

TDM Roundtable Recommendations: Sirolimus (Rapamycin) Assay Validation

TDM Roundtable Participating Organizations:

American Association for Clinical Chemistry
American Association of Bioanalysts
American Clinical Laboratory Association
American Society for Clinical Laboratory Science
American Society for Clinical Pathology
American Society for Microbiology
College of American Pathologists
Centers for Medicare and Medicaid Services
Food and Drug Administration
Pharmaceutical Research and Manufacturers of America

The concept of the TDM Roundtable was developed by the AACC TDM Renaissance Committee of the TDM-CT Division, in conjunction with the FDA, as means of fostering greater cooperation between the laboratory community and the agency to improve patient care.

Special thanks to the TDM Roundtable workgroup that drafted the document.

Robert Murray, JD, PhD, Chair

Thomas Annesley, PhD

Paula Stonemetz

Steven Wong, PhD

Kiang-Teck J. Yeo, PhD

TDM Roundtable Recommendations For Sirolimus (Rapamycin) Assay Validation

Table of Contents

1. Introduction	3
2. Background	3
3. Risks to Health	3
4. Performance Characteristics	5
General Study Recommendations	5
Specific Performance Characteristics	6
Precision	6
Recovery	7
Linearity	7
Sensitivity	8
Specificity for parent compound	8
Interference	9
Specimen collection and handling conditions	11
Method comparison	11
Studies at external sites	13
Calibrators	13
5. References	14
6. Further Related References	15

TDM Roundtable Recommendations For Sirolimus (Rapamycin) Assay Validation

1. Introduction

This guidance was developed as a recommendation to researchers and manufacturers to aid in the development and validation of Sirolimus (Rapamycin) assays. Such devices are intended to quantitatively measure sirolimus (rapamycin) concentration as an aid in the management of patients receiving therapy with this drug.

2. Background

Over the past decade there has been a dearth of new TDM immunoassays. These assays are used to optimize drug therapy. This crisis has been precipitated by a combination of factors, particularly the research and regulatory approval costs associated with developing and bringing to market new assays. The TDM Roundtable was created to explore new methods to facilitate the introduction of new TDM assays, such as Rapamycin. This document brings together in one location the most current scientific, clinical and technical information available on Rapamycin. It is our hope that this document will provide manufacturers with a blueprint for more expeditiously, and cost effectively, moving new Rapamycin assays through the regulatory review process. We hope to develop 1 technical documents on other areas in the near future.

3. Risks to Health

There are no known *direct* risks to patient health. However, failure of the test to perform as indicated or error in interpretation of results may lead to improper patient management.

A falsely low sirolimus (rapamycin) measurement could contribute to a decision to raise the dose above that which is necessary for therapeutic benefit. This could result in increased risk in the form of thrombocytopenia, leukopenia, anemia, or hyperlipidemia. A falsely high sirolimus (rapamycin) measurement could contribute to a decision to decrease the dose below that which is necessary for immunosuppression. This could result in increased risk of rejection of the transplanted organ.

An optimal concentration range for whole blood sirolimus (rapamycin) concentration, when given in combination with cyclosporine following kidney transplantation, has been suggested as 5-15 ng/mL for a trough, or pre-dose, concentration, using a microparticle enzyme immunoassay (MacDonald, 2000; Mahalati, 2001). Clinical trials have shown large inpatient variability observed in trough sirolimus (rapamycin) concentrations (Mahalati, 2001), indicating that optimal dose adjustment should be based on more than a single trough sample.

Optimal ranges for patients depend upon many factors such as patient tolerance of the drug, drug dosage, co-administered drugs, and time post-transplant, as well as metabolite cross-reactivity of the specific commercial assay used. Therefore, use of assay results to adjust a treatment regimen without consideration of other clinical factors could pose a risk. For these reasons, each institution should establish the optimal concentration based on the assay used and other factors relevant to their patient population. In addition, performance observed for a new assay relative to a gold standard (e.g. measures of bias, variability, cross-reactivity) should be clearly portrayed by the manufacturer in the labeling. If this drug is approved for transplantation of other organs in addition to kidney, the recommended concentration may be different.

Risks to health generally associated with the use of the sirolimus (rapamycin) assays are given in the table below. The measures recommended to mitigate these identified risks are given in this guidance document, as shown in the table below. You should also conduct a risk analysis to identify any other risks specific to your device and describe the risk analysis method. If you elect to use an alternative approach to address a particular risk identified in this guidance document, or have identified risks additional to those in the guidance, you should provide sufficient detail to support the approach you have used to address that risk. It would also be helpful to consult with FDA concerning your studies in such cases.

Identified Risk	Recommended Mitigation Measures
Analytical error overestimating sirolimus (rapamycin) concentration	Documented accuracy and analytical specificity throughout the measurement range
Analytical error underestimating sirolimus (rapamycin) concentration	Documented accuracy throughout the measurement range
Analytical imprecision in estimating sirolimus (rapamycin) concentration	Documented precision throughout the measurement range
Analytical interference resulting in substances other than sirolimus (rapamycin) being measured and reported	Documented crossreactivity of substances other than sirolimus (rapamycin)

There may be other patient management risks, and these should be addressed by the sponsor, for example, in the product labeling.

4. Performance Characteristics

General Study Recommendations

You should include patient samples or sample pools, derived from the intended use population (i.e., patients taking sirolimus (rapamycin)) for the analytical protocols described below. Minimally, samples from patients taking sirolimus (rapamycin) should be included in the precision and recovery studies, as well as method comparison studies. This is important because patient samples reflect the relevant proportions of free and bound drug, metabolites, and other drugs commonly co-administered to transplant patients and therefore help demonstrate robustness of the assay.

Although spiked samples can be used to supplement the studies, we caution against using spiked samples as the only matrix in the evaluations, because spiked samples may not provide an accurate assessment of the performance characteristics. We recommend that you do not use only hemolysates (often found in control or calibrator material) in the analytical studies, because these specimens may not test the effects of all preparatory steps on test performance. Studies which require freezing of samples (between run precision studies, for example) may require use of hemolysates, but use of such samples should be limited when possible.

You should perform all of your analytical protocols in accordance with the procedures you recommend to users in the package insert, in order to reflect performance expected by the user. Therefore, ensure that all steps (e.g., cell lysis, extraction, and centrifugation) are included in each of the analytical studies and that all manufacturer recommended quality control and calibration procedures are followed.

So that results can be best interpreted, you should provide appropriate specifics concerning protocols. These specifics are also necessary to aid users in interpreting information in your labeling. For example, when referring to National Committee for Clinical Laboratory Standards (NCCLS) evaluation protocols or guidelines, you should indicate which specific aspects of the protocols or guidelines you followed.

In studies using spiked samples, you should provide information about purity of drugs, metabolites, or potential interferents used, as well as the type of sample that drug is spiked into.

Whole blood is the matrix recommended in consensus statements from major scientific groups associated with organ transplantation (Holt, 2002; Yatscoff, 1995). For assays intended for use in other matrices, you will need to demonstrate a strong correlation with the analyte in whole blood using specimens from patients on drug therapy. We recommend contacting FDA, Office of In Vitro Diagnostic Devices to discuss your protocol before initiating a study of this type. Studies intended for sirolimus (rapamycin) instrument-based assays used in central clinical laboratories are described below. Depending on indications for use, assay methodology, and test performance compared to currently marketed devices, additional studies, including clinical studies, may be appropriate.

Specific Performance Characteristics

You should assess the following performance characteristics, in order to document performance and properly label your device in conformance with 21 CFR 809.10(b)(12).

Precision

You should characterize within-run, and total precision according to guidelines provided in “Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline” (1999) National Committee for Clinical Laboratory Standards (NCCLS), Document EP05-A². That document includes guidelines for experimental design, computations, and format for statement of claims.

You should evaluate precision for at least three concentrations spanning most of the assay range. Typically these concentrations are chosen to represent (a) sub-therapeutic range or near low end of the reportable range (b) concentrations considered to be within therapeutic range and (c) near high end of reportable range or toxic range. If the assay range extends to considerably higher concentrations, the precision evaluation, including validation with samples from patients taking sirolimus (rapamycin), should include higher drug concentrations in order to span the assay range.

You should include precision validation using samples from patients taking sirolimus (rapamycin), in order to demonstrate robustness of the assay. If it is not feasible to conduct the entire precision evaluation using such samples then the precision evaluation of patient samples can be supplemented with spiked whole blood samples or pools. However, you should ensure that evaluations of subtherapeutic level samples are included in the patient sample validation.

The description of your protocol and results should include the items listed below:

- Effects of hemolysate preparation steps (when hemolysates are necessary for one or more elements of the method validation)
- sample types (e.g., pooled patient samples, spiked whole blood)
- point estimates of the concentration
- standard deviations of within-run and total precision
- sites at which precision protocol was run
- number of days, runs, and observations.

You should also identify which factors (e.g., instrument calibration, reagent lots, and operators) were held constant and which were varied during the evaluation. You should

² or the most recent approved version of this document

describe the computational methods, if they are different from that described in NCCLS EP05-A.

Recovery

As a measure of accuracy, you should characterize the percent recovery of sirolimus (rapamycin). Typically, these studies involve spiking known amounts of sirolimus (rapamycin) into samples that are either negative for these drugs or contain known drug concentrations. You should include spiking into samples from patients taking sirolimus (rapamycin), as part of the study. Final concentrations of the spiked samples should span a significant part of the reportable range and include potential medical decision levels.

You should evaluate replicates of each concentration or sample. You should choose the number of replicates so that any clinically significant differences observed will be statistically significant. Description of the study protocol should include:

- sample types and concentrations
- materials used for spiking
- number of replicates
- definition or method of calculating recovery.

When reporting results, you should indicate the range of recoveries for each concentration level evaluated since this approach is more informative than describing only average recoveries at each concentration level.

Linearity

You should characterize the linear range of the assay response by evaluating samples whose concentration levels are known relative to one another. A graphic display or table of the known concentration vs. the observed concentration should be included. The sample concentrations should be evenly distributed across the reportable range of the assay. The appropriate number of replicates and concentration levels depends on the reportable range of the assay. Diluted patient sample pools are appropriate samples for the study. "Evaluation of the Linearity of Quantitative Analytical Methods; Approved Guideline" (2003) NCCLS Document EP06-A³ describes a protocol for sample preparation, value assignment, appropriate analyte range and concentrations to test, as well as statistical design and analysis methods, and a format for statement of claims.

Some immunoassays may exhibit a "high dose hook effect," in which there is a fall in response of the assay at high concentrations. Whenever appropriate (e.g., for two-site or sandwich immunoassays), you should extend linearity studies beyond the reportable range to the highest concentrations that may be encountered in clinical settings in order to evaluate whether your device exhibits a high dose hook effect.

³ or the most recent approved version of this document

The description of your protocol should include sample types and preparation, concentrations, number of replicates and statistical methods used. When practical, the linearity of the assay should be characterized using dilutions of patient samples containing an elevated drug concentration. Spiked whole blood may be used when patient samples are not available, (for example at very high drug concentrations). The description of results should include, the acceptable maximum differences from linearity or the measured maximum differences (including confidence intervals) from linearity and the range of linearity, as described in NCCLS EP06-A . You should include data from your high-dose hook evaluation, if applicable.

You should provide information on how samples outside the reportable range should be treated. If you recommend that users dilute samples that are above the reportable range, you should provide a specific protocol for dilution and include a validation of that protocol. You should also clarify how samples with concentrations outside the range of linearity are reported to the user.

Sensitivity

In addition to the lower limit of detection, you should characterize the functional sensitivity of the assay, which is the lowest drug concentration for which acceptable assay precision is observed. Often this is considered the concentration at which the inter-assay coefficient of variation is not greater than 20%. The acceptance criteria for sensitivity of a TDM assay should take into account the lower limits of therapeutic dose and any possible patient non-compliance issues.

The description of your sensitivity evaluation should include sample type, definition of your measures of sensitivity and results. Clarify how measurements below the level of sensitivity are reported to the user.

Specificity for parent compound

As a measure of assay specificity, you should characterize cross-reactivity with sirolimus (rapamycin) metabolites. Primary known metabolites should be included for sirolimus (rapamycin) specificity studies; these include 41-O-demethyl-, 7-O-demethyl-, 12-hydroxy-, 16-O-demethyl-, 39-O-demethyl-, 27, 39-O-di-demethyl-, and dihydroxy-sirolimus (Mahalati, 2001). When metabolites of high purity are available, drug free whole blood should be spiked with the metabolites to a final concentration consistent with the highest concentration expected based on experience with the intended use population. When such metabolites are not available in high purity, the metabolites present in patient specimens should be measured by an appropriate method, and their effect on the proposed assay estimated. Specimens from patients with elevated creatinine concentration should be included, when available, because such patients typically show higher than average metabolite concentrations. In either case, replicates should be evaluated, and the exact protocol, along with details of the metabolite purity, should be described. It may be helpful to consult with FDA prior to undertaking this alternative type of study.

The description of your evaluation should include description of types of samples used for spiking, number of replicates, concentration of metabolite, computation or definition of cross-reactivity used and percent cross-reactivity for each metabolite.

Interference

You should characterize the effects of potential interferents on assay performance. Potential sources of interference that you should test include, but are not limited to, the following:

(1) endogenous compounds, such as (where applicable, the recommended upper limit concentration is given in parentheses):

- bilirubin (60 mg/dL)
- triglycerides (1500 mg/dL)
- cholesterol (500 mg/dL)
- uric acid (20 mg/dL)
- rheumatoid factor (500 IU/ml)
- hematocrit (15-60%)
- albumin (12 g/dL)
- gamma globulin (12 g/dL)
- human anti-mouse antibodies, HAMA

(2) commonly co-administered drugs including, but not limited to:

- cyclosporine
- mycophenolic acid and its metabolite, MPAG
- acyclovir
- amphotericin B
- ciprofloxacin
- erythromycin
- fluconazole
- flucytosine
- gentamicin
- itraconazole
- ketoconazole
- gancyclovir (and pro-drugs)
- rifampin
- tacrolimus
- tobramycin
- vancomycin.]
- common over-the-counter drugs

(3) anticoagulants or preservatives with which the sample is likely to come in contact, such as EDTA.

When testing these interferents, you should adjust sirolimus (rapamycin) concentrations in the sample to near medical decision levels. Typically, interference studies involve adding potential interferent to the sample containing the drug and determining any bias in recovery of sirolimus (rapamycin), relative to a control sample (to which no interferent has been added). Recommended guidelines for interference testing are described in detail in “Interference Testing in Clinical Chemistry; Approved Guideline” (2002) NCCLS Document EP07-A⁴. This document includes guidelines for setting decision criteria as well as for protocol designs, statistical methods, evaluating interference using patient specimens and establishing validating and verifying interference claims. The following classes of potential interferents should be tested:

- For endogenous substances, test at the highest concentration expected based on experience with the intended use population. Interference studies using samples naturally high in the endogenous compound being tested can be informative and this approach should be considered when such samples are available.
- For drug levels, test to levels 3 times the highest acute peak concentration reported following therapeutic dosage.
- For specimen additives, test up to levels five times the recommended concentration.

If you observe interference at the concentration levels tested, you should test lower levels in order to determine the lowest concentration that could cause interference. You should test replicate samples in these protocols.

The description of your evaluation should include the following items (if description of the protocol refers to NCCLS EP07-A, clarify which aspects of the guidelines were followed):

- types and levels of interferents tested
- sample type (e.g., spiked whole blood pools, samples naturally high in endogenous compounds)
- concentrations of sirolimus (rapamycin) in the sample
- number of replicates tested
- definition or method of computing interference.

When reporting results, you should identify any observed trends in bias (negative or positive) across the concentration range of interferent tested. Include the standard error of the observed recoveries at each concentration or the range of observed recoveries at each concentration evaluated for a potential interferent. This approach is more informative than listing average recoveries alone.


⁴ or the most recent approved version of this document

For substances listed as non-interfering, you should state the criteria on which this is based, e.g., inaccuracies due to these substances are less than 10% at sirolimus (rapamycin) concentrations of 15 ng/ml. If any potential interferents are known from the literature or other sources to interfere with the test system, you should include this information in the labeling. You may not need to perform any additional interference testing with these known interferents.

Specimen collection and handling conditions

You should substantiate the labeled recommendations for specimen storage and transport, by assessing whether the device can maintain acceptable performance (e.g., precision, accuracy) over the storage times and temperatures (including freeze/thaw cycles) recommended to users. An appropriate study includes analysis of sample aliquots stored under the conditions of time, temperature, or allowed number of freeze/thaw cycles recommended in the package insert. You should state the criteria for acceptable range of recoveries under the recommended storage and handling conditions. Any other sources of preanalytical error, such as binding to a specimen container or gel, should be identified.

Method comparison

Sirolimus (rapamycin) assays vary significantly in terms of cross-reactivity patterns with metabolites whose therapeutic and toxic effects are not well-defined (Gallant-Haidner, 2000). Therefore, you should compare the new assay to a candidate reference method, specific for the parent compound. Carefully validated high performance liquid chromatography methods that measure parent drug specifically, such as methods described as reference procedures should be used as comparator in the method comparison study (Salm, 2000; Streit, 2002). If the discordance exceeds 25% relative to the reference procedure, you should address the reasons for the discordance, and describe steps to be taken to minimize risk of patient mismanagement which is based on the results of such tests. If other commercially marketed sirolimus immunoassays become available, it may be beneficial to evaluate comparison to these, in addition. 

You should follow the guidelines provided in the document, “Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline” (1995) National Committee for Clinical Laboratory Standards, Document EP09-A⁵ concerning experimental guidelines and statement of claims. You should evaluate kidney transplant patient samples with drug concentrations distributed across the reportable range of the assay when used in applications for which the drug is approved. Banked (retrospective) samples are appropriate for these studies as long as the information listed below concerning sample characterization is available. We recommend including samples from multiple geographic sites or clinical centers.

Appropriate sample size depends on factors such as precision, interference, range, and other performance characteristics of the test. The number of patients should also be large enough so that inter-individual variation would be observed. A statistical justification to support the

⁵ or the most recent approved version of this document


study sample size should be provided in the protocol description. We expect that the sample size target, however supported, will include a minimum of 100 samples distributed fairly evenly over a minimum of 50 *individual patients*.

If you choose to include multiple measurements from individual patients, you should summarize your results of appropriate statistical analyses such as Analysis of Variance, Generalized Estimating Equations, or Bootstrapping, to account for correlation of repeat measurements within patients in the study. If you choose to include multiple measurements from individuals it would be beneficial if they range over time post-transplant.

For your results to be properly interpreted you should provide all relevant information on the sample population in the package insert. Information on the sample population should include:

- the number of individual patients represented by the samples;
- the number of data points;
- the number of clinical sites; and
- information regarding the time of last dose.

You should state any specific selection (inclusion/exclusion) criteria for samples. You should also indicate whether samples were collected from patients with specific clinical outcomes, or from centers using atypical or novel drug regimens. Factors such as age range (e.g., adults), time post-transplant (e.g., chronic, acute), and time of blood draw with respect to drug administration (e.g., trough, peak) can influence drug-to-metabolite ratios and consequently, assay bias (Gallant-Haidner, 2000; Lampen, 1998; Kaplan, 1998; Kelly, 2002). Therefore, you should describe these features of the general sample population, whenever possible.⁶

You should clarify the HPLC method used, and include references to validation of the procedure from the literature. You should conduct separate analyses of data for each organ transplant group for which the test is indicated. If samples evaluated in the study include both trough and other times of blood draw relative to drug administration, you should conduct separate statistical analyses for these groups as well. If samples in the study are known to include patients at various times post-transplant, it would be helpful to conduct statistical evaluations, to address this parameter, as well.  When providing the results of the method comparison study, you should include the following information:

- Scatterplots of the new assay versus the reference (e.g., LC-MS) method. The plots should contain all data points, the estimated regression line and the line of identity. Data points in the plot should represent individual measurements.

⁶ Currently evaluation of trough samples is considered sufficient for method comparison, as long as these samples sufficiently span the claimed therapeutic range.

- A description of the method used to fit the regression line and results of regression analysis including the slope and intercept with their 95% confidence limits, the standard error of the estimate (calculated in the y direction), and correlation coefficient should be included. In cases where parameters are not consistent throughout the reportable range, estimates of more than a single range may be appropriate. If the comparator, as well as the new assay is subject to measurement error, a regression method such as the Deming method may be appropriate, rather than Least Squares.
- To illustrate the degree of inter-individual variations, you should include graphs of difference in measurements (i.e., new device minus reference HPLC method) versus the reference HPLC method. Appropriate representations include a bias plot of difference in measurements (y - x) versus the reference method (x), as recommended in NCCLS Document EP09-A , or versus the mean of y and x, as recommended by Bland and Altman (Bland, 1995).

Studies at external sites

You should demonstrate performance at external laboratory sites in addition to that of the manufacturer's site by evaluating the assay in at least three external sites. You may choose to include this as part of the method comparison study described above. Data from individual sites should initially be analyzed separately to evaluate any inter-site variation. Method comparison results from the individual sites can be pooled in the package insert, if you demonstrate that there are no significant differences in results among sites.

Calibrators

You should provide the following information about the calibrators in the assay kit in your summary report:

- Protocol and acceptance criteria for real-time or accelerated stability studies for opened and unopened calibrators.
- Protocol and acceptance criteria for value assignment and validation, including any specific instrument applications or statistical analyses used.
- Identification of traceability to a domestic or international standard reference material.
- Protocol and acceptance criteria for the transfer of performance of a primary calibrator to a secondary calibrator.

For information about calibrators marketed separately as class II devices under 862.1150, see the guidance "Abbreviated 510k Submissions for *In Vitro* Diagnostic Calibrators," <http://www.fda.gov/cdrh/ode/calibrator.html>.

5. References

Bland, JM, Altman, DG, Comparing methods of measurement: Why plotting difference against standard method is misleading, *Lancet* 1995; 346:1085-1087.

Gallant-Haidner HL, Trepanier DJ, Freitag DG, Yatscoff RW. Pharmacokinetics and metabolism of sirolimus. *Ther Drug Monit* 2000; 22:31-5

Holt, DW, Lee, T, Jones, K, Johnston, A, Validation of an Assay for Routine Monitoring of Sirolimus Using HPLC with Mass Spectrometric Detection, *Clinical Chemistry* 2000;46:1179-1183

Kaplan B, Meier-Kriesche H, Napoli KL, Kahan BD. The effects of relative timing of sirolimus and cyclosporine microemulsion formulation coadministration on the pharmacokinetics of each agent *Clin Pharmacol Ther* 1998; 63: 48-53.

Kelly P, Kahan BD Review: metabolism of immunosuppressant drugs. *Curr Drug Metab* 2002 Jun;3(3):275-87

Lampen A, Zhang Y, Hackbarth I, Benet LZ, Sewing KF, Christians U. Metabolism and transport of the macrolide immunosuppressant sirolimus in the small intestine. *J Pharmacol Exp Ther* 1998; 285: 1104-12.

MacDonald, A, Scarola, J, Burke, JT, Zimmerman, JJ, Clinical Pharmacokinetics and Therapeutic Drug Monitoring of Sirolimus, in Shaw, LM (ed.), *Advances in Therapeutic Drug Monitoring for Immunosuppressants: A review of Sirolimus, Clinical Therapeutics* 2000;22 (Suppl B): B101-B121

Mahalati, K, Kahan, BD, Clinical Pharmacokinetics of Sirolimus, *Clinical Pharmacokinetics* 2001;40: 573-585

Salm, P, Taylor, PJ, Pillans, PI, The Quantification of Sirolimus by High-Performance Liquid Chromatography-Tandem Mass Spectrometry and Microparticle Enzyme Immunoassay in Renal Transplant Recipients, in Shaw, LM (ed.), *Advances in Therapeutic Drug Monitoring for Immunosuppressants: A review of Sirolimus, Clinical Therapeutics* 2000;22 (Suppl B): B71-B85

Streit, G, Armstrong, VW, Oellerich, M, Rapid Liquid Chromatography-Tandem Mass Spectrometry Routine Method for Simultaneous Determination of Sirolimus, Everolimus, Tacrolimus, and Cyclosporin A in Whole Blood, *Clinical Chemistry* 2002;48:955-958

Yatscoff RW, Boeckx R, Holt DW, Kahan BD, LeGatt DF, Sehgal S, Soldin SJ, Napoli K, Stiller C. Consensus guidelines for therapeutic drug monitoring of rapamycin: report of the consensus panel. *Ther Drug Monit* 1995 Dec;17(6):676-80.

6. Further Related References

Aspeslet, LJ, Yatscoff, RW, Requirements for Therapeutic Drug Monitoring of Sirolimus, an Immunosuppressive Agent Used in Renal Transplantation, in Shaw, LM (ed.), *Advances in Therapeutic Drug Monitoring for Immunosuppressants: A review of Sirolimus*, *Clinical Therapeutics* 2000;22 (Suppl B): B86-B92

Davis, DL, Soldin, SJ, An Immunophilin-Binding Assay for Sirolimus, in Shaw, LM (ed.), *Advances in Therapeutic Drug Monitoring for Immunosuppressants: A review of Sirolimus*, *Clinical Therapeutics* 2000;22 (Suppl B): B62-B70

French, DC, Saltzgueber, M, Hicks, DR, Cowper, AL, Holt, DW, HPLC Assay with Ultraviolet Detection for Therapeutic Drug Monitoring of Sirolimus, *Clinical Chemistry* 2001;47: 1316-1319

French, DC, Saltzgueber, M, Hicks, DR, Cowper, AL, Holt, DW, HPLC Assay with Ultraviolet Detection for Therapeutic Drug Monitoring of Sirolimus (Rapamycin), *Clinical Chemistry* 2001;47:1316-1319 [Reference to his reference for therapeutic target.]

Holt DW, Armstrong VW, Griesmacher A, Morris RG, Napoli KL, Shaw LM International Federation of Clinical Chemistry/International Association of Therapeutic Drug Monitoring and Clinical Toxicology working group on immunosuppressive drug monitoring. *Ther Drug Monit* 2002 Feb;24(1):59-67

Holt, DW, Lee, T, Johnston, A, Measurement of Sirolimus in Whole Blood Using High-Performance Liquid Chromatography with Ultraviolet Detection, in Shaw, LM (ed.), *Advances in Therapeutic Drug Monitoring for Immunosuppressants: A review of Sirolimus*, *Clinical Therapeutics* 2000;22 (Suppl B): B38-B48

Jones, K, Johnston, A, Holt, DW, Proficiency-Testing Issues Relating to Sirolimus, in Shaw, LM (ed.), *Advances in Therapeutic Drug Monitoring for Immunosuppressants: A review of Sirolimus*, *Clinical Therapeutics* 2000;22 (Suppl B): 122-132

Jones, K, Saadat-Lajevardi, S, Lee, T, Horwatt, R, Hicks, D, Johnston, A, Holt, DW, An Immunoassay for the Measurement of Sirolimus, in Shaw, LM (ed.), *Advances in Therapeutic Drug Monitoring for Immunosuppressants: A review of Sirolimus*, *Clinical Therapeutics* 2000;22 (Suppl B): B49-B61

Kaplan, B, Meier-Kriesche, H-U, Napoli, K, Kahan, BD, A Limited Sampling Strategy for Estimating Sirolimus Area-Under-the-Concentration Curve, *Clinical Chemistry* 1997;43: 539-540

Maleki, S, Graves, S, Becker, S, Horwatt, R, Hicks, D, Stroshane, RM, Kincaid, H, Therapeutic Monitoring of Sirolimus in Human Whole-Blood Samples by High-Performance Liquid Chromatography, in Shaw, LM (ed.), *Advances in Therapeutic Drug Monitoring for Immunosuppressants: A review of Sirolimus*, *Clinical Therapeutics* 2000;22 (Suppl B): B25-B37

Meier-Kriesche, H-U, Kaplan, B, Toxicity and Efficacy of Sirolimus: Relationship to Whole-Blood Concentrations, in Shaw, LM (ed.), Advances in Therapeutic Drug Monitoring for Immunosuppressants: A review of Sirolimus, *Clinical Therapeutics* 2000;22 (Suppl B): B93-B100

Napoli, KL, A Practical Guide to the Analysis of Sirolimus Using High-Performance Liquid Chromatography with Ultraviolet Detection, in Shaw, LM (ed.), Advances in Therapeutic Drug Monitoring for Immunosuppressants: A review of Sirolimus, *Clinical Therapeutics* 2000;22 (Suppl B): B14-B24

Salm, P, Taylor, PJ, Pillans, PI, Analytical Performance of Microparticle Enzyme Immunoassay and HPLC-Tandem Mass Spectrometry in the Determination of Sirolimus in Whole Blood, *Clinical Chemistry* 1999;45: 2278-2250

Sehgal, SN, Rapamune® (RAPA, rapamycin, sirolimus): Mechanism of Action Immunosuppressive Effect Results From Blockade of Signal Transduction and Inhibition of Cell Cycle Progression, *Clinical Biochemistry* 1998;31: 335-340

Shaw, LM, Kaplan, B, Brayman, KL, Introduction and Overview, in Shaw, LM (ed.), Advances in Therapeutic Drug Monitoring for Immunosuppressants: A review of Sirolimus, *Clinical Therapeutics* 2000;22 (Suppl B): B1-B13

Taylor, PJ, Johnson, AG, Quantitative Analysis of Sirolimus (Rapamycin) in Blood by High-Performance Liquid Chromatography-Electrospray Tandem Mass Spectrometry, *Journal of Chromatography B* 1998;718: 251-257