0.398)	1.055 (1.020 to 1.096)	-0.093 (-0.316 to 0.102)	0.9746	0.766

Bias Analysis (Liver Transplant Sample Data)

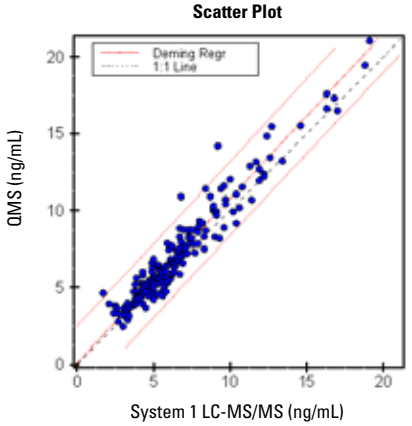
	Avg Bias (ng/mL)	Bias SD	Avg. % Bias
System 1 LC-MS/MS vs. QMS (Rgt Lot 1)	0.568	0.910	8%
System 1 LC-MS/MS vs. QMS (Rgt Lot 2)	0.201	0.768	3%
System 3 LC-MS/MS vs. QMS (Rgt Lot 1)	0.661	0.863	10%
System 3 LC-MS/MS vs. QMS (Rgt Lot 2)	0.294	0.759	4%

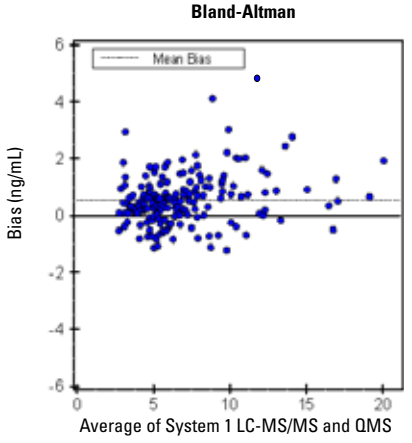
Scatter and Bland-Altman Plots of QMS Everolimus Assay vs. System 1 LC-MS/MS (Kidney Transplant Patient Samples)



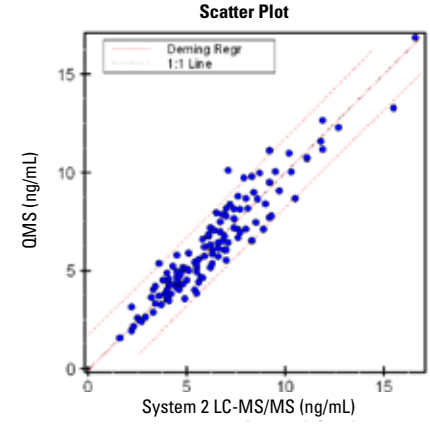


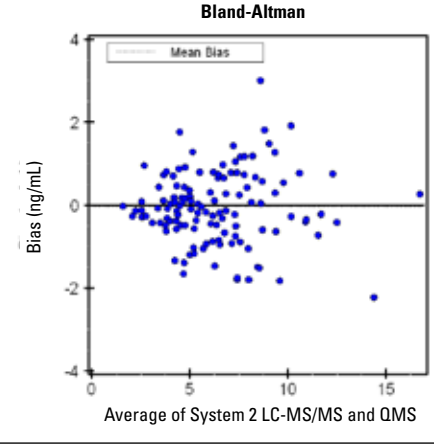
Scatter and Bland-Altman Plots of QMS Everolimus Assay (Rgt Lot 1) vs. System 1 LC-MS/MS (Liver Transplant Patient Samples)



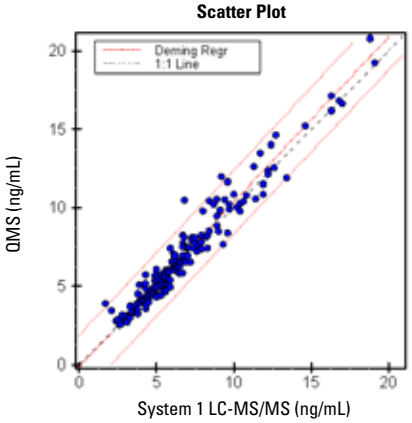


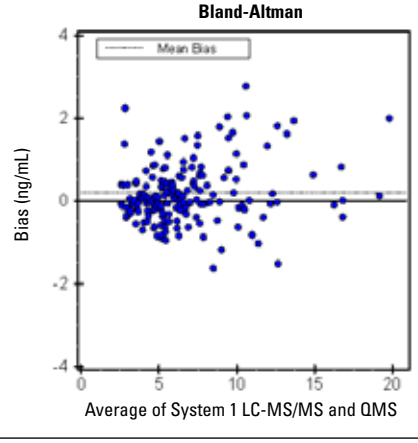
Scatter and Bland-Altman Plots of QMS Everolimus Assay vs. System 2 LC-MS/MS (Kidney Transplant Patient Samples)



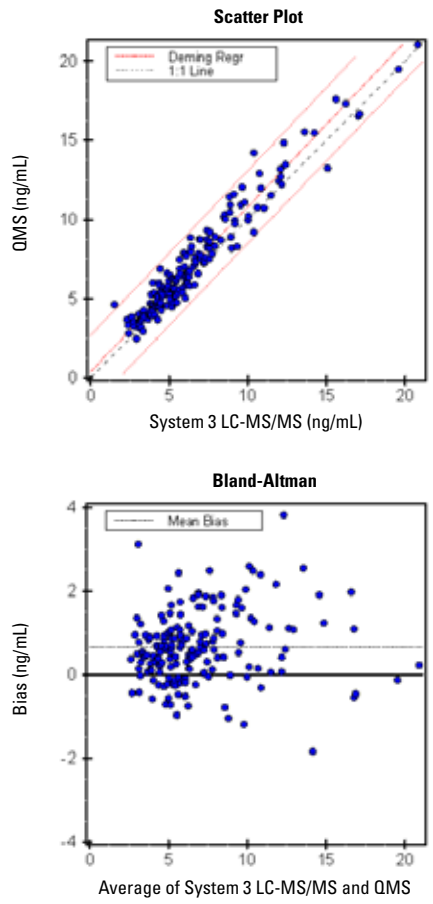


Scatter and Bland-Altman Plots of QMS Everolimus Assay (Rgt Lot 2) vs. System 1 LC-MS/MS (Liver Transplant Patient Samples)

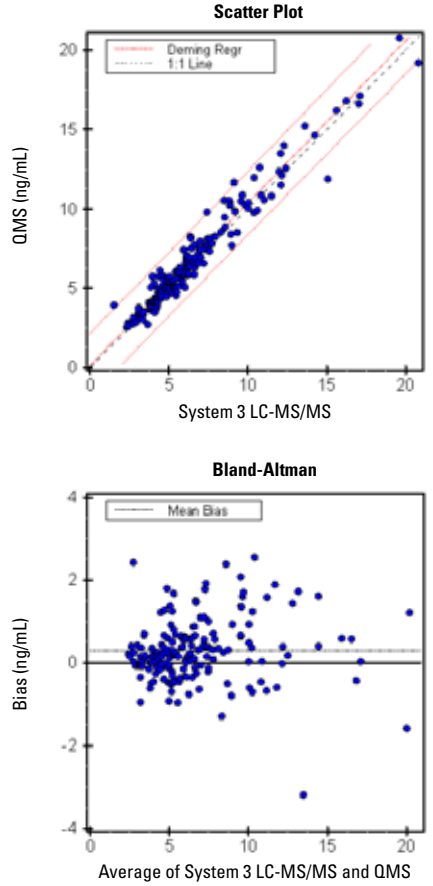




Scatter and Bland-Altman Plots of QMS Everolimus Assay (Rgt Lot 1) vs. System 3 LC-MS/MS (Liver Transplant Patient Samples)



Scatter and Bland-Altman Plots of QMS Everolimus Assay (Rgt Lot 2) vs. System 3 LC-MS/MS (Liver Transplant Patient Samples)



Precision

Precision was determined as described in CLSI protocol EP5-A2.[12]

A tri-level human blood based control containing everolimus and a tri-level patient sample pool was used in the study. Each level was assayed in duplicate twice a day for 20 days. Each of the runs per day was separated by at least two hours. The means and the within run, between run, between day and total CV were calculated. Representative results are shown below.

20-Day Precision Study: Kidney Transplant Patient Samples (Reagent Kit Lot 1)

Sample	Expected Value (ng/mL)	Total N	Mean (ng/mL)	Within Run %CV	Between Run %CV	Between Day %CV	Total %CV
Control Level 1	4.0	80	4.1	3.9	2.8	2.7	5.6
Control Level 2	8.0	80	8.1	3.8	2.6	3.4	5.7
Control Level 3	15.0	80	16.3	3.6	3.4	3.7	6.2
Patient Pool 1	2.9	80	2.8	5.6	4.8	4.5	8.6
Patient Pool 2	6.0	80	5.8	2.3	3.6	1.9	4.7
Patient Pool 3	10.5	80	10.1	2.4	2.1	2.8	4.2

20-Day Precision Study: Kidney Transplant Patient Samples (Reagent Kit Lot 2)

Sample	Expected Value (ng/mL)	Total N	Mean (ng/mL)	Within Run %CV	Between Run %CV	Between Day %CV	Total %CV
Control Level 1	4.0	80	4.3	3.4	2.2	5.2	6.6
Control Level 2	8.0	80	8.3	3.1	2.0	5.1	6.3
Control Level 3	15.0	80	15.9	2.3	3.3	4.4	6.0
Patient Pool 1	2.9	80	2.9	4.8	4.1	6.4	9.0
Patient Pool 2	6.0	80	6.1	2.6	3.5	4.8	6.5
Patient Pool 3	10.5	80	10.2	2.1	2.6	4.8	5.9

Another in-house precision study was performed using 3 lots of reagent kits. The results for the combined in-house precision study with kidney transplant sample pools are shown below.

20-Day Precision Study (Combined In-House Precision Study): Kidney Transplant Patient Samples*

Sample	Expected Value (ng/mL)	Total N	Mean (ng/mL)	Within Run %CV	Between Run %CV	Between Day %CV	Total %CV
Control Level 1	4.0	240	3.9	5.9	3.8	5.4	8.8
Control Level 2	8.0	240	8.0	3.8	3.2	4.2	6.5
Control Level 3	15.0	240	14.5	4.9	3.0	4.7	7.4
Patient Pool 1	4.0	240	3.9	11.7	5.2	0.6	12.8
Patient Pool 2	8.0	240	8.3	2.6	4.7	6.3	8.2
Patient Pool 3	12.0	240	11.6	3.6	4.6	5.7	8.2

* The within-run component of this evaluation represents an individual sample extraction for each set of duplicates

20-Day Precision Study: Liver Transplant Patient Samples (Reagent Kit Lot 1)

Sample	Expected Value (ng/mL)	Total N	Mean (ng/mL)	Within Run % CV	Between Run % CV	Between Day % CV	Total % CV
Control Level 1	4.4	80	4.4	3.7	4.0	2.4	6.0
Control Level 2	8.4	80	8.5	3.6	0.7	2.7	4.6
Control Level 3	15.2	80	15.6	2.3	1.1	1.8	3.2
Patient Pool 1	2.9	80	2.7	4.4	4.9	4.8	8.1
Patient Pool 2	5.1	80	5.4	2.5	3.0	2.5	4.7
Patient Pool 3	12.5	80	13.0	2.3	0.3	2.1	3.2

20-Day Precision Study: Liver Transplant Patient Samples (Reagent Kit Lot 2)

Sample	Expected Value (ng/mL)	Total N	Mean (ng/mL)	Within Run % CV	Between Run % CV	Between Day % CV	Total % CV
Control Level 1	4.4	80	4.4	3.4	3.1	4.5	6.4
Control Level 2	8.4	80	8.4	2.8	1.9	2.4	4.1
Control Level 3	15.2	80	15.6	2.5	0.8	2.1	3.4
Patient Pool 1	2.9	80	2.9	5.3	3.6	6.6	9.2
Patient Pool 2	5.1	80	5.5	2.9	1.0	3.9	5.0
Patient Pool 3	12.5	80	13.0	2.4	0.6	2.3	3.4

Interfering Substances

Specificity

Interference studies were conducted using CLSI protocol EP7-A2 as a guideline.[13] Cross-reactivity was tested for the major metabolites of everolimus listed in the table below. 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.

Metabolites

Studies were conducted to examine the cross-reactivity of the QMS Everolimus antiserum to major everolimus metabolites. The compounds tested were added in two concentrations to human blood hemolysate containing 5 ng/mL of everolimus drug and tested using the QMS Everolimus assay. Percent cross-reactivity was calculated. The results are shown below.

Everolimus Metabolites			
Compound Tested	**Concentration Tested (ng/mL)**	**Recovered Concentration (ng/mL)**	**% Cross-Reactivity**
RAD SA	5	6.08	7
RAD SA	20	6.07	2
RAD PSA	5	6.33	12
RAD PSA	20	9.00	16
RAD PC	5	8.86	63
RAD PC	20	17.61	59
45/46 OH RAD	5	5.82	ND
45/46 OH RAD	20	6.13	2
24 OH RAD	5	6.23	9
24 OH RAD	20	6.81	5
25 OH RAD	5	6.56	15
25 OH RAD	20	10.24	22

ND = Not Detectable. The cross-reactivity is considered not-detectable (ND) if the difference between the spiked sample and the control is less than the standard deviation of the control replicates.

In addition, studies were conducted to examine the cross-reactivity of the QMS Everolimus antiserum to sirolimus and its major metabolites. The compounds tested were added to human blood hemolysate containing 5.5 ng/mL of everolimus drug and tested using the QMS Everolimus assay. Percent cross-reactivity was calculated. The results are shown below.

Sirolimus and Sirolimus Metabolites			
Compound Tested	**Concentration Tested (ng/mL)**	**Recovered Concentration (ng/mL)**	**% Cross-Reactivity**
Sirolimus	10	9.94	46
Trihydroxy-sirolimus; 7,41-O-didesmethyl sirolimus	90	9.34	4
41-O-desmethyl-hydroxy sirolimus	90	8.55	3
41-O-desmethyl-hydroxy sirolimus; 7-O-desmethyl sirolimus	90	7.29	2
11-hydroxy sirolimus	90	16.43	12
Isomer of 11-hydroxy sirolimus	90	11.00	6
Hydroxy sirolimus	90	6.96	2
N-oxide sirolimus	90	12.10	7
Isomer of hydroxyl sirolimus or N-oxide sirolimus	90	6.71	1
41-O-desmethyl sirolimus; 32-O-desmethyl sirolimus	30	18.32	45

Endogenous Substances

The following compounds, when tested with the QMS Everolimus assay at the concentrations indicated, resulted in less than 10% error in detecting everolimus. The test samples contained 4.0-5.8 ng/mL everolimus. The results are shown below.

Interfering Substance Tested	Concentration Tested	N	Measured Test Sample (ng/mL)	% Recovery
Bilirubin	60 mg/dL	10	4.35	96
Cholesterol	500 mg/dL	15	5.61	97
Creatinine	5 mg/dL	15	5.40	100
Gamma Globulin	12 g/dL	3	3.77	93
HAMA type 1*	Normal Human Level	3	4.34	103
HAMA type 2*	Normal Human Level	3	4.01	95
Hematocrit	60%	10	4.26	102
Rheumatoid Factor	1350 IU	3	4.28	101
Total Protein	12 g/dL	3	4.27	105
Triglyceride	1500 mg/dL	3	4.24	101
Uric Acid	40 mg/dL	3	4.20	100

*HAMA = human anti-mouse antibodies

Drug Interference

Recovery and cross-reactivity were tested with drugs that are routinely administered with everolimus. Cross-reactants were analyzed in an everolimus-spiked hemolysate at 5-6 ng/mL. The following compounds were tested and found to have greater than ±10% interference.

Compound Tested	Concentration Tested (µg/mL)	% Cross-Reactivity	% Recovery
Amikacin	150	0.0	113
Ciprofloxacin	250	0.0	123
Triamterene	600	0.0	74

No significant interference was observed for the following compounds.

Compound Tested	Concentration Tested (µg/mL)	% Cross-Reactivity	% Recovery
Acetaminophen	200	ND	103
N-Acetylprocainamide	120	ND	106
Acyclovir	1000	0.0	105
Albuterol	0.18	ND	101
Allopurinol	60	ND	104
Amphotericin B	100	0.0	103
Ascorbic Acid	30	ND	101
Atenolol	40	ND	103
Azathioprine	10	ND	99
Bactrim (5:1 Sulfamethoxazole:Trimethoprim)	525 Sulfamethoxazole 45 Trimethoprim	0.0	106
Caffeine	100	ND	100
Captopril	50	0.0	105
Carbamazepine	120	0.0	109
Cefaclor	230	ND	102
Chloramphenicol	250	ND	106
Cimetidine	100	ND	104
Cyclosporin A	1	ND	103
Digoxin	0.01	-2.0	97
Disopyramide	30	0.0	96
Erythromycin	200	0.0	96
Ethanol	3500	ND	105
Fluconazole	75	0.0	98
Flucytosine	300	0.0	102
Folic Acid	0.01	ND	101
Furosemide	100	ND	101
Ganciclovir	1000	ND	101
Gemfibrozil	75	ND	103
Gentamicin	20	ND	106
Glipizide	60	ND	103
Glyburide	40	ND	99
Heparin	16	0.0	108
Hydralazine	32	ND	103
Hydrochlorothiazide	40	ND	102
Ibuprofen	400	ND	99
Insulin	0.0167	1	104
Intralipid	15000	ND	105
Isoniazid	70	ND	102
Isoproterenol HCl	0.06	ND	102
Itraconazole	17	ND	98
Kanamycin A	100	ND	101
Kanamycin B	100	ND	101
Ketoconazole	10	ND	106
Labetalol	200	ND	103
Lidocaine	100	ND	101
Lithium	22.2	ND	100
Lovastatin	4	0.0	94
Metformin HCl	5100	ND	106
Methicillin	240	ND	100
Methotrexate	910	ND	100
Metoclopramide	4	ND	104

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Compound Tested	Concentration Tested (µg/mL)	% Cross-Reactivity	% Recovery
Misoprostol	0.015	ND	100
Morphine Sulfate	6	ND	98
Mycophenolic Acid	250	ND	98
Nadolol	333	ND	98
Naproxen	1000	0.0	93
Niacin	800	ND	101
Nifedipine	120	0.0	104
Omeprazole	14	ND	105
Pantoprazole Sodium	15	0.0	107
Penicillin G	100	0.0	105
Phenobarbital	150	ND	102
Phenytoin	100	0.0	103
Piperacillin	8	ND	103
Prazosin	25	ND	100
Prednisone	12	ND	100
Prednisolone	12	ND	101
Primidone	100	0.0	102
Procainamide	25	ND	106
Propranolol	0.5	ND	102
Quinidine	100	ND	98
Ranitidine	200	ND	101
Rifampin	50	0.0	107
Salicylic Acid	500	ND	100
Sotrastaurin	40	0.0	107
Spectinomycin	100	ND	101
Sulfamethoxazole	400	0.0	103
Tacrolimus	0.04	1.0	107
Theophylline	250	ND	101
Tobramycin	20	ND	99
Trimethoprim	20	ND	100
Valganciclovir HCl	36	0.0	107
Valproic Acid	1000	0.0	96
Vancomycin	630	ND	102
Verapamil	10	ND	98

ND = Not Detectable. The cross-reactivity is considered not-detectable if the difference between the spiked sample and the control is less than the standard deviation of the control replicates.

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