Guidance for Industry and FDA Staff

Class II Special Controls Guidance Document: CFTR Gene Mutation Detection Systems

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

Office of In Vitro Diagnostic Device Evaluation and Safety
Division of Hematology and Immunology Devices

Preface

Public Comment

Written comments and suggestions may be submitted at any time for Agency consideration to 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 you submit comments, please refer to Docket No. 2005D-0392. Comments may not be acted upon by the Agency until the document is next revised or updated.

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Contains Nonbinding Recommendations

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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 document was developed as a special control to support the classification of CFTR (cystic fibrosis transmembrane conductance regulator) gene mutation detection systems into class II (special controls). The CFTR gene mutation detection system is a device used to simultaneously detect and identify a panel of mutations and variants in the CFTR gene. It is intended as an aid in confirmatory diagnostic testing of individuals with suspected cystic fibrosis (CF), in carrier identification, and in newborn screening. This device is not intended for stand-alone diagnostic purposes, prenatal diagnostic, pre-implantation or general population screening. CFTR gene mutation detection systems may consist of various reagents and instruments, including polymerase chain reaction (PCR) primers, hybridization matrices, thermal cyclers, sequencers, signal detection instruments, and software packages.

This guidance is issued in conjunction with a *Federal Register* notice announcing the classification of CFTR gene mutation detection systems. Any firm submitting a 510(k) premarket notification for a CFTR gene mutation detection system 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.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances 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 guidances 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 should 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 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/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 CFTR gene mutation detection systems. A manufacturer who intends to market a device of this 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 CFTR gene mutation detection 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 CFTR gene mutation detection systems (Refer to Section 4 – **Scope**). In addition, other sections of this 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 these assays 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 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 once 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 generally 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 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 10 for specific information that you should include in the labeling for this type of device.)

Summary report

We recommend that the summary report contain the following:

- A description of the device and its intended use. You should also submit an "indications for use" enclosure.[2] Refer to Section 6 for specific information that you should include in the intended use and device description for this type of device.
- A description of the device design. We recommend that the description include a complete discussion of the performance specifications and, when appropriate, detailed, labeled drawings of the device.
- 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.)
- A 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.

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

- A description of the test method(s) you use to address each performance aspect identified in Sections 7 and 8 of this 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) present the data resulting from the test in clear and concise form, such as a table, **or** (2) describe the acceptance criteria that you apply to your test results. (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.[3] 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 CFTR gene mutation detection systems.

4. Scope

The scope of this document is limited to the following devices (product code NUA):

21 CFR 866.5900 Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation detection system

[3] 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.

The CFTR gene mutation detection system is a device used to simultaneously detect and identify a panel of mutations and variants in the CFTR gene. It is intended as an aid in confirmatory diagnostic testing of individuals with suspected cystic fibrosis (CF), carrier identification, and newborn screening. This device is not intended for stand-alone diagnostic purposes, prenatal diagnostic, pre-implantation or population screening.

5. Risks to Health

Failure of the CFTR mutation detection system to perform as indicated or errors in interpretation of results may lead to improper clinical recommendations and medical patient management. In the context of an aid to carrier-screening in adults, a false-negative or false-positive result or interpretation could lead to inaccurate estimates of a couple's risk of having a child with cystic fibrosis. In the context of assisting in the diagnosis of CF in newborns and children, a false-negative could lead to a delay in the definitive diagnosis and treatment; a false-positive could lead to unnecessary or inappropriate treatment.

Interpretation of test results depends on many factors, such as patient demographics, family history, and mutation or variants associated with infertility. To aid in a test interpretation manufacturers should recommend in their labeling that test results should be accompanied by genetic counseling. This will enable individuals and couples to receive guidance and information about risks and prognostic factors. CF has a wide clinical variability, with inconsistency of genotype-phenotype correlations for particular mutations. In addition, not all CFTR mutations cause cystic fibrosis. Possible test results that would benefit from interpretation by specialists include results for individuals who have a family history of CF, CFTR mutation carriers including couples where one or both partners are carriers, and otherwise healthy males who carry mutations associated with infertility[4].

Over 1300 mutations have been identified in CFTR the gene. Therefore genotypic CFTR tests, which typically detect a limited number of mutations, should not be used alone to diagnose cystic fibrosis. Assay results should be interpreted only in the context of other laboratory findings and the total clinical status of the patient. Manufacturers should clarify these limitations in the device labeling.

[4] For references regarding evaluation of benefits and risks to health of CFTR testing, see the following: W. W. Grody et al (Subcommittee on Cystic Fibrosis Screening, Accreditation of Genetic Services Committee, ACMG): Laboratory Standards and Guidelines for Population-based Cystic Fibrosis Carrier Screening, Genetics in Medicine, March/April 2001, **3** (2): 149-154 http://www.acmg.net/resources/policies/pol-005.asp; American College of Medical Genetics (ACMG) / American College of Obstetricians and Gynecologists http://www.acmg.net ; 2001, 2002, 2004 Technical Standards and Guidelines for CFTR Mutation Testing http://www.acmg.net/Pages/ACMG_Activities/stds-2002/cf.htm ; ACMG 2004 Standards and Guidelines for Clinical Genetics Laboratories http://www.acmg.net/Pages/ACMG_Activities/stds-2002/stdsmenu-n.htm ; 2004 Cystic Fibrosis Foundation (CFF) / Center for Disease Control (CDC) Recommendations on Newborn Screening for Cystic Fibrosis (http://www.cff.org, http://www.cdc.gov/.

In the table below, FDA has identified the risks to health generally associated with the use of CFTR gene mutation detection systems addressed in this document. The measures recommended to mitigate these identified risks are given in this guidance document, as shown in the table below. We recommend that you conduct a risk analysis, prior to submitting your premarket notification, to identify any other risks specific to your device. The premarket notification should describe the risk analysis method. If you elect to use an alternative approach to address a particular risk 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.

Identified risk	Recommended mitigation measures
Inaccurate estimate of risk of CF; delay in diagnosis and treatment; unnecessary, or inappropriate treatment due to the following:	
Failure of the test to perform properly	Performance, Method Comparison, Software Sections 7-9
Error in interpretation	Labeling Section 10

6. Device Description

Intended Use

The intended use should specify the mutations the test is intended to detect, the general clinical utility of detecting specified mutations, and the specific populations to which the test is targeted. The intended use should clearly specify whether detection of CFTR mutations is being performed as an aid in carrier identification, newborn screening, or confirmatory diagnostic testing of individuals with suspected cystic fibrosis (CF). References to professional society recommendations are acceptable.

Test Methodology

You should describe in detail the methodology used by your device to detect mutations or polymorphisms. For example, you should describe the following elements, where applicable for your device:

- Overall design of the test, including quality control of feature identity and placement, where applicable.
- Platform[5] (e.g., flow cytometry, instrumentation for clinical multiplex test systems).
- Sequence or identity of oligonucleotides, primers, probes, or other capture elements.

[5] Some of the elements listed in this section are applicable to instrumentation. If you are submitting a separate 510(k) for your instrumentation, you can describe the elements specific for instrumentation in that 510(k).

- Composition and spatial layout of arrays or other spatially fixed platforms.
- Methods used in attaching the probe material to a solid surface, if applicable.
- For multiplexed tests in which many probes are handled during the manufacturing process, the quality control design specifications that are in place to prevent probe cross-contamination.
- Specificity of probes for locus of interest, especially important when pseudogenes or sequence-related genes exist.
- Assay components such as buffers, enzymes, fluorescent dyes, chemiluminescent reagents, other signaling and signal amplification reagents, instruments.
- Internal controls and external controls used.
- Range of input sample concentrations that meet performance specifications.
- Hybridization conditions, washing procedures and drying conditions (e.g., temperature, length of time), and variations thereof, where 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 (design and functional testing).
- Stability and reproducibility of the platform when used for its intended use.

Illustrations or photographs of non-standard equipment or methods can be helpful in understanding novel methodologies. You should describe any additional process controls that guard against the possibility of inaccurate genotype determinations (miscalls) resulting from genotypes that are not detected by your device.

We recommend that you include a description of the method and technology used in CFTR gene mutation detection, and of the system components of the kit.

Instrumentation

You may submit a 510(k) for a CFTR gene mutation detection assay intended to be run on previously cleared instrumentation. Alternatively, the instrumentation may be a part of the new system included in your 510(k). You should analyze any instrumentation that is specific and dedicated to the device according to the "Guidance for FDA Reviewers and Industry: *Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices*" document available at http://www.fda.gov/cdrh/ode/guidance/337.html. If the CFTR gene mutation detection system is (or contains) a multiplex assay, it may be run on instrumentation for clinical multiplex test systems. 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*." If your device includes a CFTR gene mutation detection assay with instrumentation for clinical multiplex test systems for that assay, you may submit the information for both devices within one 510(k).

The guidance document, "*Replacement Reagent and Instrument Family Policy*", does not apply to CFTR gene mutation detection systems for use with instrumentation for clinical multiplex test systems.

You should include a copy of instrumentation manuals for specified instrumentation. If general purpose instrumentation is to be used, you should provide specifications for the required instruments to be used in labeling.

Validation of instrumentation

You should provide specifications in your labeling for any generic instrument needed to run your assay, so that the user may select an instrument that is suitable for their purposes. If your device includes proprietary instrumentation, whether manufactured by you or by another company, you should include specific information about the instrument(s) in your submission. We recommend that you address the following:

- Characterization: You should include information on how the instrument assigns values or interprets assay variables such as feature location, size, concentration, volume, drying of small samples, effect of small volume reactions and its impact on test results.
- Calibration: You should describe how the instrument is calibrated and the materials used in calibration.
- Uncertainties: You should describe potential sources and estimates of uncertainties in results introduced by hardware components, such as scanners.

If you specify a particular instrument (by manufacturer or brand) for use with your assay you should assure that any changes made to the instrument (by you or the instrument manufacturer) are tracked. If changes introduce new or different assay performance issues, you should validate your device under the changed conditions.

Test Results/Reporting

You should provide examples of test reports (e.g., printouts) as would be supplied to the healthcare provider. Reports should be consistent with current recommendations of genetics professional societies, and should contain adequate interpretation guidelines for the use of the ordering physician/counselor.

Where applicable, reports should include information on the mutations/polymorphisms identified and the methodology and technology used for detection. You should identify appropriate literature references to genotypic interpretations, or phenotypic predictions, if applicable, to enable users to access information about specific genotypes. We expect that for most 510(k) applications, appropriate literature references will be available. If such supporting information is not available, you should provide information based on the clinical studies you conducted to address clinical validity of your device.

7. Performance Characteristics

In your 510(k), you should detail the study design you used to evaluate each of the performance characteristics outlined below. This should include information such as:

- Description of the samples you used in testing (including types of samples, preparation and origin of samples, number of samples, and how the samples specifically represent your intended use samples). For all samples, you should include starting material, extraction method, concentration, and purity.

- Descriptions of the computations and statistical analyses you used to evaluate your data.
- Explanations, if there were any deviations from your protocol.

For each of the performance characteristics described below, you should also provide a clear description of all results and acceptance criteria in your 510(k).

The issues described below generally apply, regardless of the technology used by the device to detect the CF mutations. If you make additional claims for other performance characteristics not mentioned below, you should describe the study designs and results you used to support them.

Preanalytical Factors

Consideration of preanalytical factors is critical for high-quality genetic tests. If your device includes reagents for extraction and preparation of DNA for testing, you should validate each step in the preanalytical process for its effects on analytical parameters such as reproducibility, accuracy, and stability of product and describe study design and results addressing this issue in the 510(k). Your external site studies (e.g., reproducibility, method comparison) should include evaluation of the preanalytical process.

If your device does not include reagents for DNA extraction and preparation as part of the assay, you should provide specifications for the reagents needed and for assessing the quality of the assay input DNA, so that the user can select appropriate reagents. You should describe, in your 510(k), the study design and results you used to determine these specifications. In this case, we recommend that in studies establishing the performance of your device, you permit sites to use any extraction method they choose, providing that meets your specifications. In this way, you can evaluate any effect of variations in preanalytical processes on your device performance.

You should evaluate the accuracy and precision of your assay, including DNA extraction, from all the sources of DNA that you recommend for your assay (e.g., blood, peripheral blood mononuclear cells, buccal swab). You should also evaluate all sample collection and storage options you recommend (e.g., heparin-preserved vs. EDTA-preserved blood, stored vs. fresh sample). Your validation of appropriate storage conditions should include both the sample and the extracted product.

You should evaluate the stability of all of your reagents, and recommended samples.

Quality Control

CFTR gene mutation detection systems should include both positive and negative controls. Controls should approximate sample DNA concentration in order to adequately challenge the system.

You should describe the following concerning quality control and calibration material:

- The nature and the function of the various controls that you include with, or recommend for, your system. These controls may differ between individual technologies, but they should enable the user to determine if all steps and critical reactions have proceeded properly and without contamination or cross-hybridization.

- Your methods for value assignment and validation of control and calibrator material.
- You methods for establishing quality control and calibration procedures, including your recommended frequency.

We recommend that you implement the calibration of CFTR gene mutation detection systems where appropriate.

Analytical Factors

You should perform analytical studies that demonstrate that the device detects the mutations it claims to detect, and does not detect mutations when none are present. You should describe any process controls that guard against the possibility of miscalls (e.g., inaccurate genotype determinations) resulting from genotypes that are not detected by the assay, and report this as a limitation, if applicable.

Samples

Samples used to perform analytical studies should be patient samples derived from the intended use population, in order to show that the device will perform as claimed in clinical setting. We recommend that you evaluate test samples that encompass all genotypes in the test system. In cases where you have limited samples of a rare genotype, you should test a statistically determined number of replicates of the real clinical samples so that you can calculate a meaningful reproducibility result for that allele. In cases where samples with rare mutant alleles cannot be obtained, genomic DNA samples from cell lines or clone blends may be used. However, you should ensure that the composition of the test samples generated from these clones resembles, as closely as possible, the protein and DNA content and concentrations of real clinical samples. DNA containing the mutation should be added at a copy number that would be found in a natural sample. In addition, you should test clones blended in combination with various other alleles or genetic backgrounds, so that they reflect heterozygous, as well as homozygous samples.

Appropriate sample size depends on factors such as reproducibility, interference, and other performance characteristics of the test. You should choose the sample size to achieve a stated statistical confidence that the test performs as expected. Both heterozygous and homozygous mutant samples are acceptable. However, a pair of alleles from the same sample is not statistically independent, and therefore does not generate as much information as a pair of alleles from two independent samples.

Analytical Sensitivity / Analyte Concentration Specifications

We recommend that you establish the range of analyte concentrations that are measurable, or detectable by your assay as appropriate for your intended use. Once you have established this, you should validate analyte concentrations at the limits of these ranges in order to adequately stress the system. We recommend that you determine the 95% limit of detection (LOD) of analyte, as well as the possibility of a saturation limit at high levels of analyte. For further guidance concerning determination of LOD, see "Protocols for Determination of Limits of Detection and Limits of Quantitation;" Approved Guideline, NCCLS, EP17-A.

Interference

You should assess interference with CFTR gene mutation detection from any input into the system. Potential interfering substances may not always be removed by sample preparation and may also interfere with sample preparation. Therefore, we recommend that you characterize the effects of potential interfering substances on assay performance. Examples of experimental designs, including guidelines for selecting interfering substances for testing, are described in detail in "Interference Testing in Clinical Chemistry; Approved Guideline" (2002), CLSI document EP7-A. Potential interfering substances can include compounds normally found in blood, such as triglycerides, hemoglobin, bilirubin, lipids, and exogenous compounds such as common drugs.

Where applicable, you should evaluate cross-contamination of your device. In particular, you should perform studies to characterize potential carryover by alternating specimens of known genotype. You should also evaluate homologous gene sequences for cross-reactivity.

Precision (Repeatability/Reproducibility)

You should fully examine the reproducibility of your CFTR gene mutation detection system. "Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline" (2004), CLSI Document EP5-A, and "User Protocol for Evaluation of Qualitative Test Performance" (2002) CLSI Document EP12-A include guidelines for experimental design, computations, and a format for stating performance claims. We recommend that you incorporate the following in the design of your reproducibility studies:

- Design the study so that you can characterize intra- and inter-assay reproducibility.
- Use appropriate test samples at multiple DNA concentrations, similar to the concentrations in the procedure you recommend to users. You should include both wild-type and mutation sequences. In addition, the genotype of samples or sample panels you test should, as much as possible, reflect all the alleles that are included in the test.
- Ensure that samples used in reproducibility testing are processed from real clinical samples (e.g. whole blood, buccal swabs, or other intended use matrices) at the test site, and that processing mimics the procedure you plan to recommend in the test labeling.
- Include three or more sites, with multiple operators at each site conducting the study over multiple days. Sites should include at least two external laboratories that reflect potential users of the assay. Operators should reflect potential users of the assay, in terms of education and experience. If you will provide training for users to perform the test once it is marketed, you should provide information on operator training. If such training is not expected to be provided for users, you should not provide additional training (other than the proposed labeling, such as the package insert) at the testing sites.
- Ensure that procedures used in the reproducibility studies are the same as the procedure that you will recommend to users in the package insert.
- Include multiple product lots, and multiple instruments (if instruments are part of the test system), to adequately test the expected performance of the system.

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. You should also describe the computations and statistical analyses used to evaluate the data.

An analysis of precision should be made for each mutation tested and overall. Because the outcome is dichotomous (presence or absence of mutation) and precision is expected to be high for CF mutations, a standard variance components analysis (such as described in CLSI EP5-A2), reporting the coefficient of variation by factor, may not be informative and may not be possible due to computational difficulties. Instead, the percent of correct sequence calls (as determined by bi-directional sequencing) stratified by the levels of each factor (e.g., site, operator, lot, instrument) may suffice as a reasonable alternative.

8. Method Comparison

You should perform method comparison studies that demonstrate that your device detects the genotypes it claims to detect, and does not detect mutations or polymorphisms when none are present. Samples used in these studies should be patient samples derived from the intended use population, in order to show that the device will perform as claimed in a clinical setting.

Because of the abundance of technologies that could be used to detect CFTR gene mutations, assays may vary significantly in terms of methodology, instrumentation, and sample source, making direct comparison difficult. You should compare results of your device to bidirectional DNA sequence analysis.

Description of Study Results

You should describe the protocol and results of your method comparison in your 510(k). You should submit, along with your comparative sequence data, a measure of sequence quality such as a phred score or percent correct sequence calls. You can then use this information to calculate the percent correct genotype call rate of your device, relative to the bidirectional sequencing data. We recommend that you tabulate all results and indicate the percent correct calls for the various genotypes. We recommend that you resolve and explain all discrepant results in the 510(k); however, you should use original unresolved results for all performance calculations, in order to avoid bias. You should include failed assays (e.g., inability to correctly determine genotype within a sample, reporting of an incorrect result, instrument failure, or reagent failure) in your description of results. Any incorrect or absent genotype determinations should be considered disagreements for the purposes of reporting performance characteristics.

When conducting studies, samples should be masked so that operators are unaware of the sequencing results, in order to ensure that the validity of the study results is not compromised. You should report results (e.g., phred score or percent correct sequence calls) with 95% confidence intervals. Exact confidence intervals should be computed instead of standard asymptotic confidence intervals when the latter poorly approximate the former (e.g., for an observed percent of correct sequence calls near 100%).

If, in addition, you wish to provide additional data on other performance characteristics of your device, we recommend that you provide a description of the testing in your submission.

Sample selection, inclusion and exclusion criteria

While prospective samples are preferred, well-characterized samples from banks can be used in your method comparison study, if clinical utility and validity are already established in the literature. You should use clinical samples from all matrices you claim in your intended use to demonstrate that correct results can be obtained from clinical material. You should fully describe selection (inclusion/exclusion) criteria, and characterize any relevant features of the samples (whether prospective or retrospective).

You should provide clear information supporting sample integrity. We recommend that you validate statements in your labeling about sample storage and transport by assessing sample stability and recovery over the storage times and temperatures recommended to users. For example, an appropriate study may include an analysis of aliquots stored under the conditions of time, temperature, or specified number of freeze/thaw cycles. We recommend that you state your acceptance criteria for the sample stability parameters.

The following is a rough guideline for sample size calculation: If the sample size is N and the observed sensitivity is N/N or 1.0, then the exact one-sided 95% confidence interval is (N – 3)/N (Van Belle, Statistical Rules of Thumb, Wiley, 2002). For example, if N=100, the 95% lower bound is 97/100 or 0.97. Clearly for small sample sizes such as N=5, the sample size is too small because then the sensitivity lower bound is only 2/5 or 0.40.

Clinical Validation

Prospective clinical testing to determine clinical validity and utility may not be necessary for validation of CFTR gene mutation detection systems, as there may be an established scientific framework and sufficient body of evidence supporting the clinical validity and utility of your device. In this case, you should provide the relevant peer-reviewed references. 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. If clinical testing is performed, we recommend that you select patients prospectively in order to maximize the number of mutations detected. In either case, you should use clinical samples to demonstrate that correct results can be obtained from clinical material.

9. Software

If your system includes software, you should submit software documentation detailed in accordance with the level of concern (See: *Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices* http://www.fda.gov/cdrh/ode/guidance/337.html. You should determine the Level of Concern prior to the mitigation of hazards. In vitro diagnostic devices of this type are considered a moderate level of concern, because software flaws could indirectly affect the patient and potentially result in injury because of the action or inaction of a healthcare provider who does 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 cyber-security 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, identification of 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; available on the FDA Web site at: http://www.fda.gov/cdrh/comp/guidance/938.pdf.
- Guidance for Off-the-Shelf Software Use in Medical Devices; Final; available on the FDA Web site at: http://www.fda.gov/cdrh/ode/guidance/585.pdf.
- 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.

10. 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). Although final labeling is not required for 510(k) clearance, final labeling for in vitro diagnostic devices must comply with the requirements of 21 CFR 809.10 before an *in vitro* diagnostic device is introduced into interstate commerce.

Directions for use

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 local/institutional training programs, if available, that are designed to familiarize users with the features of the device and how to use it in a safe and effective manner.

If your assay will not include reagents for DNA extraction and preparation, you should provide specifications for the extraction and preparation reagents that users will need to run the test. You should also provide specifications for assessing the quality of the assay input DNA, so that users can select appropriate reagents.

Specimen collection and handling conditions/stability

You should state the acceptance criteria for the specimen stability and integrity parameters. For example, labeling should clearly state the validated conditions for specimen transport, storage, temperature, and specified number of freeze/thaw cycles.

Quality Control

You should include a description of quality control recommendations in the package insert. This should include a clear explanation of the control material to be used in the assay and expected results for this control material.

Instrumentation

We recommend that you provide a user manual that addresses all components of the specified instrumentation. Your user manual should provide an adequate description of the role of the software, the user interface with the software, as well as results of performance testing to demonstrate that the software functions as designed. We recommend pictorial representations of computer screens, graphical user interfaces (GUIs), and other elements that aid the user in correctly using the software.

The user manual, where possible, should also include descriptions of how the user can recognize incorrect operation or failure of the instrumentation, and a troubleshooting guide.

If general purpose instrumentation is to be used, you should provide specifications for the required instruments to be used in labeling.

Performance

You should include in the package insert all study designs and results for studies described in Sections 7 and 8 of this guidance document that would aid users in interpreting test results. For the method comparison, you should describe device performance in comparison to bidirectional DNA sequencing. We recommend presentation of your results in the form of tables (e.g., n x n tables), descriptions of percent correct genotype calls relative to sequence analysis, and a list of the nature of any miscalls (e.g., correct sequence versus one predicted by device). You should include descriptions of percent agreement, or diagnostic sensitivity and specificity, as appropriate.

You may provide the diagnostic sensitivity and specificity of your system in your labeling, if applicable. Diagnostic sensitivity is the proportion of patients with a well-defined clinical disorder whose test values are positive or exceed a defined decision limit

(i.e., a positive result and identification of the patients who have a disease). Diagnostic specificity is the proportion of subjects who do not have a specified clinical disorder whose test results are negative or within the defined decision limit.

Expected Values

You should provide data concerning prevalence for each CFTR mutation or polymorphism, including, where appropriate, prevalence according to ethnicity and race.

Interpretation of Results

You should clearly reference any phenotype definitions. We recommend that you provide a section in your package insert to aid users in interpreting test results. The result reports should be consistent with current recommendations of genetics professional societies, if applicable, and should contain adequate interpretation guidelines for the use of the ordering physician/counselor. To aid in a test interpretation manufacturers should recommend in their labeling that test results should be accompanied by genetic counseling. See also the section on Test Results/Reporting, above.

Precautions for interpretations

Any assay limitations should be clearly described in the labeling. Most CFTR gene mutation detection systems should contain the following limitation:

- Assay results should be interpreted only in the context of other laboratory findings and the total clinical status of the patient.
- The presence of rare mutations in the CFTR gene (not tested by the device) may result in false results/miscalls.
- This test should not be used alone to diagnose cystic fibrosis.

Standards and Guidances

The following additional standards, guidelines, and guidances may be helpful when preparing your CFTR gene mutation detection system submission:

- CLSI Guidelines
  - Molecular Diagnostic Methods for Genetic Diseases; Approved Guideline (2000) CLSI document MM1-A
  - Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine; Approved Guideline (2004) CLSI document MM9-A
- FDA Guidances
  - 27 Feb. 2003 CDRH Draft Guidance for Industry and FDA Reviewers: *Multiplex Tests for Heritable DNA Markers, Mutations and Expression Patterns* http://www.fda.gov/cdrh/oivd/guidance/1210.pdf
  - 12 Mar 2003 CDRH Draft Guidance for Industry and FDA Reviewers: *Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests* http://www.fda.gov/cdrh/osb/guidance/1428.pdf