Guidance for Industry

Class II Special Controls Guidance Document: In Vitro HIV Drug Resistance Genotype Assay

Additional copies of this guidance are available from the Office of Communication, Training and Manufacturers Assistance (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or from the Internet at http://www.fda.gov/cber/guidelines.htm.

For questions on the content of this guidance, contact Office of Blood Research and Review (OBRR) at 301-827-3524.

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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 appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

We, FDA, are issuing this guidance document in conjunction with a *Federal Register* final rule reclassifying from class III to class II the in vitro HIV drug resistance genotype assay, an in vitro diagnostic device (IVD), to use in detecting HIV genomic mutations that confer resistance to specific antiretroviral drugs, as an aid in monitoring and treating HIV infection. This guidance document serves as the special control to support the reclassification. Special controls, when combined with general controls, provide reasonable assurance of the safety and effectiveness of the device.

Following the effective date of a final rule classifying the device, any manufacturer submitting a 510(k) premarket notification for an in vitro HIV drug resistance assay will need to address the issues covered in this special controls guidance. The manufacturer must show that its device meets the recommendations of this guidance or in some other way provides equivalent assurances of safety and effectiveness.

You should contact the Division of Blood Applications, Center for Biologics Evaluation and Research (CBER) at (301-827-3524) for information on filing your submission or for any questions you may have.

The issues identified in this guidance document represent those that we believe need to be addressed before your device can be marketed. 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 in the document, "A Suggested Approach to Resolving Least Burdensome Issues."[1]

[1] See http://www.fda.gov/cdrh/ode/guidance/1188.html.

This guidance document finalizes the draft guidance entitled "Guidance for Industry: Premarket Notifications [510(k)s] for In Vitro HIV Drug Resistance Genotype Assays" dated August 2001 (66 FR 45682, August 29, 2001).

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the FDA'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 FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

HIV drug resistance testing provides information about specific mutations present in the virus infecting an individual. Because the presence of mutations may affect the efficacy of certain HIV drugs, this information has been shown to be clinically useful by providing therapeutic guidance in monitoring or treating HIV-infected individuals. The mutations listed in Tables A and B below are associated with HIV drug resistance and we recognize the clinical significance of these mutations. Other mutations, including those listed in Tables C through E below, are suspected of being associated with HIV drug resistance, but their significance has not been as widely accepted. We consider those listed in Tables C through E to be relevant to HIV drug resistance, but not of confirmed clinical significance, however.

In this document, we describe two pathways for you to seek clearance of your assay as a Class II device for detecting HIV mutations. Under the first pathway, you would demonstrate the analytical sensitivity of your test in identifying mutations in Tables A through E, below. An applicant following the second pathway would develop a combination of analytical data and clinical data showing the performance of the test as an aid in treatment of subjects with HIV. We recognize that as the field progresses, additional mutations may become widely recognized as clinically significant. We have based the information in this document on current science.

FDA believes that special controls, when combined with general controls, provide reasonable assurance of the safety and effectiveness of the in vitro HIV drug resistance genotype assay. Thus, a manufacturer who intends to market a device of this type must:

- conform to the general controls of the Federal Food, Drug & Cosmetic Act (the Act), including the premarket notification requirements described in 21 Code of Federal Regulations (CFR) Part 807, Subpart E;
- address the specific risks to health associated with in vitro HIV drug resistance genotype assays identified in this guidance; and
- obtain a substantial equivalence determination from FDA prior to marketing the device.

Section IV of this guidance document identifies the classification regulation and product code for this in vitro HIV drug resistance genotype assay. 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 807.81-807.100 and other information on this topic developed by FDA and available on the FDA website, such as Device Advice: Premarket Notification 510(k)[2] Under The New 510(k) Paradigm–guidance (Ref. 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 once FDA has issued a guidance document that provides recommendations about what should be addressed in a submission for the device. Alternatively, manufacturers considering modifications to their own cleared devices may reduce their burden by submitting a Special 510(k) (Ref.1).

III. 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). We therefore recommend you include a report summarizing how this special controls guidance document was used during device development and testing (Ref. 2). The summary report should include a summary of the test data or description of the acceptance criteria applied to address the device risks identified in this guidance document, as well as any additional risks specific to your device. Additional information on an abbreviated 510(k) submission is provided in the "Guidance for Industry and FDA Staff: Format for Traditional and Abbreviated 510(k)s,"[3] issued August 12, 2005.

IV. SCOPE

The scope of this document is limited to the following devices as described in 21 CFR 866.3950 In Vitro HIV Drug Resistance Genotype Assay (NHS):

21 CFR 866.3950 – Assay, Genotype, HIV Drug Resistance, In Vitro

(a) Identification. In vitro HIV drug resistance genotype assays are devices that consist of nucleic acid reagent primers and probes together with software for predicting drug resistance/susceptibility based on results obtained with these primers and probes, for use in detecting HIV genomic mutations that confer resistance to specific antiretroviral drugs, as an aid in monitoring and treating HIV infection.

[2] See http://www.fda.gov/cdrh/devadvice/314.html.

[3] See http://www.fda.gov/cdrh/ode/guidance/1567.pdf

V. RISKS TO HEALTH

Failure of the test to perform as indicated, or errors in interpretation of results, may lead to improper patient management and the failure to optimize drug treatment, specifically by subjecting a patient to unnecessarily high viral loads or to unnecessary risks of side effects due to administration of inappropriate drugs. Furthermore, the difficulty of detecting low levels of resistant mutants dictates that assay results must be used in conjunction with other clinical information. Adjusting a treatment regimen solely on the basis of assay results and without consideration of other clinical factors could pose a risk.

Current standards for care of HIV-infected patients rely heavily upon tests for viral load (copies/ml of virus in serum/plasma). Therapy is designed, in part, to decrease the viral load as much as possible (generally, to below detectability). High viral loads and/or viral load rebound during HAART (highly active antiretroviral therapy - currently three and even four drug regimens) are taken as an indication of treatment failure. One of the most common causes of treatment failure is the existence or emergence of virus species resistant to the drugs included in the regimen (Ref. 3).

Assays have been developed to identify the genotypes of virus present in infection. These assays identify the nucleic acid sequences in specific portions of the HIV genomes (e.g., the protease (PR), and reverse transcriptase (RT), genes)) that make up the viral population in a patient and are being used to guide treatment choices for patients. Multiple problems are associated with the use of such genotyping assays, however. They generally detect only the most prevalent members of the viral "swarm." So-called "archived" species, which may have accumulated during development of resistance to previous antiretroviral therapy and which may remain at significant levels, may be undetectable by genotyping assays. Furthermore, the correlation between viral sequence and clinical resistance may be poorly determined. Some "resistance" mutations may appear early in antiretroviral therapy and may, indeed, herald the onset of resistance, but may have only minimally detectable effects in various in vitro drug resistance assays. Absolute IC50 or IC90 (50% or 90% inhibitory concentration) levels may vary from assay to assay and may be difficult to relate to in vivo drug levels. Cross-resistance, interference, and the existence of phenotypes based on changes at multiple viral genetic loci may further confound the significance of genotyping data (Ref. 4).

We are providing this guidance to help you ensure the reliability of drug resistance genotype assays for recognized mutations and to show you how such assays may be developed for review by the FDA as Class II medical devices.

In the table below, we have identified the risks to health generally associated with the use of in vitro HIV drug resistance genotype assays. 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 510(k) 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
Subjecting patients to unnecessarily high viral loads or to unnecessary risks of side effects due to administration of inappropriate drugs.	See sections VI & VII

VI. PERFORMANCE CHARACTERISTICS

Generally, drug resistance genotype tests have two critical components: (1) the assay that determines and reports the genotype; and (2) the interpretation algorithm, which is a data analysis method by which the genotype is interpreted to predict the phenotype of the infecting viral swarm. Both components contribute to overall assay performance.

You may use a minimal interpretation algorithm outlined in this document (Tables A and B, below) or you may submit data supporting the use of additional interpretation rules or alternative interpretation methodologies.

You should submit as part of your 510(k) submission scientific data to support the performance characteristics of the device, including documented protocols for in-house and external testing and summaries of results and explanations of unexpected results, charts (scatter grams, histograms, etc.).

Under 21 CFR 807.92(d), we will request unprocessed laboratory data, including line listings and actual data sheets, if the summaries appear to contain erroneous interpretation of raw data.

We have outlined the types of data and/or performance characteristics that you should include in a 510(k) submission to characterize the performance of an in vitro HIV drug resistance genotype assay.

We believe that certain mutations in the HIV genome have been convincingly associated with viral resistance to specific antiretroviral drugs used to treat HIV infection. We recognize that other mutations of interest in the HIV genome have been implicated but not proven to be associated with viral resistance to certain antiretroviral drugs. You should provide analytical data sufficient to demonstrate the ability of your tests to detect mutations in both these categories. We realize, however, that clinical data showing the performance of the test as an aid in treatment of subjects with HIV may reduce the nature and extent of analytical studies necessary to assure assay effectiveness when such data demonstrate the ability of an assay to predict drug resistance genotypes. For this reason, you may submit less extensive analytical data on “established” and “implicated” mutations when you also submit such supporting clinical trial data.

This document provides two pathways to 510(k) clearance of in vitro HIV drug resistance genotype assays. Under the first pathway, you may obtain clearance of an investigational assay by submitting extensive analytical data alone. Under the second pathway, you may obtain clearance by submitting supportive clinical data showing the performance of the test as an aid in treatment of subjects with HIV and less extensive analytical data. This guidance recognizes that, due to the lack of extensive analytical data when an applicant follows the second option, the indications for use of the device may be more limited.

A. Performance of the Interpretation Algorithm

1. Validation of Phenotypes Predicted by Genotyping: In Vitro Studies

   You should support any phenotypic prediction based on genotypic information either by reference to Tables A and B, below, or by additional analytical verification studies. If you address mutations not listed in Tables A or B, include in your verification studies for such mutations in vitro assays measuring the binding of the active form of the antiretroviral inhibitor to its target substrate, and in vitro viral replication assays (including determination of the effect of the given genotype on IC50 or IC90), if available. You should further support phenotypic predictions not listed in Tables A or B by including in vivo data, as outlined below in VI.A.2. You may submit verification studies derived in whole or in part from data previously published in peer-reviewed journals. When relying upon previously published data, you should provide legible copies of all publications used to support your claims, together with individual summaries, in English, of individual publications and an overall summary of all the literature cited.

2. Verification of Phenotypes Predicted by Genotyping: In Vivo Studies

   Any phenotypic prediction that is not listed in Tables A or B should be verified by in vivo studies that correlate the existence and/or appearance of the corresponding genotype in patients with the existence and/or development of partial or complete resistance to specific therapy. You may use mutations in determining algorithm rules for which supportive in vivo data have not been acquired (and assuming contradictory in vivo data are not known) if the supportive in vitro data are compelling and the lack of in vivo data is prominently included in the report to the end user whenever the rule is used. Patient viral burden should be determined throughout these studies. You may submit verification studies derived in whole or in part from data previously published in peer-reviewed journals. When relying upon previously published data, you should provide legible copies of all publications used to support the correlation between genotype and phenotype, together with individual summaries, in English, of individual publications and an overall summary of all the literature cited.

B. Performance of the Assay in Determining Genotype

1. Analytical Sensitivity

a. You should test panels of virions from cloned virus or patient specimens containing known, common, single-locus (e.g., a particular amino acid or sequence at a particular mutation's locus) or multiple-locus mutations, to determine analytical sensitivity.

- In the Specific Performance Characteristics section of the package insert, you should list all mutations that you can demonstrate the assay successfully detects according to the criteria laid down in this section and the immediately following section of this guidance document (VI.B.1.b). Throughout this guidance document, we will refer to this list as the Fully Verified Performance list.

- You should test all mutations that will be listed in the Fully Verified Performance list of the package insert (see IX.B, below), as well as all mutations listed in Tables A and B at a level within fourfold (copies/ml) of the limit of detection (LOD). If data from clinical trials showing the performance of the test as an aid in treatment of subjects with HIV (see section VI.E) support the clinical utility of the assay, we will accept data from an incomplete subset of the studies described in this and the immediately following section (VI.B.1.b). In all cases, however, the Fully Verified Performance list should include only mutations detected with 90% sensitivity (point estimate, at least 10 measurements) at or near the LOD, in the studies described in sections VI.B.1.a and b.

- You may test multiple related or unrelated mutations together in the context of a single genomic clone. In cases where codon degeneracy (i.e., alternative sequences coding for the same amino acids) allows different sequences to code for identical amino acid mutations, you may test any single nucleic acid sequence that codes for the amino acids in question. In the package insert, list the actual codon sequences that you tested.

- You should submit to FDA the identity of any specific mutations at the nucleic acid level that are known to be unusually difficult to sequence if they contribute to the interpretation algorithm you use in reporting assay results.

- You may construct panels by spiking methods, using well-characterized HIV-1 clones.

- You should test each mutation at least 10 times in these studies, at or near the lowest viral level that the assay can reliably detect. When using clinical specimens for these studies, you should determine the sequence of each specimen's viral "swarm" by sequencing at least 10 molecular subclones.

- You should use three different lots of the assay in these analytical sensitivity studies.

- You should include in your submission a brief study description and well-organized data presentation including:

    1. the identity and number of loci tested;
    2. the number of times each was tested;
    3. the genetic context in which each was tested;
    4. the viral load tested (copies/ml);
    5. the overall sensitivity (number correctly identified /total); and
    6. a summary of lot distribution over the studies.

Table A (Ref. 5)

Mutations Recognized to Confer Clinical Resistance to Reverse Transcriptase Inhibitors

Mutation	Resistance Profile	Interpretation
M41L	ZDV	Confers resistance in combination with other ZDV mutations
A62V	Multi-NRTI*	Uncommon, only confers resistance in combination with F75I, F77L, F116Y, and/or Q151M
K65R	DDC, DDI, ABC	Confers resistance to DDI and ABC usually in combination with other mutations. As a single mutation may cause resistance to DDC
D67N	ZDV	Confers resistance in combination with other ZDV mutations
S68G	Multi-NRTI*	Uncommon but usually confers resistance in combination with A62V, F77L, F116Y, and/or Q151M
T69D	DDC	As a single mutation may confer resistance
69INS	Multi-NRTI*	Confers resistance usually in combination with ZDV resistance mutations (M41L, D67N, K70R, L210W, T215Y/F, K219Q/E)
K70R	ZDV	Confers resistance in combination with other ZDV mutations
L74V	DDI, DDC, ABC	As a single mutation may cause clinical resistance to DDI and DDC. Additional mutations may be required for ABC
F751	Multi-NRTI*	Uncommon, only confers resistance in combination with A62V, F77L, F116Y, and/or Q151M
F77L	Multi-NRTI*	Uncommon, only confers resistance in combination with A62V, F75I, F116Y, and/or Q151M
L100I	NVP, EFV	Often found in combination with other mutations
K103N	NNRTI (all)**	As a single mutation confers resistance
V106A	NVP, DLV	As a single mutation confers resistance
V108I	NVP, EFV	Often found in combination with other mutations
Y115F	ABC	Confers resistance in combination with other ABC mutations or with ZDV mutations
F116Y	Multi-NRTI*	Uncommon, only confers resistance in combination with A62V, F75I, F77L, and/or Q151M
Q151M	Multi-NRTI*	Usually confers resistance in combination with A62V, F75I, F77L, F116Y
Y181C/I	NVP, DLV	As a single mutation confers resistance
M184 I/V	3TC, ABC, DDC, DDI	As a single mutation confers resistance to 3TC and DDC, the addition of other mutations may be required for clinical resistance to DDI or ABC
Y188C/L	NNRTI (all)**	As a single mutation confers clinical resistance
L210W	ZDV	Confers resistance in combination with other ZDV mutations
T215Y/F	ZDV	Confers resistance in combination with other ZDV mutations
K219Q/E	ZDV	Confers resistance in combination with other ZDV mutations

*Multi-NRTI refers to zidovudine (ZDV), didanosine (DDI), zalcitabine (DDC), abacavir (ABC), and stavudine (D4T).

**All NNRTI equals nevirapine (NVP), delavirdine (DLV), and efavirenz (EFV).

Table B (Ref. 5)

Mutations Recognized to Confer Clinical Resistance to Protease Inhibitors

Mutation	**Resistance Profile**	**Interpretation**
D30N	NFV	As a single mutation confers resistance to NFV
M46I	ALL PIS*	Confers resistance in combination with other mutations associated with clinical resistance
G48V	SQV	Confers resistance in combination with other mutations associated with clinical resistance
I50V	APV	Confers resistance usually in combination with other mutations
I54V	ALL PIS*	Confers resistance in combination with other mutations associated with clinical resistance
V82 (A/F/T/S)	RTV, IDV, LPV/RTV, NFV, SQV	More strongly associated with IDV, RTV, and LPV; Confers resistance usually in combination with other mutations
I84V	ALL PIS*	Confers resistance usually in combination with other mutations
N88D	NFV	As a single mutation confers resistance to NFV
L90M	ALL PIS*	More strongly associated with SQV or NFV but in combination with other mutations may confer resistance to all PI

*ALL PIS equals APV (amprenavir), IDV (indinavir), LPV/RTV (lopinavir/ritonavir), NFV (nelfinavir), SQV (saquinavir), and RTV (ritonavir).

b. You should also test panels that include clones with known, preferably common, multiple mutations (i.e., multiple mutations that need to be simultaneously present in order to allow resistance predictions).

You may obtain these clones from patients or by using site-directed mutagenesis.

You should test each of these clones at least 10 times, using three different lots of the assay, at clinically relevant viral loads.

You should clearly characterize the clones, particularly with respect to the identities of the mutations in each clone.

c. You should conduct studies similar to those described in VI.B.1 a and b, immediately above, to show that the assay can detect all mutations listed in Tables C, D, and E (below), as well as all mutations that are used in the interpretation algorithm.

For these studies, you should demonstrate the ability of your assay to detect at least one common mutation codon (at the corresponding locus) for each mutation listed and at one level of virus (copies per ml.), as specified in this section (VI.B.1.c).

You should demonstrate that mutations listed as "Primary" in these tables can be routinely detected at viral levels within tenfold of the minimum levels for which a claim is sought.

You may demonstrate the detectability of "Secondary" mutations at any level within the usable range of the assay.

For mutations listed in Tables C through E, or other mutations used in the interpretation algorithm that are also listed in Tables A or B or in the Fully Verified Performance list, you should perform the studies described above in sections VI.B.1.a and b.

If clinical data showing the performance of the test as an aid in treatment of subjects with HIV (see section VI.E) support the clinical utility of the assay, we will accept data from an incomplete subset of the studies described in this section (VI.B.1.c). However, in such cases we may require precautionary labeling in the Limitations for Use section of the package insert indicating which mutations have been incompletely tested and verified.

Table C (Ref. 5)

Mutations in the Protease Gene Selected by Protease Inhibitors

(Primary mutations generally cause decreased inhibitor binding and are the first mutations selected during therapy with the associated antiretroviral. Secondary mutations may also contribute to drug resistance, although they may have less direct effect on inhibitor binding in vitro than primary mutations).

Drug	**Degree**	**Associated Mutations**
Indinavir	Primary	M46I; V82A, or F, or T, or S
	Secondary	L10I, or R, or V; K20M, or R; L24I; V32I; M36I; I54V; A71V, or T; G73S, or A; V77I; I84V; L90M
Ritonavir	Primary	V82A, or F, or T, or S
	Secondary	K20M, or R; V32I; L33F; M36I; M46I, or L; I54V, or L; A71V, or T; V77I; I84V; L90M
Saquinavir	Primary	G48V; L90M
	Secondary	L10I, or R, or V; I54V, or L; A71V, or T; G73S; V77I; V82A; I84V
Nelfinavir	Primary	D30N; L90M
	Secondary	L10F, or I; M36I; M46I, or L; A71V, or T; V77I; V82A, or F, or T, or S; I84V; N88D
Amprenavir	Primary	I50V; I84V
	Secondary	L10F, or I, or R, or V; V32I; M46I; I47V; I54V

Table D (Ref. 5)

Mutations in the Reverse Transcriptase Gene Selected by Nucleoside Reverse Transcriptase Inhibitors

(Primary mutations generally cause decreased inhibitor binding and are the first mutations selected during therapy with the associated antiretroviral. Secondary mutations may also contribute to drug resistance, although they may have less direct effect on inhibitor binding in vitro than primary mutations).

Drug	**Degree**	**Associated Mutations**
Zidovudine	Primary	K70R; T215Y, or F
	Secondary	M41L; D67N; L210W; K219Q
Stavudine	Primary	V75T
Didanosine	Primary	L74V
	Secondary	K65R; M184V, or I
Zalcitabine	Secondary	K65R; T69D; L74V; M184V, or I
Lamivudine	Primary	E44D; V118I; M184V, or I
Abacavir	Primary	K65R; L74V; M184V
	Secondary	M41L; D67N; K70R; Y115F; L210W; T215Y, or F; K219Q
Multi-nRTI Resistance-A	Primary	Q151M
	Secondary	A62V; V75I; F77L; F116Y
Multi-nRTI Resistance-B	Primary	T69S and 2 amino acids encoded by an insertion between RT codons 69 and 70 (69 Insertion)
	Secondary	M41L; A62V; D67N; K70R; L210W; T215Y, or F; K219Q

Table E (Ref. 5)

Mutations in the Reverse Transcriptase Gene Selected by Non-Nucleoside Reverse Transcriptase Inhibitors

(Primary mutations generally cause decreased inhibitor binding and are the first mutations selected during therapy with the associated antiretroviral. Secondary mutations may also contribute to drug resistance, although they may have less direct effect on inhibitor binding in vitro than primary mutations).

Drug	**Degree**	**Associated Mutations**
Nevirapine	Primary	K103N; V106A; V108I; Y181C, or I; Y188C, or L, or H; G190A
	Secondary	L100I
Delavirdine	Primary	K103N; Y181C
	Secondary	P236L
Efavirenz	Primary	K103N; Y188L; G190S, or A
	Secondary	L100I; V108I; P225H

d. Generally, assays should correctly identify the amino acids at all codons known or suspected to be involved in conferring drug resistance.

2. Range of Detectability

You should define the overall plasma/serum concentration of virus (viral burden) at which these tests are effective. The assay should be effective at a viral burden that is clinically relevant.

You should determine assay performance (sensitivity and specificity for specific genotypes) over the entire range of the assay, both with respect to overall viral levels (copies/ml) and with respect to the percent representation of specific mutations (e.g., 25% of total).

In general, it is important to determine assay performance as overall viral levels and/or mutant proportions decrease.

Although you should determine the accuracy at all loci specifically listed in the Fully Verified Performance list of the package insert (see IX.B, below), you need to fully evaluate only a representative set of 30 total loci for each parameter (viral level and mutant proportion) according to the criteria described in these sections (VI.B.2.a, b, and c). These 30 loci may consist of any of the loci listed in Tables A or B or in the Fully Verified Performance list.

If you submit supportive clinical data showing the performance of the test as an aid in treatment of subjects with HIV (as described in Section VI.E), without the studies described in these sections (VI.B.2.a, b, and c), the Fully Verified Performance list should be limited to the subset of the mutations in Tables A and B for which you have provided the analytical data described in this section (VI.B.2.a, b, and c).

a. Using the minimal proportions of mutant species in the range of detectability, you should determine assay performance at minimum detectable levels of virus and at one half log and 1.0 log below minimum. Thus, if 30% is the minimal proportion of mutant species that the assay can reliably detect and 1,000 copies/ml is the minimal viral level at which the assay can reliably obtain sequences, you should test the following levels of virus mixtures: 100; 300, and 1,000 (copies/ml, containing 70% wild type and 30% mutant). You should provide data describing any maximal level of viral burden at which mutant detectability decreases, if such a maximal level is known.

b. Using the minimal viral levels in the range of detectability, you should determine assay performance at approximately 100% and 50% of the minimal mutant proportions in the range of detectability. You should also test mutant species at a proportion of 100%. (For example, if you seek a claim for 25% mutant/75% wild type, the following proportions of mutant would be tested: 100%, 25%, and 12.5%).

c. You should test at each data point described in the above paragraphs VI.B.2.a and b, at least in triplicate.

d. You should report the mutant/wild type ratios tested and sensitivity at each level.

3. Precision

For assays that claim to determine the quantitative levels or proportions of viral mutants (rather than just presence or failure to detect), precision studies should define the coefficients of variation for the HIV resistance assay within one experiment using one product lot and also across three product lots. You should include in your study at least 20 10-aliquot sample sets (20 different validated mutations at 20 different loci, 10 replicates, for each lot). You should do your studies at the lowest level in the range of detectability and also at higher levels, at your discretion.

4. Reproducibility

You should determine assay reproducibility by testing three lots at different sites, on different days, and by different investigators. You should analyze samples in triplicate, including a subset of mutations for which claims are sought. You should include in these studies tests of viral mixtures (either clinical samples or mixtures of clones).

5. Lot Acceptance Testing

You should perform lot acceptance testing to ensure adequate performance of each lot of assay produced. Lot acceptance testing should include data indicating adequate performance with panel members at the lowest levels/proportions in the range of detectability. You should include in this testing mutations with a range of detectabilities.

6. Specificity

During the course of analytical sensitivity studies, we expect that many defined analytes with various combinations of wild type loci and resistance mutations will be tested. You should accumulate, analyze, and report data from these experiments concerning the non-specificity of the assay (i.e., how often the assay reports an incorrect result at wild type loci).

7. Assay Interference

Most assays are subject to interference from specific components. These components may be introduced during sample collection and handling or they may be present in the patient as a result of the patient's therapy or condition. You should determine the effects on the assay of a variety of substances and conditions that are likely to cause interference. You may test for interference using spiking methodology in addition to testing original clinical specimens. Some conditions that may cause interference include:

- other infections including HIV-2, human T-cell lymphotrophic virus type I/II (HTLV-I/II), cytomegalovirus (CMV), Epstein-Barr Virus (EBV), hepatitis B virus (HBV), hepatitis C virus (HCV), yeast infections, pneumocystis, M. tuberculosis, M. Avium and M. intracellulare;

- samples collected in various anticoagulants or other collection media;

- hemolyzed, icteric, lipemic, and bacterially contaminated samples;

- chemicals, drugs, and heated-, and detergent-treated samples;
- samples subjected to multiple freeze/thaw cycles;
- fresh vs. frozen samples, serum vs. plasma, and single specimen vs. plasma pool;
- samples from patients with autoimmune diseases including systemic lupus erythematosus (SLE), antinuclear antibodies (ANA), rheumatoid arthritis mixed cryoglobulinemia;
- nucleic acid-based drugs, metabolites, and binding substances, particularly those known or suspected to have inhibitory effects on reverse transcription; and
- drugs commonly used for treatment of opportunistic infections associated with HIV, including ganciclovir, foscarnet, antimycobacterials, ribavirin, and alpha-interferons.

8. Reagent Characterization

   You should characterize the nucleic acid sequences (primers, probes, etc.), capture agents, enzymes, controls, and calibrators used in the assay. You should describe the rationale and methods used to qualify each lot of critical components. Please refer to the December 1999 Guidance in the Manufacture and Clinical Evaluation of In Vitro Tests to Detect Nucleic Acid Sequences of Human Immunodeficiency Virus Types 1 and 2, section VI, for further guidance (Ref. 6).

9. Sample Collection and Handling Conditions

   If two or more types of specimens are recommended for testing, you should determine the performance characteristics for each type of specimen, unless you can demonstrate that different specimen matrices, anticoagulants, etc., do not affect assay results differentially.

C. Stability

You should submit summary data verifying the stability of critical components (nucleic acid sequences, capture agents, enzymes, controls, calibrators, clones, or transcripts, as applicable). We will request unprocessed laboratory data, including line listings and actual data sheets, if the summary appears to portray the raw data erroneously (21 CFR 807.92(d)).

D. Assay Performance on Clinical Samples

1. Sensitivity on Clinical Samples

- In your sensitivity studies you should include a panel of 20 unspiked, specimens selected to have viral loads between the lower LOD and fourfold of the LOD (LOD X 4) whose genetic makeup has been determined by molecularly subcloning and sequencing 40 subclones each (or by equivalent techniques). You should determine the performance of the assay in these studies for as many of the mutations listed in Tables A and B as possible. To test the performance on Table A and B mutations that are rare, you should use specimens which do represent them and test them both neat and diluted to between LOD and LOD X 4 copies per ml. We recognize there may be a few cases where certain specific mutations are very rare and unable to be obtained. You need not perform the studies outlined in this section (VI.D.1) if you submit supportive clinical trial data showing the performance of the test as an aid in treatment of subjects with HIV (as described in Section VI.E).

- The Fully Verified Performance List should include only mutations detected in these studies (VI.D.1) at greater than or equal to 90% sensitivity (point estimate based on at least 10 measurements).

2. Population Sensitivity Studies

You should also determine how frequently, in a target population, the assay gives interpretable data. You should include in such studies 100 random clinical specimens with viral loads distributed throughout the clinically relevant, usable range of the assay, including a substantial number within the range of approximately LOD to LOD X 4.

3. Specificity on Clinical Samples

During the course of the clinical sensitivity testing, described above in VI.D.1, you should test a variety of defined samples, representing various combinations of wild type loci and resistance mutations. You should accumulate, analyze, and report data from these experiments concerning the nonspecificity of the assay (i.e., how often the assay reports an incorrect result at wild type loci).

4. Reproducibility on Clinical Samples

You should determine clinical reproducibility using specimens (as described in paragraph VI.D.1, above). Each specimen should be tested in triplicate, on different days, at different sites, by different investigators and using three different lots.

E. Clinical Trial Data Showing the Performance of the Assay as an Aid in Treatment of Subjects with HIV

You do not need to submit clinical trial data showing the performance of the test as an aid in treatment of subjects with HIV when you submit complete sets of analytical data, as described in sections VI.B.1 and 2, and VI.D.1, above. You may lessen the nature and extent of analytical studies, however, as described in sections VI.B.1 and 2, and VI.D.1, above, if you submit clinical data showing the performance of your test as an aid in treatment of subjects with HIV and if your labeling claims recognize this limitation. An example of an appropriate clinical trial would be a study comparing use vs. nonuse of the investigational assay, measuring clinical endpoints. Clinical endpoints could be AIDS-defining events, death, or acceptable surrogate markers, such as viral burden. Thus, we have identified two tracks for clearance, one relying on extensive analytical data and the other relying on limited analytical data in combination with clinical trial data showing the performance of the test as an aid in treatment of subjects with HIV, with specific limitations on claims made in the labeling. A summary chart that highlights the differences between these two tracks is presented in Table F.

Table F

Highlights of the Different Elements of the "Analytical-Data" and "Clinical Trial" Tracks for Clearance of HIV Drug Resistance Genotype Assays.

(Only differing elements are listed. See the body of the document for full discussion).

Section	**"Analytical Data" Track**	**"Clinical Trial Track," with data showing the performance of the test as an aid in treatment of subjects with HIV**
VI.E	No clinical trial data showing the performance of the test as an aid in treatment of subjects with HIV	Clinical trial data demonstrating use of sponsor's assay has benefit in treatment of subjects with HIV, as defined by clinical disease progression or surrogate markers.
VI.B.1.a and b	Stringent analytical sensitivity on cloned isolates or clinical samples, covering all mutations in Tables A and B.	Stringent analytical sensitivity studies on a subset of the mutations in Tables A and B. Mutations in Tables A and B not covered by these studies should be omitted from the list of mutations in the Fully Verified Performance list.
VI.B.1.c	Less stringent analytical studies on all mutations in Tables C, D, and E.	Less stringent analytical studies on mutations in Tables C, D, and E are desirable. Incompletely verified mutations should be listed in the Limitations for Use section.
VI.B.2. a and b	Titration of assay performance across various viral levels and wild type/mutant proportions on a subset of 30 of the mutations listed in Tables A and B, and the Fully Verified Performance list.	Mutations in Tables A and B not covered by studies on the titration of assay performance (across various viral levels and wild type/mutant proportions) should be omitted from the Fully Verified Performance list.

IX.B and VI.D.1	Test 20 clinical isolates whose swarms have been characterized by sequencing of multiple subclones. Mutations not successfully detected in these studies should not be listed in the Fully Verified Performance List.	You do not need to test the clinical isolates whose swarms have been determined by sequencing of multiple subclones. Mutations not successfully detected by such studies, however, should not be listed in the Fully Verified Performance List.

VII. OTHER CONSIDERATIONS

A. Design Controls

You should consult the FDA document "Design Control Guidance for Medical Device Manufacturers" (March 11, 1997).[4] You must adhere to the "Quality System Regulation" (QSR), found in 21 CFR Part 820, including design controls, to ensure adequate design of the entire system, from sample acquisition through data interpretation and reporting at sites of intended use.

B. Statistical Methods

All statistical methods used to prepare a 510(k) premarket notification should be appropriate for the study protocol, types of data collected, and intended use of the device. You should select statistical methods from recognized sources and properly reference them in the submission. We encourage you to discuss statistical methods with us during the planning phases of your studies.

C. Devices Used for Generating Data for Submission

You should perform all studies either with a product that is representative of the final product that will be marketed or one that can be related to that product through concurrent testing.

[4] See www.fda.gov/cdrh/comp/designgd.html.

D. Instruments and Software

You should include in your submission information about instruments that are components of the assay. You should describe the function, operating characteristics, and manuals for each instrument. You should validate all instruments and software for their intended use. You should provide instrument and software specifications; verification, validation, and testing documents; and a hazard analysis. You must follow the QSR during development of devices automated with software (see CFR 820.30 (a) (2) (i)). You should also refer to the "General Principles of Software Validation; Final Guidance for Industry and FDA Staff," January 11, 2002.[5] When submitting software/instrument applications to CBER, you should follow the "Guidance for Industry and FDA Staff: Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices," May 11, 2005.[6]

E. Pre-submission Meetings

We encourage you to meet with us prior to filing your submission to clarify current FDA policy, to discuss plans of proposed submissions, and to resolve any other questions.

VIII. PRODUCT MODIFICATION

When a product has been cleared for marketing through a 510(k) premarket notification mechanism, and you change or modify the device in a way that could significantly affect the safety or effectiveness of the device (21 CFR 807.81 (a)(3)(i)), or you make a major change or modification in the intended use of the device (21 CFR 807.81 (a)(3)(ii)), you must submit a new 510(k) premarket notification for the change and obtain clearance to market the changed device (807.81 and 807.100). Specific examples of when a new 510(k) premarket notification should be filed include, but are not limited to, changes to the indications for use, as expressed in the "Fully Verified Performance List,"changes in the technological characteristics, modifications of the interpretation algorithm, and changes in oligonucleotide components. Modifications to the interpretation algorithm may be handled as Special 510(k) submissions. If you are considering a change to your product, you should consult CDRH's Office of Device Evaluation's memorandum entitled "Deciding When to submit a 510(k) for a Change to an Existing Device," January 10, 1997, (Ref. 7). You may also contact CBER.

[5] See http://www.fda.gov/cdrh/comp/guidance/938.html
[6] See http://www.fda.gov/cdrh/ode/guidance/337.html.

IX. LABELING

The 510(k) premarket notification must include labels, labeling, and advertisements sufficient to describe the device, its intended use, and the directions for its use (21 CFR 807.87(e)). You must label your product in accordance with 21 CFR 809.10. We recommend the following in preparing labeling.

A. Intended Use Statement

The intended use statement should read, ". . . for use in detecting HIV genomic mutations that confer resistance to specific types of antiretroviral drugs, as an aid in monitoring and treating HIV infection."

- You should not mention specific mutations or loci in the intended use statement.

- Describe: the types of specimen(s) to be tested (serum, plasma, cells, etc.); method of collection (including whether any anticoagulants should be used); the analyte to be studied (DNA or RNA); the effective range of concentration of virus detectable; the viral subtypes for which a claim is sought; and the clinical situations in which use of the assay is appropriate.

B. Specific Performance Characteristics

- Include in this section of the package insert (21 CFR 809.10(b)(12)) the Fully Verified Performance list. This should be a list of all mutations for which analytical studies have been successfully completed as described in sections VI.B.1.a and b, VI.B.2.a, b, and c, and VI.D.1 of this guidance document.

- You should include in this "Fully Verified Performance List," only those mutations that your assay detects with sensitivity greater than or equal to 90% (point estimate based on at least 10 measurements) in the analysis of clinical isolates (Section VI.D.1) and at a sensitivity of at least 90% (point estimate, at least ten measurements) in the mixture studies performed on samples at the LOD (LOD for mutant level and proportion, VI.B.2). Thus, if you seek a claim for detecting down to 1,000 copies per ml. at a 40:60 ratio (mutant: wild type), then any mutations with sensitivity of detection below 90% when measured in a 40:60 ratio at 1,000 copies per ml. should not be listed in the Fully Verified Performance list.

- You should include in this list mutations not listed in Tables A and B if you perform full analytical studies on them as described in sections VI.B.1.a and b; and if you submit data that verify their clinical significance, to the extent the data justify the use of the mutations in the interpretation algorithm without associated precautionary labeling or disclaimers.

C. Reporting of Results

Interpretation and Reporting of Assay Results

For assays that require any kind of "rules-based" interpretation algorithm, you should provide, in the package insert, an interpretation algorithm to translate raw data into drug resistance profiles, and you should clearly describe the entire algorithm. The information provided in Tables A and B serves as an interpretation algorithm. You should incorporate in the assay interpretation algorithm all mutations listed in Tables A and B, above, without modification, together with their listed interpretations. You should incorporate in the assay interpretation algorithm mutations not listed in Tables A or B, above, or modifications of interpretations listed in Table A or B, above, if you present and summarize the data supporting each such proposed interpretation in the submission. You should include in such supporting data, original data, or data cited from peer-reviewed literature as described in section VI.A.1 and 2. In this section of the labeling, you should list any interpretation rule that is used in the algorithm.

Include in the interpretation algorithm incompletely verified interpretations if your report to the end user includes an indication that incompletely verified data was used whenever an interpretation is based on such information. You should also include in the package insert disclaimers warning that the clinical significance of the interpretations in question has not been fully verified. Furthermore, you should summarize the justification for such rules in the package insert, with references to the supporting literature and/or summaries of original, submitted data, as appropriate. If you use interpretation methodologies other than ones that are "rules-based," you should contact FDA for guidance on what information to provide in the labeling.

D. Limitations for Use

List all applicable limitations. If the device has software-generated interpretations, these limitations should be incorporated into the software. The following are examples of specific limitation statements that should be included in the package insert for your device:

- You should prominently list in this section, mutations in Tables A through E for which you have not performed the analytical studies outlined in this guidance document.

- You should also prominently list in this section, any mutations used in the interpretation algorithm for which you have not performed analytical studies outlined in this guidance document.

- You should indicate the approximate minimum detectable proportion of virus in the total population (e.g., a mutant at a level of 25% against a background of 75% wild type can be detected, but the same mutant at a level of 10% is not detected). You should also indicate the approximate minimum viral level (copies per ml.) at which the assay can give reliable data.

- You should also describe in the limitations section any interfering substances, conditions, or other factors that can affect the performance characteristics of the assay.

- You should state that assay results should be interpreted only in the context of other laboratory findings and the total clinical status of the patient.

X. REFERENCES

1. U.S. Food and Drug Administration, Center for Devices and Radiological Health, "The New 510(k) Paradigm - Alternate Approaches to Demonstrating Substantial Equivalence in Premarket Notifications - Final Guidance," March 20, 1998. http:/www.fda.gov/cdrh/ode/parad510.html.

2. U.S. Food and Drug Administration, Center for Devices and Radiological Health, "Device Advice, Content of a 510(k); 510(k) Summary or Statement"http://www.fda.gov/cdrh/devadvice/314312.html#link_7.

3. Perrin, L. and Telenti, A. (1998). HIV Treatment Failure: Testing for HIV Resistance in Clinical Practice, *Science, 280*, 1871-1873.

4. Hirsch, M.S. et al. (1998). Antiretroviral Drug Resistance Testing in Adults with HIV Infection: Implications for Clinical Management, *JAMA, 279(24)*, 1984-1991.

5. Hirsch, M.S. et al. (2000). Antiretroviral drug resistance testing in adult HIV-1 infection: Recommendations of an International AIDS Society-USA panel, *JAMA 283(18)*, 2417-26.

6. U.S. Food and Drug Administration, Center for Biologics Evaluation and Research, "Guidance for Industry In the Manufacture and Clinical Evaluation of In Vitro Tests to Detect Nucleic Acid Sequences of Human Immunodeficiency Virus Types 1 and 2," December 1999. http://www.fda.gov/cber/gdlns/hivnas.pdf.

7. U.S. Food and Drug Administration, Center for Devices and Radiological Health, Office of Device Evaluation, "Deciding When to Submit a 510(k) for a Change to an Existing Device," January 10, 1997, memorandum. http://www.fda.gov/cdrh/ode/510kmod.html.