Guidance for Industry

Premarket Notifications [510(k)s] for In Vitro HIV Drug Resistance Genotype Assays: Special Controls

DRAFT GUIDANCE

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research (CBER)
August 2001

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GUIDANCE FOR INDUSTRY
Premarket Notifications [510(k)s] for In Vitro HIV Drug Resistance Genotype Assays

This guidance document represents FDA's current thinking on special controls for HIV drug resistance assay premarket notifications [510(k)s]. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statutes and regulations.

I. INTRODUCTION

A. Purpose

We, the Center for Biologics Evaluation and Research (CBER), Food and Drug Administration (FDA) have issued this draft guidance to assist you, manufacturers and sponsors of HIV Drug Resistance Assays, to comply with the requirement of special controls for class II devices, if the HIV Drug Resistance Assay devices are reclassified from Class III. Designation of this guidance document as a special control would mean that you must establish that your device complies with either the specific recommendations of this guidance or some alternative control that provides equivalent assurances of safety and effectiveness [§513(f) (21 U.S.C 360c(f)]. You will help ensure the production of standardized, reliable, and reproducible tests for detecting HIV mutations known to be associated with HIV drug resistance, if you follow the recommendations in this document.

B. Definition

An HIV Drug Resistance Genotype Assay is an in vitro diagnostic device (IVD) intended for clinical laboratories to use in detecting HIV genomic mutations that confer resistance to specific anti-retroviral drugs, as an aid in monitoring and treating HIV infection.

C. Background

Clinically, HIV drug resistance testing has been shown to be useful for therapeutic guidance in monitoring or treating HIV infected individuals. We recognize that the mutations listed in Tables A and B (see below) are associated with HIV drug resistance. Other mutations, including those listed in Tables C-E (see below), are suspected of being associated with HIV drug resistance, but their significance has not been widely accepted.

HIV Drug Resistance Assays for which clinical trials have shown a clear medical benefit need further validation by analytical studies only to the degree necessary to characterize the scientific basis of the assay.

In this document, we describe two pathways for you to seek clearance of your assay as a Class II device for detecting HIV mutations. The first pathway is based on your

demonstrating rigorously the analytical sensitivity of your test for mutations in Tables A through E, below. The second pathway allows you to perform a less rigorous demonstration of the analytical sensitivity of your test for the mutations listed in Tables A-E, provided that data from clinical studies give evidence that use of the test will provide a medical benefit. We recognize that as the field progresses, additional mutations may become widely recognized as clinically significant. As advances are made in science and technology, we will amend the guidance as appropriate.

This guidance does not supersede other publications, but provides additional clarification on the information you should provide to us for review. You may refer to 21 CFR 807.87 for information that you must include in a premarket notification 510(k) for a medical device and to 21 CFR 809.10 for information about the labeling of in vitro devices. You are responsible for complying with the 21 CFR Part 820, Quality System Regulation for Class II or Class III devices, which includes Design Controls and Corrective and Preventive Action.

D. Regulatory Jurisdiction

Devices approved after 1976 for which there is no predicate device are generally classified as class III devices. However, FDA may reclassify such devices by using appropriate mechanisms. We believe that HIV Drug Resistance Assays may be suitable for reclassification and regulation as class II devices subject to special controls. This draft guidance document may serve as a special control if we reclassify these devices to class II. You should contact the Division of Blood Applications at CBER (301-827-3524) for information on filing your submission and for any questions you may have.

Analyte Specific Reagents

This guidance applies to HIV Drug Resistance Assays, but not to Analyte Specific Reagents (ASRs). ASRs are substances that are intended for use in a diagnostic application for identification and quantification of an individual chemical substance or ligand in biological specimens. ASRs are Class III devices when they are intended as a component in a test for use in the diagnosis of a contagious condition, such as HIV. We consider commercially distributed ASRs used in genotyping systems to detect HIV mutations to be class III devices requiring premarket approval.

II. SCIENTIFIC AND CLINICAL BACKGROUND

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 Anti-Retroviral Therapy - currently three and even four drug regimens) is 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. 1). 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. However, multiple problems are associated with the use of such genotyping assays. Generally they detect only the most prevalent members of the viral "swarm." So called "archived" species, which may have accumulated during development of resistance to previous anti-retroviral therapy and which may be remnant 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 anti-retroviral 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. 2).

We are providing this guidance to help you to assure the reliability of drug resistance genotype assays for recognized mutations and to show you how such assays may be developed and verified for review by the FDA as Class II medical devices. We are willing to work with you to determine the correlation between use of the assay and benefit to the patient for mutations that are currently not generally recognized as being associated with HIV resistance to anti-retroviral drugs.

III. DATA CONSIDERATIONS

We may request data and statistical analysis in premarket notification submissions to market in vitro diagnostic devices. The types of data and analysis that we may request depend on the technological characteristics of the new device, how you intend the device to be used, and the claims you intend to make for it. You can establish the performance of the device by comparison to any legally marketed medical device with the same intended use and/or by other studies to determine the operating characteristics of the device.

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.

You should submit:

- scientific data to support the performance characteristics of the device;
- documented protocols for in house and external testing;
- test results including analyses and conclusions; and
- summaries of results and explanations of unexpected results, charts (scatter grams, histograms, etc.).

You should submit unprocessed laboratory data, including line listings and actual data sheets when we specifically request them.

While it is not possible to list all scientific data that you might need to submit for a particular device, we have outlined the types of data and/or performance characteristics that you should consider including in a 510(k) submission to characterize the performance of the HIV Drug Resistance Genotype assay.

We believe that certain mutations in the HIV genome have been proven to be associated with viral resistance to specific anti-retroviral drugs used to treat HIV infection. We recognize that other mutations of interest in the HIV genome have not been proven to be associated with viral resistance to certain anti-retroviral drugs. We believe that you need only to provide analytical data demonstrating the ability of your tests to detect mutations in both these categories. However, we realize that the existence of supportive clinical trial data can increase confidence in the ability of an assay to be of benefit to the patient and that this increased confidence may reduce the nature and extent of analytical studies to assure assay effectiveness. For this reason, we are willing to accept less extensive analytical data on "established" and "implicated" mutations when supporting clinical trial data is submitted.

Thus, this document provides for two pathways to 510(k) clearance. In the first option, you may obtain clearance with extensive analytical data alone. In the second option, when you submit strongly supportive clinical data from trials using the investigational assay you may elect to submit limited analytical data. However, reliance on clinical data and less extensive analytical data may limit the claims of intended use set out in the labeling. (See Section VI.B of this guidance.)

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. You should include in your verification studies for mutations not listed in Tables A or B in vitro assays measuring the binding of the active form of the anti-retroviral inhibitor to its target substrate and in vitro viral replication assays (including determination of the effect of the given genotype on IC_{50} or IC_{90}). You should further support phenotypic predictions not

listed in Tables A or B by including clinical data, as outlined below in III.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: Clinical Studies

You should verify any phenotypic prediction not listed in Tables A or B, by clinical 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. 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 your claims, 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. Analytic Sensitivity

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

- In the Specific Performance Characteristics section of the package insert, you should list all mutations which 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 (III.B.1, a & 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 VI.B, below) as well as all mutations listed in Tables A and B. We may clear submissions that present data from an incomplete subset of the studies described in this and the immediately following sections (III.B.1.a & b) specifically limiting the Fully Verified Performance list to a subset of mutations for which sufficient analytical data has been provided, if data from clinical trials (see section III.E) using the assay support the clinical utility of the assay.
- 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. However, in the package insert, you must list all relevant potential codons (i.e., alternative sequences coding for the same amino acid) that were not specifically 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 ten (10) times in these studies, at or near the lowest viral level that the assay can reliably detect. When using a clinical specimen for these studies, you should determine the sequence of the 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:

i. the identity and number of loci tested;
ii. the number of times each was tested;
iii. the genetic context in which each was tested;
iv. the viral load tested (copies/ml);
(1) the overall sensitivity (number correctly identified /total); and

(2) a summary of lot distribution over the studies.

b. You should also test panels that include clones with known, preferably common, multiple mutations (i.e., multiple mutations which 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 ten times, using three different lots of the assay, at clinically relevant viral loads.

c. You should clearly characterize the clones particularly with respect to the identities of the mutations in each clone.
You should conduct studies similar to those described in III. B. 1. a & b, immediately above, to show that the assay can detect all mutations listed in Tables C, D & 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 paragraph.
You should demonstrate that mutations listed as "Primary" in these tables can be routinely detected at viral levels within four fold of the minimum levels for which a claim is sought.

You may demonstrate the detectability of "Secondary" mutations at any level within the useable range of the assay.
For mutations listed in Tables C – 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 III.B.1. a. & b.
We may clear your submissions presenting data from an incomplete subset of the studies described in this section (III.B.1.c.) if your data from clinical trials (see section III.E) using the investigational assay support the clinical utility of the assay. 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.

d. Generally, assays should correctly identify the amino acids at all codons in Protease and Reverse Transcriptase known or suspected to be involved in conferring drug resistance. We will consider for clearance on a case by case basis assays that fail to do so if they serve a specific and demonstrable public health need. In such cases, labeling for the assays should describe the device's more limited uses. A precautionary statement warning that the device has reduced sensitivity may be necessary, even if the codons that the device fails to read are only suspected of conferring resistance and are not fully verified.

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 levels and/or mutant proportions decrease.
Although you should determine the sensitivity and specificity at all loci specifically listed in the Fully Verified Performance list of the package insert (see VI.B, below), you need to fully evaluate only a representative set of 30 total loci for each parameter (viral level & mutant proportion) according to the criteria described in these sections (III.B.2.a, b & c). These 30 loci may consist of any of the loci listed in Tables A or B or in the Fully Verified Performance list.
We may clear submissions lacking studies described in these sections (III.B.2.a, b & c), and limit the Fully Verified Performance list to a subset of the mutations in Tables A and B for which you have provided sufficient analytical data, if there is strongly supportive data from clinical trials using the investigational assay.

a) Using the minimal proportions of mutant species in the range of detectability, you should determine assay performance at two-log (or smaller) intervals above the minimal viral levels in the range of detectability and up to the maximum level in the range of detectability. Similarly, you should determine assay performance at half-log intervals (or smaller) below (and including) the minimal level in the range of detectability, and down to 1.0 log below minimum. Thus, if 30% is the minimal proportion of mutant species that the assay can reliably detect and 1000 copies/ml is the minimal viral level at which the assay can reliably obtain sequences, you should test the following levels of virus mixtures (in copies/ml, containing 70% wild type and 30% mutant): 100; 300; 1000; 100,000 (or less); 10,000,000 (or less, but not to exceed 100 times the next lower level tested, nor to exceed the useable range of the assay).

b) Using the minimal viral levels in the range of detectability, you should determine assay performance at approximately 100%, 80% and 50% of the minimal mutant proportions in the range of detectability, as well as at least two higher levels selected to be equally spaced (linearly) between the minimal proportion and 100% proportion. You should also test mutant at a proportion of 100%. (Thus, for example, if you seek a claim for 25% mutant the following proportions of mutant would be tested: 100%, 75%, 50%, 25%, 20% and 12.5%.)

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

3. Precision

For assays which 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 additionally at higher levels, at your discretion.

4. Reproducibility

You should determine assay reproducibility 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.

5. Lot acceptance testing

You should perform lot acceptance testing to assure 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. All amino acid mutations for which a claim is sought should be tested, at least singly.

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 we may expect to cause interference include:

a. 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*;

b. Samples collected in various anticoagulants, or other collection media;

c. Hemolyzed, icteric, lipemic, and bacterially contaminated samples;

d. Chemicals, drugs, heated, and detergent treated samples;

e. Samples subjected to multiple freeze thaw cycles;

f. Fresh vs. frozen samples, serum vs. plasma, and single specimen vs. plasma pool;

g. Samples from patients with autoimmune diseases including Systemic Lupus Erythematosus (SLE), Anti-Nuclear Antibodies(ANA), Rheumatoid Arthritis mixed cryoglobulinemia;

h. Nucleic acid based drugs, metabolites and binding substances, particularly those known or suspected to have inhibitory effects on reverse transcription.

i. Drugs commonly used for treatment of opportunistic infections associated with HIV, including ganciclovir, foscarnet, anti-mycobacterials, 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 III. for further guidance. (Ref. 4)

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 determine the stability of critical components (nucleic acid sequences, capture agents, enzymes, controls, calibrators, clones or transcripts, as applicable) and should maintain files with the raw data for inspection by FDA. You do not have to submit this data to us unless specifically requested to do so, but may provide the data in summary form.

D. Assay Performance on Clinical Samples

1. Sensitivity on Clinical Samples.

In your Sensitivity studies you should include a panel of 50 unspiked, repository specimens selected to have viral loads between the lower limit of detection (LoD) and fourfold of the LoD (LoD X 4) whose genetic make up has been determined by molecularly subcloning and sequencing 40 subclones each (or by equivalent

techniques). Selection of the clinical specimens should be random for characteristics other than viral load. You should determine the performance of the assay in these studies for all mutations listed in Tables A and B. To test the performance on Table A and B mutations not adequately represented in the randomly selected panel of 50 clinical samples, you should acquire specimens which do represent them and test them both neat and diluted to between LoD and LoD X 4 copies per ml. We will consider exceptions in cases where certain specific mutations are very rare and unlikely to be obtained. We may clear submissions lacking the studies described in this section (III.D.1) with the Fully Verified Performance list limited to the subset of the mutations in Tables A and B for which sufficient analytical data has been provided, but only if there is strongly supportive data from clinical trials using the investigational assay included in the submission.

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, useable 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 immediately above in III.E.2, 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 non-specificity 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 these repository specimens (as described in paragraph III.D.1, above). Each specimen should be determined in triplicate, on different days, at different sites, by different investigators and using three different lots.

E. Clinical Trial Data Supporting Efficacy

You do not need to submit clinical trial data demonstrating the efficacy of your assays for clearance when you submit complete sets of analytical data, as described in sections III.B.1 & 2 and III.D.1, above. However, you may lessen the nature and extent of analytical studies, as described in sections III.B.1 & 2 and III.D.1, above, if you submit supportive data from clinical trials directly demonstrating that the use of your assay results in clinical benefit and if you are willing to accept specific restrictions on certain claims made in the labeling. An example of an appropriate clinical trial would be a study comparing use vs.

non-use 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 requiring extensive analytical data and the other requiring limited analytical data in combination with clinical trials and entailing specific limitations on claims made in the labeling. A summary chart that highlights the differences between these two tracks is presented in Table F.

F. Modifications of Criteria for Special Purpose Assays

We may clear "special purpose" assays with limited claims for subsets of the mutations listed in Tables A and B, if you successfully demonstrate that doing so would benefit the public health.

Table A (Ref. 3)

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
V75I	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 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), stavudine (D4T) **All NNRTI = nevirapine (NVP), delavirdine (DLV), efavirenz (EFV)

Table B (Ref. 3)

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	
L90M	ALL PIS	More strongly associated with SQV or NFV but in combination with other mutations may confer resistance to all PI

ALL PIS = APV (amprenavir), IDV (indinavir), LPV/RTV (lopinavir/ritoanvir), NFV (nelfinavir), SQV (saquinavir), RTV (ritonavir)

Table C (Ref. 3)

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 -Ref. 3).

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. 3)

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 -Ref. 3).

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. 3)

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 -Ref. 3).

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

Table F

Highlights of the different requirements required by the "Analytical-Data-Only" and "Clinical Trial" tracks to clearance.

[Only differing requirements are listed. See the body of the document for full requirements.]

Section	"Analytical Data-Only" Track	"Clinical Trial Track"
III.E	No clinical trial data	Clinical trial data demonstrating use of sponsor's assay has benefit defined by clinical progression or surrogate markers.
III.B.1 a & b	Stringent analytical sensitivity on cloned isolates or clinical samples, covering all mutations in Tables A & B.	Stringent analytical sensitivity studies on a subset of the mutations in Tables A & B. FDA may require mutations in Tables A & B not covered by these studies to be omitted from the list of mutations in the Fully Verified Performance list.
III.B.1.c	Less stringent analytical studies on all mutations in Tables C, D & E.	Less stringent analytical studies on mutations in Tables C, D & E are desirable, but not required. FDA may require incompletely verified mutations to be listed in the Limitations for Use section.
III.B.2. a &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&B and the Fully Verified Performance list.	FDA may require mutations in Tables A & B not covered by studies on the titration of assay performance (across various viral levels and wild type/mutant proportions) to be omitted from the Fully Verified Performance list.
III.D.1	Analytical sensitivity on a panel of 50 clinical specimens (characterized by sequencing 40 subclones each), covering all mutations in Tables A & B.	Full set of 50 panel members not required. FDA may require mutations in Tables A & B not covered by these studies to be omitted from the Fully Verified Performance list.
VI.B	All mutations in Tables A and B may be listed in the "Indications for Use" statement.	The only mutations listed in the "Fully Verified Performance list will be those for which the sponsor has submitted full analytical data, equivalent to that required in the "Analytical-Data-Only" track.

IV. OTHER CONSIDERATIONS

A. Design Controls.

You should consult the FDA document "Design Control Guidance for Medical Device Manufacturers" (March 11, 1997); and the regulations in 21 CFR Part 820 to assure adequate design of the entire system, from sample acquisition through data interpretation and reporting at sites of intended use.

B. Statistical Methods.

You should ensure that all statistical methods in a 510(k) are appropriate for the study protocol, type 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 these aspects 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 which is representative of the final product that will be marketed or one that can be related to that product through concurrent testing.

D. Instruments.

You should make information about instruments that are dedicated components of the assay part of the submission. You should describe the function, operating characteristics, and manuals for each instrument.

E. Pre-submission meetings.

We encourage you to meet with us prior to filing your submission to clarify current FDA policy and to resolve questions not met by current guidance.

V. PRODUCT MODIFICATION

When a product has been cleared for marketing through a 510(k) mechanism, and modifications are made to the product that alter its indications for use and/or change the fundamental scientific technology, you must submit a new 510(k) for the change and obtain clearance to market the changed device. Specific examples of when a new 510(k) should be filed include, but are not limited to, new labeling for genotypic prediction of phenotypic resistance for new anti-viral drugs, new labeling for newly discovered mutations or mutations with newly documented phenotypes, or material changes in the interpretation algorithm. You should consult the 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. 5) if you are considering a change to your product.

VI. LABELING

A. Intended Use

- The intended use statement should read, "…for use in detecting HIV genomic mutations that confer resistance to specific types of anti-retroviral drugs, as an aid in monitoring and treating HIV infection."
- You should not mention specific mutations or loci in the intended use statement.
- You should label your product in accordance with 21 CFR 809.10 including: the types of samples (serum, plasma, cells, etc.); method of collection (anticoagulants); 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

- You should include in this section of the package insert the "Fully Verified Performance" list. This should be a list of all mutations for which full analytical studies have been successfully completed according to the requirements of sections III.B.1 a & b of this guidance document.
- You may include in this list mutations not listed in Tables A and B if you perform full analytical studies on them as described in sections III.B.1 a & b and, if you submit data that verifies their clinical significance to the extent that it justifies their use in the interpretation algorithm without associated precautionary labeling or disclaimers.

- We may reduce the scope of this list if you have not successfully completed all of the studies recommended in sections III.B.2 and III.D.1 of this guidance document.

C. Directions for use

Interpretation and Reporting of Assay Results

You should provide, in the package insert, an interpretation algorithm to translate raw data into indications of drug resistance profiles. You should clearly describe the entire algorithm. At a minimum, the information provided in Tables A and B may serve as an interpretation algorithm. You may incorporate in the assay interpretation algorithm all mutations listed in Tables A and B, above, without modification, together with their listed interpretations. You may 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 may include in such supporting data, original data, or data cited from peer reviewed literature as is described in section III.A.1 & 2. In this section of the labeling, you should list any interpretation rule that is used in the algorithm, but that is not specifically listed in Tables A or B, or that relies upon a mutation that has not been otherwise qualified to be included in the Fully Verified Performance list. In some cases, it may be appropriate for you to include in the interpretation algorithm an incompletely verified interpretation rule if the package insert identifies that interpretation rule and states (1) that incompletely verified data were used to support it; and (2) that the clinical significance of the interpretation rule has not been fully verified. Furthermore, you should summarize the justification for any such rule in the package insert, with references to the supporting literature and/or summaries of original, submitted data, as appropriate.

D. Limitations for Use

You should prominently list in this section, mutations in Tables A-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.

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

VII. REFERENCES

1. Perrin, L. & Telenti, A (1998); Science 280, 1871-1873.

2. Hirsch, M.S. et al. (1998); JAMA 279, 1984-1991.

3. Hirsch MS, Brun-Vezinet F, D'Aquila RT, Hammer SM, Johnson VA, Kuritzkes DR, Loveday C, Mellors JW, Clotet B, Conway B, Demeter LM, Vella S, Jacobsen DM, Richman DD "Antiretroviral drug resistance testing in adult HIV-1 infection: recommendations of an International AIDS Society-USA Panel." JAMA. 2000 May 10, 283(18), 2417-26.

4. Guidance 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.

5. "Deciding when to submit a 510(k) for a Change to an Existing Device," January 10, 1997, Office of Device Evaluation, CDRH, FDA. http://www.fda.gov/cdrh/ode/510kmod.html.

6. "Design Control Guidance for Medical Device Manufacturers", March 11, 1997, Office of Device Evaluation, CDRH, FDA. http://www.fda.gov/cdrh/comp/designgd.pdf.