Guidance for Industry and FDA Staff

Class II Special Controls Guidance Document: Cardiac Allograft Gene Expression Profiling Test Systems

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Food and Drug Administration
Center for Devices and Radiological Health

Office of In Vitro Diagnostic Device Evaluation and Safety
Division of Chemistry and Toxicology Devices

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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.regulations.gov. Please identify your comments with the docket number listed in the notice of availability that publishes in the *Federal Register* announcing the availability of this guidance document. Comments may not be acted upon by the Agency until the document is next revised or updated.

Additional Copies

Additional copies are available from the Internet at: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/default.htm. You may also send an e-mail request to dsmica@fda.hhs.gov to receive an electronic copy of the guidance or send a FAX request to 301-847-8149 to receive a hard copy. Please use the document number 1686 to identify the guidance you are requesting.

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Guidance for Industry and FDA Staff

Class II Special Controls Guidance Document: Cardiac Allograft Gene Expression Profiling Test Systems

1. Introduction

This guidance document was developed as a special controls guidance to support the classification of cardiac allograft gene expression profiling test systems into class II (special controls). A cardiac allograft gene expression profiling test system is a device that measures the RNA expression level of multiple genes and combine this information to yield a signature (pattern, classifier, index, score) to aid in the identification of a low probability of acute cellular rejection (ACR) in heart transplant recipients with stable allograft function.

This guidance provides recommendations to manufacturers regarding preparation of premarket notifications and labeling for cardiac allograft gene expression profiling test systems. The recommendations in this document are applicable to RNA expression assays such as real time polymerase chain reaction (qRT-PCR) and gene expression microarrays to be used as an aid in the identification of a low probability of acute cellular rejection in heart transplant recipients with stable allograft function. In cardiac allograft gene expression profiling test systems, an algorithm is applied to such measurements to yield a result that can be used by physicians to aid in the management of stable cardiac allograft recipients.

A cardiac allograft gene expression profiling test system is not intended for diagnosis of ACR, or to predict or detect response to therapy, or to select the optimal therapy for cardiac allograft recipients.

This guidance is issued in conjunction with a Federal Register notice announcing the classification of cardiac allograft gene expression profiling test systems. Any firm submitting a 510(k) premarket notification for a cardiac allograft gene expression profiling test system will need to address the issues covered in this special controls guidance. The firm must show that its device addresses the issues of safety and effectiveness identified in this guidance, either by meeting the recommendations of this guidance or by some other means that provides equivalent assurances of safety and effectiveness.

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 guidance and 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/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm073704.pdf.

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2. Background – Premarket Notifications

A manufacturer who intends to market a device of this generic type must

- 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,
- conform to the special control developed for this device, by addressing the specific risks to health associated with the cardiac allograft gene expression profiling test systems identified in this guidance, and
- obtain a substantial equivalence determination from FDA prior to marketing the device. (See also 21 CFR 807.81 and 807.87).

FDA believes that special controls, when combined with the general controls of the act, are sufficient to provide reasonable assurance of the safety and effectiveness of these devices.

This special control guidance document identifies the classification regulation and product codes for the cardiac allograft gene expression profiling test system (please refer to Section 3. Scope). Other sections of this guidance document provide recommendations to manufacturers on addressing risks related to these devices.

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 the guidance entitled **Format for Traditional and Abbreviated 510(k)s**[1]. As described in **The New 510(k) Paradigm - Alternate Approaches to Demonstrating Substantial Equivalence in Premarket Notifications; Final Guidance**,[2] a manufacturer may submit a Traditional 510(k), an Abbreviated 510(k), or a Special 510(k). A manufacturer may choose to submit an Abbreviated 510(k) when a guidance document exists, when special controls have been established, or when FDA has recognized a relevant consensus standard for the device. Manufacturers considering certain modifications to their own cleared devices may submit a Special 510(k).

[1] http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm084365.htm
[2] http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm080187.htm

3. Scope

The scope of this document is limited to the following devices described in 21 CFR 862.1163 (product code OJC).

21 CFR 862.1163 – A cardiac allograft gene expression profiling test system is a device that measures the RNA expression level of multiple genes and combines this information to yield a signature (pattern, classifier, index, score) to aid in the identification of a low probability of acute cellular rejection (ACR) in heart transplant recipients with stable allograft function.

A cardiac allograft gene expression profiling test system may require instrumentation for clinical multiplex test systems. Instrumentation for clinical multiplex test systems is regulated under 21 CFR 862.2570. Guidance for such instrumentation is available in the FDA Guidance for Industry and FDA Staff, **Class II Special Controls Guidance Document: Instrumentation for Clinical Multiplex Test Systems**.[3] If your cardiac allograft gene expression profiling test system includes instrumentation for clinical multiplex test systems for that assay, you may submit the information for both the assay and the instrumentation within one 510(k). If instrument manufacturers prefer to submit a 510(k) for instrumentation only, they may submit it in conjunction with the assay premarket notification.

A cardiac allograft gene expression profiling test system is not intended for diagnosis of ACR, or to predict or detect response to therapy, or to select the optimal therapy for cardiac allograft recipients.

4. Risks to Health

A cardiac allograft gene expression profiling test system is intended to aid in the identification of a low probability of acute cellular rejection in heart transplant recipients with stable allograft function. Failure of this device to perform as indicated may lead to erroneous test results. False positive results will misclassify the patient into a higher risk group and false negative results will misclassify the patient into a lower risk group. Misclassification of ACR may lead to incorrect patient management with attendant psychological distress, inaccurate counseling, and suboptimal patient care.

In the table below, FDA has identified the risks to health generally associated with the use of this device. The measures recommended to mitigate the identified risks are described in this guidance document, as shown in the table below. You should conduct a risk analysis, prior to submitting your premarket notification, to identify any other risks specific to your device. Risks may vary depending on the type of expression assays used, the intended use of the test, the sample type, and how the result will be used. The premarket notification should describe the risk analysis method. If you elect to use an alternative approach to address the risks identified in this document, or have identified risks additional to those in this document, you should provide sufficient detail to support the approach you have used to address that risk.

[3] http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm077819.htm

Identified risk	Recommended mitigation measures
Failure of the test to perform properly, for example, results are inaccurate or absent due to failure of reagents, instrumentation, data management, or software, which may lead to false positive results, or false negative results, and incorrect patient management (e.g., false classification as low probability of ACR may delay appropriate clinical intervention).	Sections 6-7
Failure to properly interpret test results.	Sections 5 (see **Test Results**) and 8

5. Device Description

We recommend that you identify your device by regulation and product code described in Section 3, above. You must identify a legally marketed predicate device. 21 CFR 807.87(f). In order to help FDA efficiently review all the aspects of your device compared with the predicate, you should include a table that outlines the similarities and differences between the predicate and your device.

Key issues in the review of a new device are the specific intended use, the type of specimens tested, and the technology utilized. You may submit appropriate peer-reviewed literature references relevant to the technology of the device, in addition to the descriptive information below, to adequately describe the new device.

You should include the following descriptive information to adequately characterize your cardiac allograft gene expression profiling test system.

5.A. Intended Use

The intended use should specify what the test measures, the clinical indications for which the test is to be used, and the specific population for which the test is intended. It should include applicable clinical and demographic description of patients (e.g., age, allograft function stability, time post transplant) for whom clinical performance has been demonstrated. The intended use should specify whether the test is qualitative or quantitative. If the test is intended for use at a single laboratory site, this information should be included in the intended use.

5.B. Test Methodology

You should describe in detail the methodology used by your device. For example, you should describe the following elements, as applicable to your device:

- Test platform (e.g., qRT-PCR or expression arrays).
- Composition and spatial layout of arrays or other spatially fixed platforms as applicable.
- Description of the assay elements, particularly with respect to parameters such as the genes used for normalization and quality control.

- How you evaluated the potential for sample carryover or contamination.
- Limiting factors of the assay (e.g., saturation level of hybridization, maximum cycle number).
- For arrays:
    - Methods used in attaching the probe material to a solid surface.
    - Hybridization conditions, washing procedures, and drying conditions (e.g., temperature, length of time).
- Specificity of probes for the sequence of interest, especially when pseudogenes or sequence-related genes exist.
- Sample collection requirements.
- Handling methods from the time the sample is collected until the processing of the sample.
- Methods for RNA extraction that you perform, provide, or recommend to users.
- Methods for ensuring RNA integrity in sample extracts.
- Reagent components provided or recommended for use, and their function within the system (e.g., buffers, enzymes, fluorescent dyes, chemiluminescent reagents, other signaling/amplification reagents).
- Instrumentation required for your device, including the components and their function within the system.
- Types of output generated by the instrumentation and system parameters (e.g., measurement ranges).
- The computational path from raw data to the final result (e.g., how raw signals are converted into a final test result). This would include sufficient software controls for identifying and dealing with missing values and obvious problems in the dataset. Describe adjustment for background for normalization.
- External controls that you recommend or provide to users.
- Internal controls and a description of their specific function in the system.
- Related peer-reviewed literature references describing the test methodology if applicable.
- Illustrations or photographs of non-standard equipment or methods if available.

Where applicable for your device, you should describe the quality control design specifications used to address the following concerns:

- Correct placement and identity of assay features (e.g., probes) as applicable.

- For multiplexed tests in which the target molecules will contact a number of different probes, the potential for specific and non-specific probe cross-hybridization.
- Prevention of probe cross-contamination, for multiplexed tests in which many probes are handled during the manufacturing process.

5.C. Test Algorithms

The algorithms that are used in these types of test systems to determine the probability of acute cellular rejection may often be novel, proprietary, and complex and are among the most critical elements of the test system. You should provide the following, when applicable:

- A detailed description of the algorithm architecture and implementation.
- A detailed description of the datasets that were used to discover and validate the patterns or classifiers that are used in your test (often referred to as "training" and independent "test" sets, respectively), including the principles used to select the samples from which the data were derived (such as clinical history, demography, matrix, geographical origin), the statistical justification for sample size, and any assumptions you made when assembling the datasets. Patients with specimens in the training set would not be appropriate participants for the test set.
- A detailed description of performance measures (internal validation and external validation using an independent clinical dataset) and how they were obtained.

In some cases the device and the algorithm evolve over time during product development. You should provide the data obtained using the final device and final algorithm for your device.

5.D. Test Results

You should provide examples of the test reports (e.g., printouts) that are generated for the clinician. These reports should contain adequate information to allow interpretation by the ordering physician or other healthcare professional. The test report should reference the performance of the test in the clinical validation dataset. The statistical summaries included in the test report should be consistent with the way the test should be utilized. For example, probability of ACR could be quantified by negative predictive value and complemented with positive predictive value. The report may contain other descriptive information (e.g., sensitivity and specificity of the test), when applicable, as calculated using the clinical validation data set.

6. Performance Characteristics

In your 510(k), you should detail the study design you used to evaluate each of the performance characteristics outlined below.

6.A. Preanalytical Factors

Consideration of pre-analytical factors, such as the following, is critical for high-quality genomic tests.

Specimen Collection

You should evaluate all sample collection, transport, and storage options you recommend (e.g., blood collection tube types, RNA preserving fixatives, frozen samples) using specimens that are handled in the same manner as will be recommended in the test label (e.g., collection, storage, shipment methods). This includes validating that the allowable elapsed time between sample collection and stabilization of RNA (e.g., by freezing, fixation or other methods) results in uniformly acceptable specimens. You should validate that the transport conditions you specify are adequate to ensure sample integrity, and determine the limits of transport variability that are acceptable (e.g., time in transit, quantity of coolant required).

Your validation of appropriate storage conditions should include both the sample and the extracted RNA product.

RNA Extraction

If you intend to provide reagents in your test kit for extraction and preparation of RNA, you should validate each step in the preanalytical process for its effects on reproducibility, accuracy, and stability of product and describe your study design and results in the 510(k) submission. Your external site studies (e.g., reproducibility, method comparison) should include evaluation of preanalytical processes.

If you do not intend to provide reagents in your test kit for RNA extraction and preparation, you should provide adequate specifications to ensure RNA quality sufficient to generate correct test results. Examples for specifications include OD_{260}/OD_{280} ratio, ribosomal RNA ratio (28S/18S), and measurement of RNA integrity. You should not recommend any research-use-only (RUO) reagents.

6.B. Quality Control

Several levels of quality controls should be considered for cardiac allograft gene expression profiling test system devices of this type. Controls should provide information about 1) sample quality, 2) RNA quality, quantity and purity, and 3) process quality. The process quality controls should reflect the whole process, including but not limited to, RNA extraction, RNA purification, cDNA synthesis, amplification, hybridization, scanning/detection, and normalization (as applicable).

Controls should approximate the composition and RNA concentration of a sample in order to adequately challenge the system, as well as address reproducibility across the measurement range, especially near clinically relevant values.

You should describe the following concerning quality control and calibration:

- The nature and function of the various controls that you include with, or recommend for, your system. These controls should enable the user to determine if all steps and critical reactions have proceeded properly without contamination or cross-hybridization.
- Your methods for value assignment (relative or absolute) and validation of control and calibrator material, if applicable.
- The control parameters that could be used to detect failure of the instrumentation to meet required specifications.

6.C. Analytical Performance

All analytical performance studies should be conducted using the final version of your device rather than a prototype. You should evaluate performance of your assay, including RNA extraction, from all the sources of RNA that you recommend for your assay (e.g., whole blood, tissue, peripheral blood mononuclear cell lysate). We recommend that you describe the following performance characteristics:

Specimen Requirements

You should validate that the specimen requirements you specify are sufficient to identify the diagnostic patterns or classifiers of your test within your stated accuracy and precision criteria. You should determine:

- The minimum amount of sample required to perform an acceptable assay with your device.
- The lower and upper limit of the assay, in terms of RNA/cDNA concentration, for which the device can give reliable results with a given accuracy and precision.

For assays using a complex algorithm to generate a signature (pattern or classifier or index), the upper and lower limits of RNA concentration should not compromise the assay outcome as indicated by precision measures.

Analytical Specificity/Interference

Where applicable, you should evaluate potential for non-specific amplification, non-specific hybridization, and cross-hybridization of your device.

Potential interfering substances may exist in the specimen and may be introduced during specimen collection (e.g., lipemia, hemolysis, heparinization of the sample) and sample preparation. Therefore, your RNA specifications should be adequate to exclude the presence of any effect from likely interfering substances.

Cut-off

In your submission, you should explain how the cut-off was determined and how this cut-off value was validated. The cut-off utilized in the validation study should be established prior to validation. We recommend that you choose a cut-off that provides an acceptable risk/benefit trade-off between false and true negative test results (e.g., high/low probability of ACR). You should use appropriate statistical methods to validate the cut-off for your intended use. If the assay has an equivocal zone, you should explain how you determined the limits of the equivocal zone. The performance of your device using the established cut-off (and equivocal zone, if applicable) should be validated in an independent population consistent with the defined intended use of your device.

Precision (Repeatability/Reproducibility)

You should provide data demonstrating the precision (i.e., repeatability/reproducibility) of your system. The CLSI documents, "Evaluation of Precision Performance of Clinical Chemistry Devices" (CLSI Guideline EP5-A) and "User Protocol for Evaluation of Qualitative Test Performance" (CLSI Guideline EP-12A), include guidelines that may be

helpful for developing experimental design, computations, and a format for establishing performance claims. Ideally, you should identify all sources of assay variability in the precision study. You should establish that your classifier or score is sufficiently reproducible across the range of clinical specimens likely to be encountered in your intended use population. Additional factors influencing precision that you should consider include the following:

- Ensuring that samples used in reproducibility testing are processed from clinical specimens (e.g., PBMC lysates obtained from whole blood) at the test site, using the procedure you plan to recommend in the test labeling.
- If the assay is intended to be performed in more than one laboratory, including three or more sites with multiple operators at each site. Operators should reflect potential users of the assay in terms of education and experience. You should provide training only to the same extent that you intend to train users after marketing the test.
- If the assay is intended to be performed in a single laboratory, including multiple operators from that laboratory.
- Including multiple product lots (e.g., multiple lots of reagents, multiple lots of primers and probes for RT-PCR, multiple lots of arrays), and multiple instruments.
- Using appropriate test samples representing every class that the test can detect (e.g., high probability, low probability, and equivocal zone if applicable).
- If applicable, performing dye-reverse experiments to ensure that there is no bias in dye incorporation.
- If applicable, demonstrating reproducibility of sample labeling procedures.

In the study design description in your 510(k), you should identify which factors (e.g., instrument calibration, reagent lots, and operators) were held constant and which were varied during the evaluation, and describe the computations and statistical analyses used to evaluate the data. If there are external control materials for your assay, you should include such materials in your precision study in addition to real clinical specimens.

Stability Studies

You should describe your study design for determining the real-time stability of the reagents and instruments, and if applicable, for accelerated stability and stress test conditions and results. For each study, you should describe how you selected the acceptance criteria values.

Validation of Instrumentation

For instruments and systems that measure and sort multiple signals, and other complex laboratory instrumentation that has not been previously cleared, refer to the guidance document **Class II Special Controls Guidance Document: Instrumentation for Clinical Multiplex Test Systems**[3], for details on the types of data you should provide to support instrument clearance.

6.D. Clinical Validation

You should provide data from clinical studies to support the indications for use and claims for your device. The clinical validation study should use patient samples that are derived from the intended use population and that are independent of the specimens you used to develop the signature (pattern or classifier or index). You should describe the protocol of each clinical study (including the inclusion and exclusion criteria, study endpoints, acceptance criteria, study design, statistical analysis plan, and statistical justification of the sample size), and a description of how the studies support the proposed intended use. You should submit the raw data along with the processed data (i.e., final test results) from your clinical validation studies.

For the clinical validation study, the validation dataset should consist of clinical samples collected from at least three different clinical sites in different geographical locations. Preferably, studies would be conducted within the U.S. population. If the studies are conducted outside the U.S., you should document the relevance of your studies to U.S. clinical practice and demographics.

If the clinical validity of your specific device is supported by an established scientific framework and a sufficient body of evidence, then you may submit peer-reviewed references to support your claim. These should include multiple studies that test appropriate populations. In cases where the literature does not sufficiently support your indications for use, you should conduct studies to support claims for your device. Retrospective analysis of prospectively collected banked samples may be acceptable if appropriate measures are taken to identify and either remove or mitigate substantially any biases in the study set. We recommend that you discuss with FDA your specific proposed study to determine whether it is adequate.

Accuracy Using Comparison to Clinical Outcome:

Clinical Truth: In order to allow FDA to assess the performance of your device, you should define the measure of clinical outcome used for all patients in the clinical validation study and the method by which the measure was obtained.

End Points: You should describe the appropriate clinical endpoints (e.g., biopsy score, absence or presence of ACR) for your device. These endpoints should support the intended use of the device. Appropriate performance metrics may include, for example, (1) the negative predictive value of ACR, (2) the positive predictive value of ACR, (3) the pre-test prevalence of ACR, and (4) the area under the receiver operating characteristic (ROC) curve (AUC).

Validation Strategy: You should describe the method used to validate the gene signature. The description should include a clinical protocol and a statistical analysis plan. The clinical data should be a new data set that has not been derived from patients previously used in the development of the gene signature. The patients in the data set should also be representative of the intended use population for the device. The statistical technique used to analyze data from the validation study should be tailored to the pre-defined end points of interest in the study (e.g. identification of a low probability of ACR). Prior to conducting a validation study, the protocol should include clinically relevant performance goals and a detailed statistical analysis plan for demonstrating that the goals have been met. Please be aware that statistical methods rely on assumptions that should be checked for reasonableness prior to designing your validation study. For example, if multiple specimens from the same patient are used, then the statistical analysis should not assume that the test results on the specimens

are statistically independent. Instead, the data should be analyzed in a manner consistent with this repeated measures design.

In your 510(k) submission, you should provide summaries of this clinical validation study, including descriptive statistics for patients within the study as well as estimates of probabilities of incorrect results (i.e., false positives, false negatives) associated with your end point. You should report a 95% confidence interval for each statistical performance metric you report. For negative and positive predictive values, the performance will be impacted by the prevalence of ACR in the clinical validation study. Therefore, you should report the prevalence of the target endpoint.

When applicable, you should report statistical performance of the test within clinical risk strata (e.g., age, allograft function stability, time post transplant, baseline risk factors for ACR, and inflammatory markers if routinely used) to demonstrate that your test adds value over clinical variables routinely used in the assessment of whether ACR has occurred. Alternatively, the performance of the test may be compared head-to-head with the performance of the best classifier of ACR status based solely on the clinical variables. (A third approach that may or may not be appropriate is to show a statistically significant increase in performance when the test is added to predefined clinical predictors in a statistical regression model of ACR status.)

The clinical information appropriate for consideration may vary with the study group of interest. We recommend that you discuss with FDA your specific proposed study prior to conducting your study.

Study Samples

While prospective samples are preferred, well-characterized samples from banks can be used in your clinical validation study, provided that there is no collection or selection bias, and patient history and appropriate outcome information are available.[4] You should fully describe selection (inclusion/exclusion) criteria and characterize any relevant features or limitations of the samples (whether prospective or from banks). You should describe patient demographics and disease characteristics and the prevalence of relevant outcomes in the intended use and study populations. You should select samples in a way that minimizes the sources of bias such as sample integrity and storage duration. We recommend you consult with FDA prior to performing pivotal studies using banked samples.

You should use clinical samples from all matrices you claim in your intended use (e.g., frozen or collected in any nucleic acid preservative) to demonstrate that correct results can be obtained from clinical material. Appropriate sample size depends on factors such as precision/reproducibility, interference, and other performance characteristics of the test. We recommend that you provide a justification using statistical methods to support your study sample size. For samples you use in your clinical studies, you should provide data demonstrating that storage and transport of retrospectively examined samples have not affected assay results.

[4] See the guidance document Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable, http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm078384.htm

7. Software

If your system includes software, you should provide information detailed in accordance with the level of concern. (See: **Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices.**[5]) You should determine the level of concern prior to the mitigation of hazards. In vitro diagnostic devices of this type are typically considered a moderate level of concern because software flaws could indirectly affect the patient and potentially result in injury because the healthcare provider and patient do not get accurate information.

You should include the following points, as appropriate, in preparing software documentation for FDA review:

- Full description of the software design. Your software should not include utilities that are specifically designed to support uses beyond those in your intended use. You should also consider privacy and security issues in your design. Information about some of these issues may be found at the following website regarding the Health Insurance Portability and Accountability Act (HIPAA) http://aspe.os.dhhs.gov/admnsimp.
- Hazard analysis based on critical thinking about the device design and the impact of any failure of subsystem components, such as signal detection and analysis, data storage, system communications and cybersecurity in relationship to incorrect patient reports, instrument failures, and operator safety.
- Documentation of complete verification and validation (V&V) activities for the version of software that will be submitted to demonstrate substantial equivalence. You should also submit information regarding validation of the compatibility of assay software with any instrumentation software.
- If the information you include in the 510(k) is based on a version other than the release version, identify all differences in the 510(k) and detail how these differences (including any unresolved anomalies) impact the safety and effectiveness of the device.

Below are additional references to help you develop and maintain your device under good software life cycle practices consistent with FDA regulations.

- **General Principles of Software Validation; Final Guidance for Industry and FDA Staff**.[6].
- **Guidance for Off-the-Shelf Software Use in Medical Devices; Final; available on the FDA Web site**.[7]
- 21 CFR 820.30 Subpart C – Design Controls of the Quality System Regulation.
- ISO 14971-1; Medical devices - Risk management - Part 1: Application of risk analysis.
- AAMI SW68:2001; Medical device software - Software life cycle processes.

[5] http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089543.htm.
[6] http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM085371.pdf
[7] http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm073778.htm

8. Labeling

The premarket notification must include labeling in sufficient detail to satisfy the requirements of 21 CFR 807.87(e). Final labeling for in vitro diagnostic devices must also comply with the requirements of 21 CFR 809.10 before an in vitro diagnostic device is introduced into interstate commerce. The recommendations below are aimed at assisting you in preparing labeling that satisfies these requirements.

For a test intended to be performed at one laboratory site that does not distribute a package insert as part of a packaged device, the manufacturer should provide users with a reference link to the 510(k) summary and/or decision summary documents posted at the publicly accessible FDA 510(k) database at the in their test report form.

Intended use

You must specify the product's intended use. 21 CFR 809.10(a)(2), (b)(2). The intended use should specify what the test measures, the clinical indications for which the test is to be used and the specific population for which the test is intended. It should include a description of patients (e.g., sex, age, clinical stability, time post transplant, current treatment regimen). The intended use should specify whether the test is qualitative or quantitative. If the test is intended for use at a single laboratory site, this information should be included in the intended use.

General Procedure

You should include a general description of the analysis procedure, from physician sampling up to, and including, result reporting.

Directions for use

You must provide a step by step outline of recommended procedures. 21 CFR 809.10(b)(8). You should present clear and concise instructions that delineate the technological features of the specific device and how the device is to be used. Instructions should encourage users to familiarize themselves with the features of the device and how to use it in a safe and effective manner.

You should include handling and storage instructions. You should describe stability (i.e., expiration dating) under the opened and closed storage conditions that you recommend to users.

Quality Control

The step by step outline of the procedure must include details of kinds of quality control procedures and materials required, as well as details of calibration. 21 CFR 809.10(b)(8)(v) and 21 CFR 809.10(b)(8)(vi). You should provide quality control recommendations in the package insert. This should include a clear explanation of what controls are to be used in the assay and the expected results for the control material.

Precautions, Warnings, and Limitations

You must include a statement of limitations of the procedure. 21 CFR 809.10(b)(10). We recommend you clearly describe any assay limitations in the labeling and include the appropriate limitations and warnings that a physician needs to know prior to ordering the test.

In addition to any limitations and warnings that are relevant to your assay, a cardiac allograft gene expression profiling test system should contain the following limitations:

- Results from this assay should not be used for diagnosis and should be used in conjunction with standard clinical assessment.
- Results from this assay should not be used to predict response to therapy regimens or to select the optimal therapeutic regimen.
- Results from this assay should not be used to exclude a therapeutic regimen.
- A statement explaining that the results are limited to the pool of patient samples that were used in the study.

Performance Characteristics

You must include specific performance characteristics of the assay. 21 CFR 809.10(b)(12). You should include in the package insert a summary of the study designs and the results from the studies described in Section 6 that would aid users in interpreting test results. This section should include a description of the clinical (i.e., medical) and analytical (i.e., technical) performance characteristics. Clinical performance characteristics should include clinical study validation summaries. Analytical performance characteristics should include descriptions of the results and methodologies used for the studies.

Interpretation of Results

You should clearly define the "classification," "pattern," "score," or "index" used to convey the patient-specific result. Performance metrics cited in the test report (such as negative predictive value of ACR) should be based on the results from the clinical trial that was used to clinically validate the device.

Expected Values

You must state the expected values of the test, indicate how it was established and the populations with which it was established. 21 CFR 809.10(b)(11). The description of the population should include information such as the number of samples, age, sex□

, and demographics. You should include an explanation of the result (e.g., Score '15' means…).