

Everolimus Therapeutic Drug Monitoring Variability and Bias

Thermo Fisher Scientific

Analytical Methods for Everolimus TDM

There are two analytical methods available for Everolimus TDM: LC-MS/MS and immunoassay. LC-MS/MS is the acknowledged reference method (gold standard). This method is developed and validated by each laboratory that uses it. Many laboratories prepare their own internal calibrators and controls. Some laboratories may also use commercially available calibrators and controls.

The Thermo Scientific QMS Everolimus Immunoassay is designed for use on a variety of automated clinical chemistry analysers. This assay has been validated by its manufacturer. Reagents and calibrators for this assay are supplied as kits. These kits are FDA-cleared for use on kidney samples only (heart indication is not cleared in the USA) and are CE-marked for use on kidney and heart samples outside the USA.

One factor that can impact the drug concentration 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. With the general acceptance of results based on the LC-MS/MS method, the QMS Everolimus Assay was designed to provide clinicians with an assay that would produce patient results on average similar to values obtained by the LC-MS/MS method. The bias for individual samples may vary in either direction, depending on cross-reactivities with metabolites or other potential errors.

Variability Within Methods

Each method, including the gold standard LC-MS/MS, is subject to variability or imprecision. The variability within each lab is expected to be much less than the variability among several labs. The FDA Guidance on Bioanalytical Method Validation recommends imprecision to be $\leq 15\% \text{ CV}$. An example of variability among LC-MS/MS labs can be seen in the results of proficiency testing, such as the programme administered by ASI Ltd. (www.bioanalytics.co.uk).

Sample ID	QC A	QC B	QC C
Mean (ng/mL)	2.8	6.5	7.5
SD (ng/mL)	0.4	1.0	1.2
CV%	12.6	14.9	16.1
2SD	0.8	2.0	2.4
Mean - 2SD	2.0	4.5	5.1
Mean + 2SD	3.6	8.5	9.9
% Diff: -2SD to + 2SD	44.4	47.1	41.4

ERL Ever57 Nov 2010, N=83 LCMS Labs.

Proficiency testing results show that LC-MS/MS methods can vary among laboratories by as much as 47%.



Variability of results from immunoassay methods is also expected. Variability within a lab using one immunoassay method on one analyser model is expected to be less than the variability among several labs using the same immunoassay method on a variety of analyser models. Observed imprecision of the QMS Everolimus assay on the same analyser model (Hitachi 917) ranged from 4.2 - 12.8 % CV; across analysers, imprecision was 4.9 - 18.4 % CV.

	AU 680	Synchron LX	MGC 240*	Modular P	Cobas c501	CDx 90**	Indiko*	Architect c4000
Total Precision	n=60	n=60	n=60	n=60	n=60	n=60	n=80	n=80
CI Mean (ng/mL)	4.15	3.69	3.21	4.18	4.15	3.90	3.82	3.64
CI SD (ng/mL)	0.20	0.32	0.48	0.39	0.58	0.40	0.70	0.52
CI CV%	4.87	8.81	15.06	9.33	13.93	10.10	18.40	14.20
CII Mean (ng/mL)	8.00	8.24	7.46	8.20	8.28	9.00	7.32	7.39
CII SD (ng/mL)	0.43	0.74	0.89	0.38	0.96	0.50	1.00	1.07
CII CV%	5.32	9.01	11.87	4.58	11.65	5.80	14.20	14.40
CIII Mean (ng/mL)	15.64	14.77	15.66	16.17	15.97	15.60	15.30	15.88
CIII SD (ng/mL)	0.91	1.47	1.24	0.90	1.75	0.90	2.10	1.98
CIII CV%	5.82	9.92	7.91	5.59	10.95	5.60	13.80	12.50

*Pending FDA clearance, **Available outside the USA only

Bias Between Methods

A certain amount of bias between two methods is acceptable. It is typically expected that an immunoassay method exhibits a positive bias from an LC-MS/MS method. This positive bias occurs because LC-MS/MS measures only one compound, the target analyte, while an immunoassay's antibody reagent may bind not only the target analyte, but also structurally related compounds such as the target analyte's metabolites.

Comparability of everolimus values from the QMS Everolimus Assay and LC-MS/MS using 41 heart transplant patient samples. The results of the Passing-Bablok regression analysis are shown in the table below:

Slope	1.00
Y-Intercept	-0.15
Correlation Coefficient (R^2)	0.92
Number of Samples	41

Comparability of everolimus values from the QMS Everolimus Assay and LC-MS/MS on trough samples from kidney transplant patient samples. The results are shown in the table below:

Methods	N	Deming		Passing-Bablok		R	$S_{y/x}$
		Slope (95% CI)	Intercept (95% CI)	Slope (95% CI)	Intercept (95% CI)		
QMS Hitachi 917 vs. LC-MS/MS Method 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 Hitachi 917 vs. LC-MS/MS Method 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
LC-MS/MS Methods 2 vs. 1	124	0.92 (0.87 to 0.97)	0.10 (-0.29 to 0.48)	0.91 (0.86 to 0.96)	0.29 (0.01 to 0.56)	0.95	0.83

Bias Analysis

Kidney Samples	QMS vs. LC-MS/MS Method 1	QMS vs. LC-MS/MS Method 2
Average Bias (ng/mL)	-0.5	0.0
Bias SD (ng/mL)	1.00	0.87
Average % Bias	-8.4	-2.0

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Note that the above bias between QMS and LC-MS/MS may not be exactly duplicated when using an LC-MS/MS method from a lab different from the above. In addition to the variability among different LC-MS/MS methods, there are other factors that can contribute to the bias:

1. The QMS Everolimus assay has been designed to only recover clinical patient samples accurately and not artificially spiked samples
2. 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.
3. Comparability to LC-MS/MS has not been evaluated in pediatric patients and may be different due to differences in metabolism.

Factors than can elevate QMS results compared to LC-MS/MS

1. Presence of sirolimus and sirolimus metabolites in the patient sample will cause elevated QMS Everolimus results.
2. Certain conditions, such as hepatic impairment, that affect parent compound to metabolite ratio. Presence of the following everolimus metabolites will result in elevated QMS results: RAD PC, 25-OH RAD, RAD PSA,
3. Amikacin and ciprofloxacin, drugs which are routinely administered with everolimus, can cause elevated QMS Everolimus results.

Factors that can depress QMS Everolimus results compared to LC-MS/MS

1. Triamterene, a drug which is routinely administered with everolimus, can cause depressed QMS everolimus results
2. Interfering heterophile antibodies, which occur at a low frequency in the general population, can lead to undetected erroneously low QMS Everolimus results.

*Some labs have reported negative bias greater than the amount of bias listed in the Bias Analysis table.
See the list of factors that can contribute to bias between QMS Everolimus and LC-MS/MS.*

When testing samples artificially spiked with everolimus, such as quality control materials or proficiency survey samples, QMS results will be less than the gravimetrically spiked amount of everolimus. Do not use QC materials other than the QMS Everolimus Controls. Proficiency survey samples made from patient sample pools will recover accurately.

When testing samples from patients who have recently been administered sirolimus, do not use QMS Everolimus until sirolimus parent compound and metabolites are fully cleared.

For hepatic-impaired patients, consider confirming results with an LC-MS/MS method specific to the parent compound.

How the Positive Bias Against LC-MS/MS is Minimised

The positive bias is minimised by how the QMS Everolimus calibrators were designed. The QMS Everolimus master calibrators were prepared gravimetrically by spiking everolimus in human whole blood hemolysate. In order to minimise the bias between the QMS Everolimus Assay and LC-MS/MS, the QMS Everolimus calibrators 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 the calibrators are approximately one half of their gravimetric concentrations. This value assignment of the master calibrators was conducted in 2009. Commercial QMS Everolimus calibrators are anchored to the master calibrators.

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If the QMS Calibrators are tested by LC-MS/MS, values obtained will be 2 times the value listed in the QMS Calibrator labeling. This type of testing should not be performed.

Use only the QMS calibrators and controls with the QMS Everolimus Assay.

Do not use 3rd party calibrators and controls with the QMS Everolimus Assay.

Do not use the QMS calibrators and controls in analytical methods other than the QMS Everolimus Assay.



QMS Bias Among Analyser Platforms

The QMS assay can be used on a variety of automated clinical chemistry analysers. The QMS application protocol on each analyser was developed to match results from the master analyser, Hitachi 917. The QMS application protocols were validated in accordance with the FDA Reagent Replacement Policy.

Test Method	Reference Method	Number of Samples	Slope	Intercept	Correlation
AU640	Hitachi 917	100	0.94	0.00	0.991
AU640	Hitachi 917	40	1.02	-0.20	0.996
Synchron LX	Hitachi 917	100	0.94	0.10	0.988
UniCel DxC	Hitachi 917	40	0.95	0.30	0.979
MGC240*	Hitachi 917	90	1.11	-1.00	0.983
Modular P	Hitachi 917	90	1.06	-0.70	0.983
Cobas c501	Hitachi 917	105	1.01	-0.80	0.951
CDx90**	AU680	199	1.01	0.25	0.978
Indiko*	Hitachi 917	102	1.00	-0.80	0.949
Architect c4000	Hitachi 917	101	0.97	-0.20	0.983

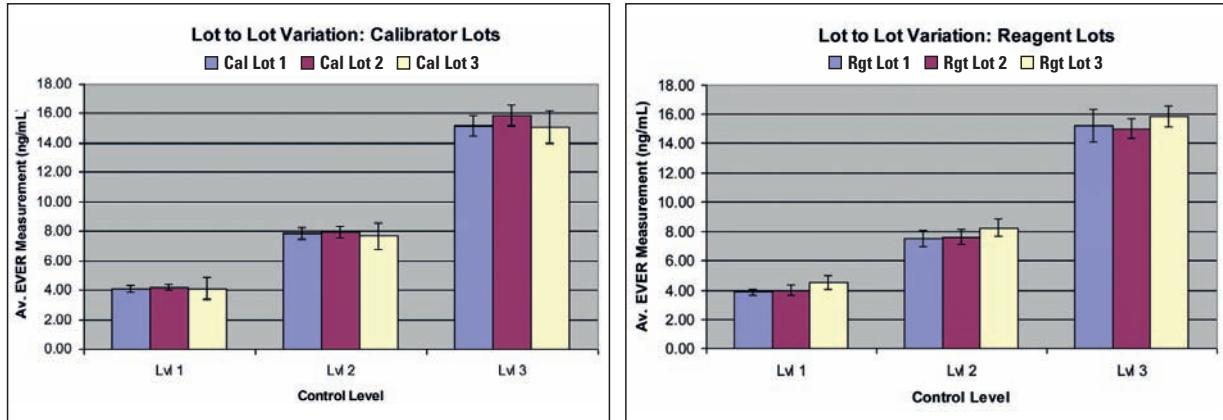
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Comparability of results between the master analyser, Hitachi 917 and the other analysers ranged from -6% (slope = 0.94) to +11% (slope = 1.11). Since QMS on the Hitachi 917 shows a negative bias of as much as -8.5% vs. LC-MS/MS, analysers showing a negative bias vs. Hitachi 917 could show greater than -8.5% bias vs. LC-MS/MS.

Consistency of QMS Results

Lot to lot consistency of QMS Everolimus calibrators is controlled by matching each commercial lot of calibrators to the master calibrator. This ensures that results from the QMS Everolimus assay will be consistent over time.

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Published reports from laboratories that have evaluated the QMS Everolimus Assay list a negative bias vs. a LC-MS/MS method of as much as 18.7%.

The QMS Everolimus assay is designed to produce similar results to LC-MS/MS on average, for trough samples from adult renal transplant patients. 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 difference in metabolite profiles.

It is common practice, when switching methods, for labs to run in parallel their current method and the new method. While there may be bias between the two methods, this process will demonstrate consistency of results from the new method. Values obtained with different methods cannot be used interchangeably. Consistent use of the same assay method for individual patients is strongly recommended.

Evaluation of QMS Everolimus Assay Using Hitachi 917 Analyzer: Comparison With Liquid Chromatography/Mass Spectrometry

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Abstract: Everolimus is an immunosuppressant requiring routine monitoring in whole blood. We evaluated the analytical performance of a new immunoassay for everolimus, Quantitative Microsphere System (QMS) everolimus (Thermo Fisher Scientific), which is CE marked and currently under review by Food and Drug Administration of the United States by comparing results with values obtained by using liquid chromatography/mass spectrometry. The total coefficient of variations (CVs) were 8.3% for low control (mean: 3.8 ng/mL), 6.1% for the medium control (mean: 8.0 ng/mL), and 7.5% for the high control (mean: 14.4 ng/mL) ($n = 80$ for each control, run over 20 non-consecutive days). The respective total CVs for patients' pool were 13.3% (mean: 4.0 ng/mL), 7.5% (mean: 8.2 ng/mL), and 8.7% (mean: 11.7 ng/mL) ($n = 80$ for each patient pool). The assay was linear from a whole blood everolimus level between 1.5 and 20 ng/mL, and the limit of quantitation was 1.3 ng/mL. Comparison was carried out using 90 renal transplant patient samples, and we observed the following Passing and Bablok linear regression plot: $y = 1.11$, slope = 20.005 ($R^2 = 0.92$). This assay was not affected by commonly used 70 drugs, but sirolimus, a drug structurally similar to everolimus, showed 46% cross-reactivity. We conclude that QMS Everolimus immunoassay has adequate sensitivity and specificity for the determination of whole-blood everolimus and can be used for routine therapeutic drug monitoring.

Key Words: everolimus, immunoassay, liquid chromatography/mass-spectrometry

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P70 Evaluation of the New Thermo Fisher QMS Everolimus Assay

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Introduction

Everolimus is an immunosuppressant for the prevention of transplant rejection. The current TDx assay has a limited life and high positive bias against the HPLC-MS/MS. We evaluated the new Thermo Fisher Scientific QMS Everolimus turbidimetric assay on the CDx90 analyser against HPLC-MS/MS.

Methods

Two pooled samples, a high control and a non-transplant patient sample were used to determine assay performance (CLSI guidelines). Both stored and fresh patient samples were quantitated with HPLC-MS/MS (SydPath). Onboard reagent stability and interference by Cyclosporine metabolites were determined.

Results

The correlation between the two methods was high ($R=0.976$) with a linearity coefficient of 0.994. Bland Altman plots showed a negative bias for the QMS assay 18.7% with an absolute difference at zero concentration of 17.4%. Intra-assay precision (CV) at levels of 3.99 ng/mL, 8.02 ng/mL and 16.18 ng/mL were 5.87%, 3.66% and 3.77% and inter-assay precision was 6.42%, 3.64% and 2.99% respectively.

Onboard reagent stability increased when capped after each run. Cyclosporine metabolites did not interfere with the assay and fresh samples correlated better ($R=0.970$) than stored refrigerated samples ($R=0.939$).

Conclusions

The new QMS Everolimus assay, yet to be released worldwide, had a constant bias but correlated well with HPLC-MS/MS. The increased reliability and accuracy of the QMS assay compared to the TDx technology provides a timely replacement. The random access assay will reduce the need to batch samples and provides a suitable alternative method to LC-MS/MS to monitor Everolimus concentrations.

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Summary

Everolimus TDM methods are accurate and reliable; however, there is inherent imprecision with each method. Bias between LC-MS/MS and the QMS method exists. The QMS Everolimus Immunoassay is designed for ease of use and consistency of results over time. This assay has undergone extensive review by the US FDA and has been cleared for clinical use. It has adequate sensitivity and specificity for the determination of whole-blood everolimus and can be used for routine therapeutic drug monitoring. Its ease-of-use features including the ability to run it in random access mode will reduce the need to batch samples and provide a suitable alternative method to LC-MS/MS to monitor everolimus concentrations.

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