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August 4, 2003

Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Room 1061
Rockville, MD 20852

Dear Sir/Madam:

The American Association for Clinical Chemistry (AACC), on behalf of the Therapeutic Drug Management (TDM) Roundtable, requests that the Food and Drug Administration (FDA) consider the enclosed Guidance Document Submission regarding Sirolimus assays. If you have any questions, please do not hesitate to contact Vince Stine, Director, Government Affairs, at 202/835-8721. Thank you for your consideration of our request.

Sincerely,

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Class II Special Controls Guidance Document: Sirolimus (Rapamycin) Assay; Guidance for Industry and FDA

Document issued on: _____

U.S. Department Of Health and Human Services
Food and Drug Administration
Center for Devices and Radiological Health

Chemistry and Toxicology Branch
Division of Clinical Laboratory Devices
Office of Device Evaluation

Preface

Public Comment

Comments and suggestions may be submitted at any time for Agency consideration to Dockets Management Branch, Division of Management Systems and Policy, Office of Human Resources and Management Services, Food and Drug Administration, 5630 Fishers Lane, Room 1061, (HFA-305), Rockville, MD, 20852. When submitting comments, please refer to the exact title of this guidance document. Comments may not be acted upon by the Agency until the document is next revised or updated.

For questions regarding the use or interpretation of this guidance contact _____ at (301) _____ or by email at mailto:_____@cdrh.fda.gov.

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Class II Special Controls Guidance Document: Sirolimus (Rapamycin) Assays; Guidance for Industry and FDA

This document is intended to provide guidance. It represents the Agency's current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind the Food and Drug Administration (FDA) or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute and regulations.

1. Introduction

This guidance was developed as a special control guidance to support the reclassification of sirolimus (rapamycin) assays into class II. The device is intended to quantitatively determine sirolimus (rapamycin) concentration as an aid in the management of patients receiving therapy with this drug. This guidance will be issued in conjunction with a Federal Register notice announcing the reclassification of this device type.

FDA may take this action after reviewing reclassification petitions from industry for sirolimus (rapamycin) test systems.

Following the effective date of this final reclassification rule, any firm submitting a 510(k) premarket notification for a sirolimus (rapamycin) assay will need to address the issues covered in the special control guidance. However, the firm need only show that its device meets the recommendations of the guidance or in some other way provides equivalent assurances of safety and effectiveness.

2. Background

FDA believes that special controls, when combined with the general controls, will be sufficient to provide reasonable assurance of the safety and effectiveness of sirolimus (rapamycin) assays. Thus, a manufacturer who intends to market a device of this generic type should (1) conform to the general controls of the Federal Food, Drug & Cosmetic Act (the Act), including the premarket notification requirements described in 21 CFR 807 Subpart E, (2) address the specific risks to health associated with sirolimus (rapamycin) assays identified in this guidance and, (3) obtain a substantial equivalence determination from FDA prior to marketing the device, unless exempt from the premarket notification requirements of the Act (refer to 21 CFR 807.85).

This special control guidance document identifies the classification regulations and product codes for the sirolimus (rapamycin) assay (Refer to Section 4 – **Scope**). In addition, other sections of this special control guidance document list the risks to health identified by FDA and describe measures that, if followed by manufacturers and combined with the general controls, will generally address the risks associated with this sirolimus (rapamycin) assay and lead to a

timely premarket notification [510(k)] review and clearance. This document supplements other FDA documents regarding the specific content requirements of a premarket notification submission. You should also refer to 21 CFR 807.87 and other FDA documents on this topic, such as the **510(k) Manual - Premarket Notification: 510(k) - Regulatory Requirements for Medical Devices**, http://www.fda.gov/cdrh/manual/510kprt1.html.

Under "**The New 510(k) Paradigm - Alternate Approaches to Demonstrating Substantial Equivalence in Premarket Notifications; Final Guidance**[1]," a manufacturer may submit a Traditional 510(k) or has the option of submitting either an Abbreviated 510(k) or a Special 510(k). FDA believes an Abbreviated 510(k) provides the least burdensome means of demonstrating substantial equivalence for a new device, particularly once a special controls guidance document has been issued. Manufacturers considering modifications to their own cleared devices may lessen the regulatory burden by submitting a Special 510(k).

The Least Burdensome Approach

The issues identified in this guidance document represent those that we believe need to be addressed before your device can be marketed. In developing the guidance, we carefully considered the relevant statutory criteria for Agency decision-making. We also considered the burden that may be incurred in your attempt to comply with the statutory and regulatory criteria in the manner suggested by the guidance and in your attempt to address the issues we have identified. We believe that we have considered the least burdensome approach to resolving the issues presented in the guidance document. If, however, you believe that there is a less burdensome way to address the issues, you should follow the procedures outlined in the "**A Suggested Approach to Resolving Least Burdensome Issues**" document. It is available on our Center web page at: http://www.fda.gov/cdrh/modact/leastburdensome.html.

3. The Content and Format of an Abbreviated 510(k) Submission

An Abbreviated 510(k) submission must include the required elements identified in 21 CFR 807.87, including the proposed labeling for the device sufficient to describe the device, its intended use, and the directions for its use. In an Abbreviated 510(k), FDA may consider the contents of a summary report to be appropriate supporting data within the meaning of 21 CFR 807.87(f) or (g); therefore, we recommend that you include a summary report. The report should describe how this special control guidance document was used during the device development and testing and should briefly describe the methods or tests used and a summary of the test data or description of the acceptance criteria applied to address the risks identified in this guidance document, as well as any additional risks specific to your device. This section suggests information to fulfill some of the requirements of 807.87 as well as some other items that we recommend you include in an Abbreviated 510(k).

[1] http://www.fda.gov/cdrh/ode/parad510.html

Coversheet

The coversheet should prominently identify the submission as an Abbreviated 510(k) and cite the title of this class II special controls guidance document.

Proposed labeling

Proposed labeling should be sufficient to describe the device, its intended use, and the directions for its use. (Refer to Section 7 for specific information that should be included in the labeling for devices of the types covered by this document.)

Summary report

The summary report should contain:

- Description of the device and its intended use. We recommend that the description include a complete discussion of the performance specifications and, when appropriate, detailed, labeled drawings of the device. You should also submit an "indications for use" enclosure.[2]

- Description of device design requirements.

- Identification of the Risk Analysis method(s) used to assess the risk profile in general as well as the specific device's design and the results of this analysis. (Refer to Section 5 for the risks to health generally associated with the use of this device that FDA has identified.)

- Discussion of the device characteristics that address the risks identified in this class II special controls guidance document, as well as any additional risks identified in your risk analysis.

  A brief description of the test method(s) you have used or intend to use to address each performance aspect identified in Section 6 of this class II special controls guidance document. If you follow a suggested test method, you may cite the method rather than describing it. If you modify a suggested test method, you may cite the method but should provide sufficient information to explain the nature of and reason for the modification. For each test, you may either (1) briefly present the data resulting from the test in clear and concise form, such as a table, **or** (2) describe the acceptance criteria that you will apply to your test results.[3] (See also 21 CFR 820.30, Subpart C - Design Controls for the Quality System Regulation.)

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[2] Refer to http://www.fda.gov/cdrh/ode/indicate.html for the recommended format.

[3] If FDA makes a substantial equivalence determination based on acceptance criteria, the subject device should be tested and shown to meet these acceptance criteria before being introduced into interstate commerce. If the finished device does not meet the acceptance criteria and, thus, differs from the device described in the cleared 510(k), FDA recommends that submitters apply the same criteria used to assess modifications to legally marketed devices (21 CFR 807.81(a)(3)) to determine whether marketing of the finished device requires clearance of a new 510(k).

- If any part of the device design or testing relies on a recognized standard, (1) a statement that testing will be conducted and meet specified acceptance criteria before the product is marketed, or (2) a declaration of conformity to the standard.[4] Please note that testing must be completed before submitting a declaration of conformity to a recognized standard. (21 USC 514(c)(2)(B)). For more information refer to the FDA guidance, **Use of Standards in Substantial Equivalence Determinations; Final Guidance for Industry and FDA**, http://www.fda.gov/cdrh/ode/guidance/1131.html.

If it is not clear how you have addressed the risks identified by FDA or additional risks identified through your risk analysis, we may request additional information about aspects of the device's performance characteristics. We may also request additional information if we need it to assess the adequacy of your acceptance criteria. (Under 21 CFR 807.87(l), we may request any additional information that is necessary to reach a determination regarding substantial equivalence.)

As an alternative to submitting an Abbreviated 510(k), you can submit a Traditional 510(k) that provides all of the information and data required under 21 CFR 807.87 and described in this guidance. A Traditional 510(k) should include all of your methods, data, acceptance criteria, and conclusions. Manufacturers considering modifications to their own cleared devices should consider submitting Special 510(k)s.

The general discussion above applies to any device subject to a special controls guidance document. The following is a specific discussion of how you should apply this special controls guidance document to a premarket notification for a sirolimus (rapamycin) assay.

4. Scope

The scope of this guidance is limited to the following devices:

FDA identifies the generic sirolimus (rapamycin) assays classified under 21 CFR 862.____. The product codes are:

____ Sirolimus (Rapamycin)

____ Sirolimus (Rapamycin) Fluorescence Polarization Immunoassay

____ Sirolimus (Rapamycin) High Performance Liquid Chromatography Assay – Ultraviolet Detection

____ Sirolimus (Rapamycin) High Performance Liquid Chromatography Assay – Mass Spectrometry

____ Sirolimus (Rapamycin) High Performance Liquid Chromatography Assay (Other Detection System)

[4] See Required Elements for a Declaration of Conformity to a Recognized Standard (Screening Checklist for All Premarket Notification [510(K)] Submissions), http://www.fda.gov/cdrh/ode/reqrecstand.html.

5. 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 intrapatient 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.

6. 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-A[5]. 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.

[5] or the most recent approved version of this document

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 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-A[6] 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.

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[2]. 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.

[6] or the most recent approved version of this document

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-demethy-, 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-A[7]. 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 (i.e., 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.

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.

[7] or the most recent approved version of this document

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-A[8] 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 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

[8] or the most recent approved version of this document

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.[9]

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.
- 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.

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

- 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.

7. Labeling

The premarket notification should include labeling in sufficient detail to satisfy the requirements of 21 CFR 807.87(e). The following suggestions are aimed at assisting you in preparing labeling that satisfies the requirements of 21 CFR 807.87(e).[10]

[10] Although final labeling is not required for 510(k) clearance, final labeling must also comply with the requirements of 21 CFR 801 or 21 CFR 809.10 before a medical device is introduced into interstate commerce. In addition, final labeling for prescription medical devices must comply with 21 CFR 801.109. Labeling recommendations in this guidance are consistent with the requirements of part 801 and section 809.10.

Specimens

You should discuss the importance of consistency of time of blood draw with respect to last dose, as well as time of day.

You should discuss any limitations or instructions related to the specimen, such as appropriate matrices or anticoagulants (in most cases, EDTA).

You should provide instructions concerning preserving integrity of the specimen, such as temperatures for collection, transport, storage (short and long term) and procedural steps of the assay necessary to maintain assay performance. Storage conditions recommended to the user should be based on the conditions you have validated for your test system. You should clearly define any acceptance criteria that you apply in determining the recommended storage conditions (e.g., inaccuracies due to instability under these conditions are less than 10% for 95% of samples tested). Additional information on storage conditions based on literature can be cited if they are applicable to your test system.

Assay procedure

You should include appropriate time limits and temperature requirements for the procedural steps. Whenever applicable, you should describe expected appearance of the specimen through various procedural steps and advise users of any signs that may indicate whether the assay is proceeding correctly.

You should advise users how to proceed for samples with concentrations above the highest calibrator. If you instruct users to dilute these samples, you should provide a validated procedure for the dilution.

You should advise users of any steps that can be taken to minimize effect of carryover, or other causes of bias or irreproducibility, based on procedures you have validated for your test system.

Quality control

You should advise users of the specifics of calibration and quality control procedures necessary to ensure the performance claims of the system and include instructions for interpretation of the results of quality control samples, satisfactory limits of performance and instructions on how to proceed if limits of performance are not satisfied. You should include recommendations for appropriate quality control specimens. Consensus documents recommend that whole blood assays should employ whole blood controls with well-characterized drug preparations.

Limitations

You should include the following limitation, when appropriate for your device type.

Patients with abnormal liver function, elevated bilirubin levels, unexpectedly high drug values, or increased time post-therapy may have impaired drug elimination and metabolite

accumulation. For such patients, use of this assay may be supported with a method more specific for the parent compound (e.g., HPLC).

You should identify any exogenous or endogenous factors known to affect results and describe the effect on results (e.g., highly lipemic samples may cause falsely low results).

References listing drugs currently known to alter metabolism of sirolimus (rapamycin) should be cited in an appropriate section of the package insert.

Optimal Concentration Range

Since the optimal concentration ranges may vary depending on the methodology used as well as the clinical state of the individual, stating one specific therapeutic range is usually not appropriate for current sirolimus (rapamycin) assays. You should include cautionary explanations concerning the lack of firm optimal concentration ranges to the user. You should discuss both patient variability and test variability. For example:

> **The optimal concentration range for sirolimus (rapamycin) in whole blood is not well established. The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic effects of sirolimus (rapamycin), co-administration of other immunosuppressants, time post-transplant and a number of other factors contribute to different requirements for optimal blood levels of sirolimus (rapamycin). Therefore, individual sirolimus (rapamycin) values cannot be used as the sole indicator for making changes in treatment regimen and each patient should be thoroughly evaluated clinically before changes in treatment regimens are made. Each user must establish his or her own ranges based on clinical experience.**
>
> **Optimal concentration ranges vary according to the commercial test used, and therefore should be established for each commercial test. Values obtained with different assay methods cannot be used interchangeably due to differences in assay methods and cross-reactivity with metabolites, nor should correction factors be applied. Therefore, consistent use of one assay for individual patients is recommended.**

Performance Characteristics

You should describe the protocol and results for each performance characteristic discussed in Section 6. Protocol descriptions and results in the package insert should include all of the information cited in Section 6, including scatterplots of the new assay versus the reference (e.g., HPLC) method and, in some cases, graphs of inter-individual variation or equivalent information, in order to best represent results of the method comparison for the user. See also applicable sections in the NCCLS guidelines cited in Section 6 concerning statements of claims.

8. New Instrument Applications

For information concerning application of cleared or approved test systems to additional analyzers, see the guidance entitled "Data for Commercialization of Original Equipment Manufacturer, Secondary and Generic Reagents for Automated Analyzers," http://www.fda.gov/cdrh/ode/odecl950.html. The approach described in that guidance is appropriate in cases when performance characteristics on the new analyzer meet pre-determined acceptance criteria specified in a protocol submitted by the manufacturer and reviewed by the FDA. If performance characteristics do not meet pre-determined acceptance criteria, a new 510(k) (which may be an Abbreviated 510(k)) is appropriate.

When the new analyzer is within the same family and does not involve any changes in reagents, sample treatment, or assay procedure that could potentially affect cross-reactivity or partitioning of metabolites, it is sufficient for the method comparison studies in the protocol to include comparison of samples on the new instrument to the previously cleared instrument. In this case, results of the method comparison study of the original test system versus the HPLC reference procedure should still be available to the user in the package insert. In contrast, when application to a new analyzer does include changes in reagents, sample treatment or procedure, a method comparison study including HPLC should be included in the protocol for the add-to and results should be included in the labeling.

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Jean's Weekly Report
8/4/03

DAT online

Last week's accomplishments

- Worked with speakers

This week's plan

- Materials due, nag speakers

Immuno Assay

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- Invited Division to co sponsor, re did brochure

This Week's Plan

- Promo, check on color of logo for sponsor

Automation Meeting

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- Did minutes and distributed

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- Begin touching base with potential sponsors

IAG

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- Make Gallwas chart of who has done what
- Distrubute book to Gallwas member

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BioRad Survey

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- Approach with plan to survey our members first and then others

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Class II Special Controls Guidance Document: Sirolimus (Rapamycin) Assay; Guidance for Industry and FDA

Document issued on: _____

U.S. Department Of Health and Human Services
Food and Drug Administration
Center for Devices and Radiological Health

Chemistry and Toxicology Branch
Division of Clinical Laboratory Devices
Office of Device Evaluation

Preface

Public Comment

Comments and suggestions may be submitted at any time for Agency consideration to Dockets Management Branch, Division of Management Systems and Policy, Office of Human Resources and Management Services, Food and Drug Administration, 5630 Fishers Lane, Room 1061, (HFA-305), Rockville, MD, 20852. When submitting comments, please refer to the exact title of this guidance document. Comments may not be acted upon by the Agency until the document is next revised or updated.

For questions regarding the use or interpretation of this guidance contact _____ at (301) _____ or by email at mailto:_____@cdrh.fda.gov.

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Class II Special Controls Guidance Document: Sirolimus (Rapamycin) Assays; Guidance for Industry and FDA

This document is intended to provide guidance. It represents the Agency's current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind the Food and Drug Administration (FDA) or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute and regulations.

1. Introduction

This guidance was developed as a special control guidance to support the reclassification of sirolimus (rapamycin) assays into class II. The device is intended to quantitatively determine sirolimus (rapamycin) concentration as an aid in the management of patients receiving therapy with this drug. This guidance will be issued in conjunction with a Federal Register notice announcing the reclassification of this device type.

FDA may take this action after reviewing reclassification petitions from industry for sirolimus (rapamycin) test systems.

Following the effective date of this final reclassification rule, any firm submitting a 510(k) premarket notification for a sirolimus (rapamycin) assay will need to address the issues covered in the special control guidance. However, the firm need only show that its device meets the recommendations of the guidance or in some other way provides equivalent assurances of safety and effectiveness.

2. Background

FDA believes that special controls, when combined with the general controls, will be sufficient to provide reasonable assurance of the safety and effectiveness of sirolimus (rapamycin) assays. Thus, a manufacturer who intends to market a device of this generic type should (1) conform to the general controls of the Federal Food, Drug & Cosmetic Act (the Act), including the premarket notification requirements described in 21 CFR 807 Subpart E, (2) address the specific risks to health associated with sirolimus (rapamycin) assays identified in this guidance and, (3) obtain a substantial equivalence determination from FDA prior to marketing the device, unless exempt from the premarket notification requirements of the Act (refer to 21 CFR 807.85).

This special control guidance document identifies the classification regulations and product codes for the sirolimus (rapamycin) assay (Refer to Section 4 – **Scope**). In addition, other sections of this special control guidance document list the risks to health identified by FDA and describe measures that, if followed by manufacturers and combined with the general controls, will generally address the risks associated with this sirolimus (rapamycin) assay and lead to a

timely premarket notification [510(k)] review and clearance. This document supplements other FDA documents regarding the specific content requirements of a premarket notification submission. You should also refer to 21 CFR 807.87 and other FDA documents on this topic, such as the **510(k) Manual - Premarket Notification: 510(k) - Regulatory Requirements for Medical Devices**, http://www.fda.gov/cdrh/manual/510kprt1.html.

Under "**The New 510(k) Paradigm - Alternate Approaches to Demonstrating Substantial Equivalence in Premarket Notifications; Final Guidance**[1]," a manufacturer may submit a Traditional 510(k) or has the option of submitting either an Abbreviated 510(k) or a Special 510(k). FDA believes an Abbreviated 510(k) provides the least burdensome means of demonstrating substantial equivalence for a new device, particularly once a special controls guidance document has been issued. Manufacturers considering modifications to their own cleared devices may lessen the regulatory burden by submitting a Special 510(k).

The Least Burdensome Approach

The issues identified in this guidance document represent those that we believe need to be addressed before your device can be marketed. In developing the guidance, we carefully considered the relevant statutory criteria for Agency decision-making. We also considered the burden that may be incurred in your attempt to comply with the statutory and regulatory criteria in the manner suggested by the guidance and in your attempt to address the issues we have identified. We believe that we have considered the least burdensome approach to resolving the issues presented in the guidance document. If, however, you believe that there is a less burdensome way to address the issues, you should follow the procedures outlined in the "**A Suggested Approach to Resolving Least Burdensome Issues**" document. It is available on our Center web page at: http://www.fda.gov/cdrh/modact/leastburdensome.html.

3. The Content and Format of an Abbreviated 510(k) Submission

An Abbreviated 510(k) submission must include the required elements identified in 21 CFR 807.87, including the proposed labeling for the device sufficient to describe the device, its intended use, and the directions for its use. In an Abbreviated 510(k), FDA may consider the contents of a summary report to be appropriate supporting data within the meaning of 21 CFR 807.87(f) or (g); therefore, we recommend that you include a summary report. The report should describe how this special control guidance document was used during the device development and testing and should briefly describe the methods or tests used and a summary of the test data or description of the acceptance criteria applied to address the risks identified in this guidance document, as well as any additional risks specific to your device. This section suggests information to fulfill some of the requirements of 807.87 as well as some other items that we recommend you include in an Abbreviated 510(k).

[1] http://www.fda.gov/cdrh/ode/parad510.html

Coversheet

The coversheet should prominently identify the submission as an Abbreviated 510(k) and cite the title of this class II special controls guidance document.

Proposed labeling

Proposed labeling should be sufficient to describe the device, its intended use, and the directions for its use. (Refer to Section 7 for specific information that should be included in the labeling for devices of the types covered by this document.)

Summary report

The summary report should contain:

- Description of the device and its intended use. We recommend that the description include a complete discussion of the performance specifications and, when appropriate, detailed, labeled drawings of the device. You should also submit an "indications for use" enclosure.[2]

- Description of device design requirements.

- Identification of the Risk Analysis method(s) used to assess the risk profile in general as well as the specific device's design and the results of this analysis. (Refer to Section 5 for the risks to health generally associated with the use of this device that FDA has identified.)

- Discussion of the device characteristics that address the risks identified in this class II special controls guidance document, as well as any additional risks identified in your risk analysis.

  A brief description of the test method(s) you have used or intend to use to address each performance aspect identified in Section 6 of this class II special controls guidance document. If you follow a suggested test method, you may cite the method rather than describing it. If you modify a suggested test method, you may cite the method but should provide sufficient information to explain the nature of and reason for the modification. For each test, you may either (1) briefly present the data resulting from the test in clear and concise form, such as a table, **or** (2) describe the acceptance criteria that you will apply to your test results.[3] (See also 21 CFR 820.30, Subpart C - Design Controls for the Quality System Regulation.)

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[2] Refer to http://www.fda.gov/cdrh/ode/indicate.html for the recommended format.

[3] If FDA makes a substantial equivalence determination based on acceptance criteria, the subject device should be tested and shown to meet these acceptance criteria before being introduced into interstate commerce. If the finished device does not meet the acceptance criteria and, thus, differs from the device described in the cleared 510(k), FDA recommends that submitters apply the same criteria used to assess modifications to legally marketed devices (21 CFR 807.81(a)(3)) to determine whether marketing of the finished device requires clearance of a new 510(k).

- If any part of the device design or testing relies on a recognized standard, (1) a statement that testing will be conducted and meet specified acceptance criteria before the product is marketed, or (2) a declaration of conformity to the standard.[4] Please note that testing must be completed before submitting a declaration of conformity to a recognized standard. (21 USC 514(c)(2)(B)). For more information refer to the FDA guidance, **Use of Standards in Substantial Equivalence Determinations; Final Guidance for Industry and FDA**, http://www.fda.gov/cdrh/ode/guidance/1131.html.

If it is not clear how you have addressed the risks identified by FDA or additional risks identified through your risk analysis, we may request additional information about aspects of the device's performance characteristics. We may also request additional information if we need it to assess the adequacy of your acceptance criteria. (Under 21 CFR 807.87(l), we may request any additional information that is necessary to reach a determination regarding substantial equivalence.)

As an alternative to submitting an Abbreviated 510(k), you can submit a Traditional 510(k) that provides all of the information and data required under 21 CFR 807.87 and described in this guidance. A Traditional 510(k) should include all of your methods, data, acceptance criteria, and conclusions. Manufacturers considering modifications to their own cleared devices should consider submitting Special 510(k)s.

The general discussion above applies to any device subject to a special controls guidance document. The following is a specific discussion of how you should apply this special controls guidance document to a premarket notification for a sirolimus (rapamycin) assay.

4. Scope

The scope of this guidance is limited to the following devices:

FDA identifies the generic sirolimus (rapamycin) assays classified under 21 CFR 862.____. The product codes are:

____ Sirolimus (Rapamycin)

____ Sirolimus (Rapamycin) Fluorescence Polarization Immunoassay

____ Sirolimus (Rapamycin) High Performance Liquid Chromatography Assay – Ultraviolet Detection

____ Sirolimus (Rapamycin) High Performance Liquid Chromatography Assay – Mass Spectrometry

____ Sirolimus (Rapamycin) High Performance Liquid Chromatography Assay (Other Detection System)

[4] See Required Elements for a Declaration of Conformity to a Recognized Standard (Screening Checklist for All Premarket Notification [510(K)] Submissions), http://www.fda.gov/cdrh/ode/reqrecstand.html.

5. 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 intrapatient 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.

6. 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-A[5]. 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.

[5] or the most recent approved version of this document

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 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-A[6] 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.

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[2]. 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.

[6] or the most recent approved version of this document

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-demethy-, 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-A[7]. 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 (i.e., 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.

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.

[7] or the most recent approved version of this document

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-A[8] 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 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

[8] or the most recent approved version of this document

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.[9]

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.
- 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.

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

- 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.

7. Labeling

The premarket notification should include labeling in sufficient detail to satisfy the requirements of 21 CFR 807.87(e). The following suggestions are aimed at assisting you in preparing labeling that satisfies the requirements of 21 CFR 807.87(e).[10]

[10] Although final labeling is not required for 510(k) clearance, final labeling must also comply with the requirements of 21 CFR 801 or 21 CFR 809.10 before a medical device is introduced into interstate commerce. In addition, final labeling for prescription medical devices must comply with 21 CFR 801.109. Labeling recommendations in this guidance are consistent with the requirements of part 801 and section 809.10.

Specimens

You should discuss the importance of consistency of time of blood draw with respect to last dose, as well as time of day.

You should discuss any limitations or instructions related to the specimen, such as appropriate matrices or anticoagulants (in most cases, EDTA).

You should provide instructions concerning preserving integrity of the specimen, such as temperatures for collection, transport, storage (short and long term) and procedural steps of the assay necessary to maintain assay performance. Storage conditions recommended to the user should be based on the conditions you have validated for your test system. You should clearly define any acceptance criteria that you apply in determining the recommended storage conditions (e.g., inaccuracies due to instability under these conditions are less than 10% for 95% of samples tested). Additional information on storage conditions based on literature can be cited if they are applicable to your test system.

Assay procedure

You should include appropriate time limits and temperature requirements for the procedural steps. Whenever applicable, you should describe expected appearance of the specimen through various procedural steps and advise users of any signs that may indicate whether the assay is proceeding correctly.

You should advise users how to proceed for samples with concentrations above the highest calibrator. If you instruct users to dilute these samples, you should provide a validated procedure for the dilution.

You should advise users of any steps that can be taken to minimize effect of carryover, or other causes of bias or irreproducibility, based on procedures you have validated for your test system.

Quality control

You should advise users of the specifics of calibration and quality control procedures necessary to ensure the performance claims of the system and include instructions for interpretation of the results of quality control samples, satisfactory limits of performance and instructions on how to proceed if limits of performance are not satisfied. You should include recommendations for appropriate quality control specimens. Consensus documents recommend that whole blood assays should employ whole blood controls with well-characterized drug preparations.

Limitations

You should include the following limitation, when appropriate for your device type.

Patients with abnormal liver function, elevated bilirubin levels, unexpectedly high drug values, or increased time post-therapy may have impaired drug elimination and metabolite

accumulation. For such patients, use of this assay may be supported with a method more specific for the parent compound (e.g., HPLC).

You should identify any exogenous or endogenous factors known to affect results and describe the effect on results (e.g., highly lipemic samples may cause falsely low results).

References listing drugs currently known to alter metabolism of sirolimus (rapamycin) should be cited in an appropriate section of the package insert.

Optimal Concentration Range

Since the optimal concentration ranges may vary depending on the methodology used as well as the clinical state of the individual, stating one specific therapeutic range is usually not appropriate for current sirolimus (rapamycin) assays. You should include cautionary explanations concerning the lack of firm optimal concentration ranges to the user. You should discuss both patient variability and test variability. For example:

> **The optimal concentration range for sirolimus (rapamycin) in whole blood is not well established. The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic effects of sirolimus (rapamycin), co-administration of other immunosuppressants, time post-transplant and a number of other factors contribute to different requirements for optimal blood levels of sirolimus (rapamycin). Therefore, individual sirolimus (rapamycin) values cannot be used as the sole indicator for making changes in treatment regimen and each patient should be thoroughly evaluated clinically before changes in treatment regimens are made. Each user must establish his or her own ranges based on clinical experience.**
>
> **Optimal concentration ranges vary according to the commercial test used, and therefore should be established for each commercial test. Values obtained with different assay methods cannot be used interchangeably due to differences in assay methods and cross-reactivity with metabolites, nor should correction factors be applied. Therefore, consistent use of one assay for individual patients is recommended.**

Performance Characteristics

You should describe the protocol and results for each performance characteristic discussed in Section 6. Protocol descriptions and results in the package insert should include all of the information cited in Section 6, including scatterplots of the new assay versus the reference (e.g., HPLC) method and, in some cases, graphs of inter-individual variation or equivalent information, in order to best represent results of the method comparison for the user. See also applicable sections in the NCCLS guidelines cited in Section 6 concerning statements of claims.

8. New Instrument Applications

For information concerning application of cleared or approved test systems to additional analyzers, see the guidance entitled "Data for Commercialization of Original Equipment Manufacturer, Secondary and Generic Reagents for Automated Analyzers," http://www.fda.gov/cdrh/ode/odecl950.html. The approach described in that guidance is appropriate in cases when performance characteristics on the new analyzer meet pre-determined acceptance criteria specified in a protocol submitted by the manufacturer and reviewed by the FDA. If performance characteristics do not meet pre-determined acceptance criteria, a new 510(k) (which may be an Abbreviated 510(k)) is appropriate.

When the new analyzer is within the same family and does not involve any changes in reagents, sample treatment, or assay procedure that could potentially affect cross-reactivity or partitioning of metabolites, it is sufficient for the method comparison studies in the protocol to include comparison of samples on the new instrument to the previously cleared instrument. In this case, results of the method comparison study of the original test system versus the HPLC reference procedure should still be available to the user in the package insert. In contrast, when application to a new analyzer does include changes in reagents, sample treatment or procedure, a method comparison study including HPLC should be included in the protocol for the add-to and results should be included in the labeling.

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Jean's Weekly Report
8/4/03

DAT online

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- Worked with speakers

This week's plan

- Materials due, nag speakers

Immuno Assay

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This Week's Plan

- Promo, check on color of logo for sponsor

Automation Meeting

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- Did minutes and distributed

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- Begin touching base with potential sponsors

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- Make Gallwas chart of who has done what
- Distrubute book to Gallwas member

Bayer Survey

This week's plan

- Work with Peggy and Ann on design, cost, etc.

BioRad Survey

This week's plan

- Approach with plan to survey our members first and then others

PR

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- AM Summary report
- Straighten out Donna's mess

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