Guidance for Industry and FDA Staff

Class II Special Controls Guidance Document: Sirolimus Test Systems

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Food and Drug Administration
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GRZAN

Preface

Public Comment

Written comments and suggestions may be submitted at any time for Agency consideration to the Division of Dockets Management, Food and Drug Administration, 5630 Fishers Lane, Room 1061, (HFA-305), Rockville, MD, 20852. Alternatively, electronic comments may be submitted to http://www.fda.gov/dockets/ecomments. When submitting comments, please refer to Docket No. 2004D-0412. Comments may not be acted upon by the Agency until the document is next revised or updated.

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This guidance represents the Food and Drug Administration's (FDA'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 FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

1. Introduction

This guidance was developed as a special controls guidance to support the classification of sirolimus (rapamycin) test systems into class II. Sirolimus test systems are intended to quantitatively determine sirolimus concentrations in whole blood as an aid in the management of transplant patients receiving therapy with sirolimus. Many aspects of this document, especially those concerning performance characteristics and risks to health, were developed using information FDA obtained from the Therapeutic Drug Management (TDM) Roundtable. This working group was composed of representatives from laboratory medicine as well as device manufacturers.

This guidance document addresses instrument-based chromatographic assays or immunoassays used in central clinical laboratories. It does not address assays that use other methodologies or point of care assays.

This guidance is issued in conjunction with a Federal Register notice announcing the classification of sirolimus test systems. Any firm submitting a premarket notification (510(k)) for a sirolimus test system will need to address the issues covered in this special controls guidance document. 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.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidance documents describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidance documents means that something is suggested or recommended, but not required.

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 follow 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 document, "A Suggested Approach to Resolving Least Burdensome Issues." It is available on our Center web page at: http://www.fda.gov/cdrh/modact/leastburdensome.html.

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 a sirolimus test system. A manufacturer who intends to market a device of this generic type should (1) conform to the general controls of the Federal Food, Drug, and 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 test systems identified in this guidance, and (3) obtain a substantial equivalence determination from FDA prior to marketing the device.

This guidance document identifies the classification regulation and product code for sirolimus test systems (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 a sirolimus test system 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.

As explained in "**The New 510(k) Paradigm - Alternate Approaches to Demonstrating Substantial Equivalence in Premarket Notifications; Final Guidance**[1]," a manufacturer may submit either a Traditional 510(k) or an Abbreviated 510(k). FDA believes an Abbreviated 510(k) provides the least burdensome means of demonstrating substantial equivalence for a new device, particularly when FDA has issued a guidance document that provides recommendations on what should be addressed in a submission for the device. Alternatively, manufacturers considering modifications to their own cleared devices may lessen the regulatory burden by submitting a Special 510(k).

[1] http://www.fda.gov/cdrh/ode/parad510.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 guidance document was used during the device development and testing and the methods or tests used. The report should also include a summary of the test data or description of the acceptance criteria applied to address the risks identified in this document, as well as any additional risks specific to your device. This section suggests information to fulfill some of the requirements of 21 CFR 807.87 as well as some other items that we recommend you include in an Abbreviated 510(k).

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

We recommend that the summary report contain the following:

- A 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]
- A description of the device design.
- An 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.)
- A discussion of the device characteristics that address the risks identified in this class II guidance document, as well as any additional risks identified in your risk analysis.

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

- A 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.)

- If you choose to rely on a recognized standard for any part of the device design or testing, you may include either: (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] Because a declaration of conformity is based on results from testing, we believe you cannot properly submit a declaration of conformity until you have completed the testing the standard describes. For more information, please refer to section 514(c)(1)(B) of the Act and 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 test system.

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[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).

[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.

4. Scope

The scope of this guidance is limited to the following devices as described in 21 CFR 862.3840 (product code NRP):

21 CFR 862.3840 Sirolimus Test System
A sirolimus test system is a device intended to quantitatively determine sirolimus concentrations in whole blood. Measurements are used as an aid in the management of transplant patients receiving therapy with sirolimus.

5. Risks to Health

There are no known *direct* risks to patient health. However, an indirect risk is that failure of the test to perform as indicated or error in interpretation of results may lead to improper patient management. For example, a falsely low sirolimus measurement could contribute to a decision to raise the dose of sirolimus above that which is necessary for therapeutic benefit. This could result in increased risk in the form of thrombocytopenia, leukopenia, anemia, or hyperlipidemia (Meier-Kriesche, 2000). A falsely high sirolimus 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.

Optimal ranges for sirolimus are expected to depend on the specific assay used because of variations in metabolite cross-reactivity among immunoassays. For example, assay biases ranging from 8-21% and up to 40% have been observed for immunoassays relative to chromatographic methods (Salm, 2000; Jones, 2000). Therefore, use of a sirolimus assay to adjust a treatment regimen without knowledge of performance of the assay used or its specific optimal ranges could lead to improper patient management due to error in interpretation. Optimal ranges also depend on other clinical factors, including patient drug tolerance, immunosuppressive regimen, and time post-transplant. To address these issues, the Therapeutic Drug Management Roundtable has recommended that each institution establish optimal ranges for sirolimus, based on the specific assay used at that institution, and other factors relevant to their patient population. This is similar to the recommendations, for other immunosuppressant drugs, in the National Academy of Clinical Biochemistry's, "Guidelines for Therapeutic Drug Monitoring Services". The manufacturer should also clearly portray performance observed for a new assay relative to a gold standard (e.g., measures of bias, variability, cross-reactivity) in the labeling.

For chromatographic methods, optimal ranges for whole blood trough sirolimus concentrations following kidney transplantation have been suggested as 5-15 ng/ml (Mahalati, 2001) when given in combination with cyclosporine and 12-24 ng/ml following cyclosporine withdrawal (Rapamune® package insert).

In the table below, we have identified the risk to health generally associated with the use of sirolimus test systems. The measures we recommend to mitigate this identified risk are given in this guidance document. 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**
Improper patient management (due to failure of the test to perform as indicated or error in interpretation).	Documented test system performance throughout the measurement range and appropriate labeling. See sections 6 and 7 (performance and labeling).

6. Performance Characteristics

General Study Recommendations

We recommend that you use samples or sample pools derived from patients taking sirolimus in precision and linearity 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. Spiked samples or control or calibrator material may be appropriate to supplement the analytical studies; however, we do not recommend using these types of samples as the only matrix in the evaluations because they may not provide an accurate assessment of the performance characteristics.

We recommend that you perform all of your analytical protocols in accordance with the procedures you plan to recommend to users in the labeling, in order to reflect performance expected by the user. We recommend that you ensure that all steps (e.g., cell lysis, extraction, and centrifugation) are included in the various analytical studies and that manufacturer recommended quality control and calibration procedures are followed. We recognize that evaluations that require freezing of samples (for example, between- run precision studies) may necessitate use of hemolyzed samples.

So that results can be correctly interpreted, you should provide appropriate specifics concerning protocols. For example, when referring to NCCLS evaluation protocols or guidelines, we recommend that you indicate which specific aspects of the guidelines you followed and which you modified.

In studies using spiked samples, we recommend that you provide information about the purity of drugs, metabolites, or potential interferents you used.

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 with matrices other than venous whole blood, it would be necessary to demonstrate a strong correlation between matrices using specimens from patients on drug therapy. We recommend that you contact FDA to discuss your protocol before initiating a study of this type.

Specific Performance Characteristics

Precision

We recommend that you characterize within-run and total precision of your test system. The document "Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline" (1999) NCCLS, Document EP05-A includes appropriate guidelines for experimental design, computations, and format for statement of claims.

We recommend that you evaluate precision for at least three concentrations spanning most of the range of the test system. Typically these concentrations are chosen to represent the (a) sub-therapeutic range or near low end of the reportable range, (b) concentrations considered to be within the therapeutic ranges, and (c) toxic range.

In order to demonstrate robustness of the test system, you should include samples from patients taking sirolimus in your evaluation of total precision at the various concentration levels. If it is not feasible to conduct the entire precision evaluation using patient samples, then we recommend that you supplement precision evaluation of patient samples with spiked whole blood samples, pools, or control material. If patient samples at sub-therapeutic levels or the low end of the assay range are not available, it may be appropriate to dilute patient samples of higher concentration.

In order to validate precision of the entire assay procedure, as it will be performed by the user, you should include evaluation of the effect of pre-treatment steps (such as extraction procedures). Therefore, we recommend performing pre-treatment steps separately for individual replicates in your evaluation of within-run and total precision.

We recommend that you include the following in the description of your precision evaluation, as relevant:

- Sample types (e.g., pooled samples from patients taking sirolimus, spiked whole blood samples or pools, control samples).
- Point estimates of the concentrations evaluated.
- Description of how you evaluated the effect of pre-treatment steps on precision (e.g., by individually extracting the replicate samples).
- Sites at which the precision protocol was run.
- Number of days, runs, and observations.
- Identification of factors that were held constant and those that were varied during the evaluation (e.g., instruments, calibration, reagent lots, and operators).
- Description of your computational methods (including equations, if they were modified from those described in NCCLS EP05-A).

- Results, e.g., coefficients of variation and standard deviations for within-run and total precision at each level.

Recovery

As a measure of accuracy, we recommend that you characterize the percent recovery (bias) of sirolimus with your assay. To address this, we recommend evaluating whole blood samples or pools spiked with known concentrations of sirolimus. You should determine the target concentrations of the samples using a reliable method that is independent of your assay. Gravimetric methods using drug material of defined high purity or well-validated reference chromatography methods are appropriate. We recommend that you evaluate samples with concentrations that span a significant part of the reportable range and include potential medical decision levels. We recommend that you include sufficient replicates at each level so that you can meaningfully evaluate your results. You should assess the effect of any pre-treatment steps on recovery in your assay. To address this, we recommend performing the pre-treatment steps separately for each target concentration.

We recommend that you include the following in the description of your recovery evaluation:

- Sample types (e.g., spiked whole blood) and preparation.
- Target concentrations of the samples and the method by which these were independently determined.
- Description of material used for spiking.
- Description of how you accounted for the effect of pretreatment steps on recovery (e.g., by individually pre-treating samples at the various levels you evaluated).
- Definition or method of calculating recovery, including number of replicates evaluated.
- Results, e.g., recoveries observed.

We recommend that you indicate the range of recoveries or mean and standard deviation for each concentration level when you report results, since this approach may be more informative than describing only average recoveries at each concentration level.

Linearity

You should characterize the linear range of the test system. We recommend serially diluting positive whole blood samples or pools from patients taking sirolimus with sirolimus-free whole blood, to generate samples evenly distributed across the entire assay range. We recommend that you evaluate a minimum of 5 sirolimus-positive levels within the claimed linear range and include multiple samples at each level. In order to evaluate whether there is any effect of pre-treatment steps on assay linearity, we recommend that you perform the pre-treatment steps separately for samples at each of the various target levels.

In cases where samples from patients taking sirolimus are not available for the high end of the assay range, we recommend that you evaluate linearity by dilution of patient samples at the highest concentration that is available. In such cases, if evaluation of patient samples does not span the assay range, results can be supplemented with data from spiked samples.

The document "Evaluation of the Linearity of Quantitative Analytical Methods; Approved Guideline" (2003) NCCLS Document EP06-A describes a protocol for sample preparation, value assignment, and a format for statement of claims, as well as one approach to statistical design and analysis methods for evaluation of the linear range of an assay.

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.

We recommend that you include the following in the description of your linearity evaluation:

- Sample types (e.g., whole blood samples from patients taking sirolimus) and preparation.
- Target concentrations and the methods or calculations you used to determine these concentrations.
- Description of how you evaluated the effect of pre-treatment steps on linearity (e.g., by individually pre-treating samples at each of the levels evaluated).
- Number of samples and replicates evaluated.
- Statistical methods you used to evaluate linearity.
- Results.

We recommend that results include a table of the target concentration versus the observed concentrations, in addition to the assay range of linearity. We also recommend that you include the acceptable maximum differences from linearity, if you used the approach described in NCCLS EP06-A. If applicable, you should also include data from your high-dose hook evaluation.

If you recommend to users that they should dilute samples that are above the reportable range, you should provide a specific protocol for dilution and include your results for validation of that protocol. The validation description should include the concentration range tested and the recoveries observed.

Sensitivity

You should evaluate bias and precision at the claimed sensitivity level of the test system and demonstrate that results meet your acceptance criteria. Therefore, we recommend that you

include samples at your claimed sensitivity level within the evaluations of recovery and total precision described above.

It may also be appropriate to report the limit of quantitation (LoQ). We define this as the lowest drug concentration that can be reliably detected and for which assay bias and precision meet your stated acceptance criteria. The document "Protocols for Determination of Limits of Detection and Limits of Quantitation; Proposed Guideline" (2004) NCCLS Document EP17-P describes a method for establishing the limit of quantitation. This document proposes a minimum of 40 replicates, from 3-5 different samples and determined from 5 or more runs. In order to assess whether your acceptance criteria for precision and accuracy at the sensitivity level can be met over time, we recommend that you perform multiple runs on separate days (preferably, non-consecutive days). If possible, we recommend use of multiple instruments to capture variability. Bias can be estimated by comparing the average concentration based on your assay to the value that you determined based on reference material or a reference method independent of your assay (and specific for sirolimus parent compound). Precision can be estimated by the total standard deviation of the samples evaluated with your assay.

You should include evaluation of the effect that pre-treatment steps might have on bias and precision at the assay sensitivity level. In order to address this, we recommend performing the pre-treatment steps separately for each of the individual replicates and levels in your evaluations, to simulate conditions for patient samples.

In the description of your evaluation of the limit of quantitation, we recommend that you include the (bulleted) points listed in the precision and recovery sections above, as applicable. You should state your acceptance criteria for bias and precision at the assay sensitivity level and provide results to demonstrate these criteria were met.

In some cases, you may find it useful to provide additional measures of sensitivity, such as the limit of blank or limit of detection (for proposed definitions, see NCCLS EP-17P).

Specificity for parent compound

As a measure of assay specificity, you should characterize cross-reactivity with sirolimus metabolites. Primary known metabolites appropriate for sirolimus specificity studies 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). We recommend that you spike sirolimus-free whole blood with the metabolites to a final concentration consistent with the highest concentration expected for the intended use population. When metabolites are not available, you may be able to estimate the effect of specific metabolites by measuring the metabolites present in multiple patient specimens using an appropriate chromatographic method and comparing results to your assay. We recommend that you include specimens from patients with elevated creatinine concentration when available because such patients typically show higher than average metabolite concentrations. We recommend that you consult with FDA prior to undertaking this alternative type of study.

We recommend that you include the following in the description of your evaluation:

- Types of samples used for spiking.

- Concentration and purity of metabolite (and parent compound, if present) in samples.
- Computational methods for determining cross-reactivity, including number of replicates evaluated.
- Results (e.g., percent cross-reactivity) for each metabolite.

Interference

You should characterize the effects of potentially interfering compounds on assay performance. We recommend that you test the compounds listed below. If other potentially interfering compounds become known during widespread use of the assay, you should test these as well.

(1) endogenous compounds, such as the following (examples of upper limit concentrations are 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 drugs listed below. If other relevant drugs become known, you should also evaluate these.

- 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.

Interference studies typically involve adjusting sirolimus concentrations in whole blood samples to near high and low medical decision levels, adding the potential interferent to these samples and determining any bias in recovery relative to control sample(s). Interference studies using samples naturally high in the endogenous compound being tested can be informative and we recommend that you consider this approach when such samples are available. Guidelines for interference testing are described in detail in "Interference Testing in Clinical Chemistry; Approved Guideline" (2002) NCCLS Document EP07-A. That document includes guidelines for setting decision criteria as well as for protocol designs and statistical methods for evaluating interference and establishing validating and verifying interference claims. We recommend that you consider the following guidelines from that document when planning interference studies.

- For endogenous substances, test at the highest concentration expected based on experience with the intended use population.
- 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. We recommend that you test replicate samples in these protocols.

We recommend that you include the following in the description of your evaluation:

- Specific compounds and concentrations tested for interference.
- Sample types and preparation (e.g., spiked whole blood pools, samples naturally high in endogenous compounds).
- Concentrations of sirolimus in the sample.
- Percent bias (relative to a control sample) for the compounds tested, and the definition/calculations you used to determine this.

When reporting results, we recommend that you identify any observed trends in bias (i.e., negative or positive) across the concentration range of the compound tested for interference. We recommend that you include the standard deviation or range of the observed recoveries at the interferent concentrations you evaluate. This approach may be more informative than listing only average recoveries.

For substances that you characterize as non-interfering in your labeling, you should state the criteria on which this is based, e.g., "inaccuracies due to these substances are less than X% at the sirolimus concentrations tested." If any compounds are known from the literature or other sources to interfere with the test system, you should also include this information.

Specimen collection and handling conditions

You should substantiate the recommendations in your package insert 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, that you recommend to users. We recommend that you evaluate 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 your recommended storage and handling conditions. You should also identify any other sources of pre-analytical error, such as binding to a specimen container or gel.

Method comparison

Sirolimus 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. We recommend that you compare your assay to a carefully validated high performance liquid chromatography method that measures parent drug specifically, such as methods described as reference procedures (e.g., Salm, 2000; Streit, 2002).

We recommend that you follow the guidelines provided in the document, "Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline" (2002), NCCLS Document EP09-A2, concerning experimental guidelines and statement of claims. Sirolimus (Rapamune®) is currently indicated for the prophylaxis of organ rejection in patients receiving renal transplants. Therefore, you should evaluate renal transplant patient samples with drug concentrations distributed across the reportable range of the assay. (If, in the future, the drug and assay are indicated for additional transplant populations, these should

also be included in the evaluation.) Banked (retrospective) samples are appropriate for these studies provided that appropriate information concerning sample characterization (listed below) is available. We recommend including samples from multiple geographic sites or clinical centers to enhance the chance that samples will represent a broad range of individuals and treatment regimens.

Appropriate sample size depends on factors such as precision, interference, assay range, and other performance characteristics of the test. The number of patients should also be large enough so that inter-individual variation can be observed. We recommend that you provide a statistical justification to support the study sample size. We recommend that the sample size target, however supported, include 100 or more samples distributed fairly evenly over 50 or more individual patients.

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

For your results to be properly interpreted, you should provide relevant information on the samples tested. We recommend that you include the following information:

- The number of individual patients represented by the samples.
- The number of data points.
- The number of clinical sites.
- Characterization regarding the time of last dose, e.g., trough samples. (We currently consider evaluation of trough samples sufficient, as long as the sample concentrations span the assay range.)
- Selection (inclusion/exclusion) criteria for samples.
- Other known sample characteristics relevant to interpretation of results.

Factors such as other co-administered immunosuppressant drugs (e.g., cyclosporine), age range (e.g., adults), and time post-transplant (e.g., chronic, acute) can affect drug metabolism and consequently, assay bias (Gallant-Haidner, 2000; Lampen, 1998; Kaplan, 1998; Kelly, 2002). Therefore, we recommend that you describe these features of the general sample population whenever possible. You should also indicate if samples were collected from patients with specific clinical outcomes, or from centers using atypical or novel drug regimens.

You should clarify the comparator reference method used, and include a summary of the validation of that method and references from the literature describing the method.

We recommend that you include the following in the results of your method comparison:

- Methods you used to statistically analyze the data.

- Scatterplots of the new assay versus the reference (e.g., LC-MS) method, including all data points, the estimated regression line, and the line of identity. Data points in the plot should represent individual measurements (unless you are instructing users to report an average of multiple 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. If both the comparator and the new assay are subject to measurement error, a regression method such as the Deming method may be appropriate, rather than Least Squares.

- Graphs of difference in measurements (i.e., new device minus reference method) versus the reference method, to illustrate variability. Appropriate representations could include a bias plot of difference in measurements (y - x) versus the reference method (x), as recommended in NCCLS Document EP9-A , or versus the mean of y and x, as recommended by Bland and Altman (Bland, 1995).

We recommend that you stratify analyses for samples representing different patient groups for whom differences in assay bias might be expected, if you included such samples in your study. Some examples of such groups include samples drawn at different time points with respect to dose (e.g., trough samples versus other time points) or samples representing patients at various times post-transplant (e.g., acute or chronic).

If the bias in your method comparison exceeds 25% relative to the reference procedure, or if the variability in results among patient samples is unusually large, you should address the reasons for the discordance and describe steps to be taken to minimize risk of patient mismanagement that is based on the results of such tests.

Studies at external sites

You should demonstrate performance (bias and precision) in at least two external sites, in addition to that of the manufacturer's site. We recommend that you include this as part of the method comparison study described above. You should initially analyze data from individual sites 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.

Calibrator and Control Material

We recommend that you provide the following information concerning assay calibrators and controls:

- Protocol description and acceptance criteria for real-time stability studies of opened and unopened calibrators and controls. This should include the methods or analyses you used and your criteria for recovery at the expiration date.

- Protocol description for value assignment and validation of the various calibrator and control levels. This should include the methods or analyses used and your results or acceptance criteria for recovery.

- 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).[5] The following suggestions are aimed at assisting you in preparing labeling that satisfies these requirements.

Specimens

We recommend that you include the following:

- Discussion of the importance of consistency and accurate recording of time of blood draw with respect to the last dose of sirolimus, if relevant for interpretation of results.

- Discussion of any limitations or instructions related to the specimen type, such as appropriate matrices or anticoagulants (in most cases, EDTA).

- Instructions concerning preserving integrity of the specimen, such as required temperatures or materials for collection, transport, storage (short and long term), and assay procedural steps. Storage conditions that you recommend 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. Additional information on storage conditions based on literature can be cited if it is applicable to your test system.

Assay procedure

We recommend that you include the following:

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

- 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.

- A validated procedure for dilution, if you instruct users to dilute samples with values above the highest calibrator.

- Steps that users can take to minimize the effect of carryover, or other causes of bias or irreproducibility, based on procedures you have validated for your test system.

Quality control

We recommend that you advise users of the specifics of calibration and quality control procedures necessary to ensure performance claims. 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

We recommend that you include limitations such as the following, when appropriate for your device type:

> Various immunoassays may yield results that differ from each other and from chromatographic assays on the same clinical sample. Therefore, it is important that the same analytical method be used consistently for monitoring immunosuppressant concentrations for an individual patient. Laboratories should identify the method used, when reporting results.
>
> Patients with impaired drug metabolism or clearance may show the most variation in measured values for immunoassays. For such patients, use of this assay may be supported with a chromatographic method more specific for the parent compound.
>
> Clinical trials have shown large intrapatient variability observed in trough sirolimus concentrations (MacDonald, 2000), indicating that optimal dose adjustment should be based on more than a single trough sample.

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).

We recommend that you cite references that list drugs currently known to alter metabolism of sirolimus and to affect blood concentrations of the parent or metabolites 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 immunoassays**.** You should include cautionary

explanations concerning the lack of firm optimal concentration ranges to the user and discuss both patient variability and test variability. For example:

> **The optimal concentration range for sirolimus in whole blood using this assay has not been established. Optimal concentration ranges vary according to the specific assay used, and therefore should be established for each specific assay. Values obtained with different assay methods should not be used interchangeably due to differences in cross-reactivity with metabolites, nor should correction factors be applied. Laboratories should include identification of the assay used in order to aid in interpretation of results. Each institution should establish the optimal ranges based on the specific assay used and other factors relevant to their patient population.**
>
> **Optimal ranges depend upon the patient's clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic effects of sirolimus, co-administration of other immunosuppressants, time post-transplant and a number of other factors. Therefore, individual sirolimus 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.**

Performance Characteristics

We recommend that you describe the protocol and results for each performance characteristic discussed in Section 6. Your representation of protocol and results in the package insert should include information cited in Section 6 that would be relevant to aid the user in understanding test performance. Results should include scatterplots of the new assay versus the reference (e.g., LCMS) method. In some cases, graphs or tables of inter-individual variation or equivalent information may also be appropriate in order to clearly 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 test systems to additional analyzers, see the guidance entitled "Guidance for Industry and FDA Staff; Replacement Reagent and Instrument Family Policy," available at http://www.fda.gov/cdrh/oivd/guidance/950.html. The approach described in that guidance is appropriate in cases when performance characteristics with a new analyzer meet your pre-defined acceptance criteria using a proper validation protocol. If performance characteristics do not meet your pre-determined acceptance criteria, a Special 510(k) is appropriate.

When the new analyzer does not involve any changes in reagents, sample treatment, or assay procedure that could affect cross-reactivity or partitioning of metabolites, you might determine that it is sufficient to compare samples using the new instrument to the previously cleared instrument. In this case, we recommend that you still include results of the original method comparison for the test system versus the LCMS reference procedure in the package insert, so that users can properly interpret results. When application to a new analyzer also includes

changes that could affect cross-reactivity, we recommend that you compare the new assay to a reference method, in order to validate that performance is not affected.

9. References

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