

# Guidance for Industry

## In the Manufacture and Clinical Evaluation of *In Vitro* Tests to Detect Nucleic Acid Sequences of Human Immunodeficiency Virus Type 1

### DRAFT GUIDANCE

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**Guidance for Industry<sup>1</sup>**  
**In the Manufacture and Clinical Evaluation of *In Vitro* Tests to  
Detect Nucleic Acid Sequences of Human Immunodeficiency  
Virus Type 1**

**I. INTRODUCTION**

In March 1985, the U.S. Food and Drug Administration (FDA) licensed the first screening test for the detection of antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) in serum and plasma from infected individuals. As of April 1998, there were 24 licensed kits and 5 premarket approvals (PMAs) for detection of antibodies to HIV-1 or Human Immunodeficiency Virus Type 2 (HIV-2) in blood, saliva, or urine, these included: 18 Enzyme-Linked Immunosorbent Assays (ELISAs), 3 Western Blots, 1 Particle Agglutination Assay, and 1 Indirect Immunofluorescence Assay (IFA) for detection of antibodies to HIV-1, as well as 3 ELISAs for detection of HIV-1 p24 antigen, 2 of which are for use in donor screening, and 4 combination ELISAs for detection of antibodies to HIV-1 and HIV-2, for use with blood specimens. FDA recently licensed the first test for the detection of HIV-1 antibodies in urine specimens. HIV ELISAs have been approved to screen blood and plasma donors. The IFA, the Rapid Latex Agglutination Assay, and the colorimetric Single Use Diagnostic System (SUDS), may be used to screen blood donors in urgent situations, but more often are used for urgent testing in hospitals, laboratories, medical clinics, physician's offices, emergency care situations, blood banks, or other health care settings when a routine ELISA is unavailable or impractical. Repeatedly reactive results from screening assays are further evaluated by additional, more specific tests which include Western Blot and IFA. In December 1994, FDA approved the first oral fluid collection device for professional use with a licensed HIV-1 antibody test kit and in June 1996, a supplemental Western Blot test to further evaluate the presence of antibodies in oral fluid was approved. Two PMAs for home blood sample collection kits, labeled as part of a HIV testing system, were also approved by FDA in 1996 (one has been voluntarily discontinued). The first PMA for a HIV-1 nucleic acid test, a quantitative HIV-1 ribonucleic acid (RNA) test based on amplification of deoxyribonucleic acid (DNA) sequences using the polymerase chain reaction, to measure viral load in plasma as an aid in determining patient prognosis, was approved by FDA in June 1996.

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<sup>1</sup>This guidance document represents the Agency's current thinking on *in vitro* testing to detect specific nucleic acid sequences of HIV-1. 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 statute, regulations, or both.

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In recent years, several technical advances have been made in methodologies for direct detection of viral nucleic acid. This document, when finalized, will provide guidance on manufacturing and clinical trial design issues pertaining to the validation of tests based on nucleic acid detection either in the presence or absence of an amplification step. Concerns regarding the procedures used for detection of amplified products will also be addressed. As may be the case with new technologies, issues may be identified during the review process, unique to the particular methodology under review or the specific configuration of the assay, that will need to be addressed on a case by case basis. It is also recognized that this area of science is in a state of rapid technological development. As advances are made, this document, when finalized, will be re-evaluated and revisions or modifications made as necessary. The criteria outlined below address both general and specific concerns for gene based detection techniques for HIV.

The reader is referred to the "Points to Consider in the Manufacture and Clinical Evaluation of *In Vitro* Tests to Detect Antibodies to the Human Immunodeficiency Virus Type 1" (1989) (1) for general information on filing of the Investigational New Drug Applications (INDs), Product License Applications (PLAs), Establishment License Applications (ELAs), and content of applications for approval and licensure of retroviral kits. The general regulations are in 21 CFR parts 312, 600-680 and 800. Other documents that may be pertinent to this topic include the "Review Criteria" document (2) published by the Center for Devices and Radiological Health (CDRH).

As set forth in the Inter-Center Agreement of 1991 between the Center for Biologics Evaluation and Research (CBER) and CDRH, *in vitro* tests for HIV which are required for blood donor screening and related blood bank practices are licensed under the Public Health Service Act (PHS Act) through the IND/PLA/ELA mechanism. *In vitro* tests for HIV which are not performed in relation to blood bank practices (e.g., other diagnostic tests) will be regulated by CBER under the Medical Device Authorities through the Investigational Device Exemption (IDE)/Premarket Approval Application (PMA) mechanism.

The scientific and regulatory concerns pertaining to validation of *in vitro* diagnostic/screening test kits can be broadly classified into three main categories: 1) intended use; 2) manufacturing; and 3) clinical validation of assay performance.

## **II. INTENDED USE**

The sponsor should state clearly in the IND and PLA, the intended use, the labeling claims, and the clinical utility for the product. The proposed clinical trial design should demonstrate satisfactory assay performance to validate the intended use claim in the target patient population and the specific test setting. It is recommended that the sponsors/manufacturers meet with CBER to obtain guidance early in the development process in order to resolve any issues with regard to an approvable claim for the product, special concerns for the product,

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and questions manufacturers may have. This should include a proposed clinical utility including equivalence or superiority to existent methods or licensed tests, if available, for detection and/or quantitation of the agent.

### **III. MANUFACTURING**

The manufacturing issues that may impact on product design and performance are: 1) rationale of assay design; 2) assay optimization; 3) sample collection, extraction, storage and stability; 4) manufacture of primers, probes, reagent buffers, enzymes, calibrators, controls, and quantitation standards; 5) anchoring components, i.e., beads, plates, chips; 6) kit stability; and 7) instrumentation. This document, when finalized, will attempt to address concerns pertaining to each of the points outlined above.

#### **A. Rationale and Design**

The sponsor should provide in the IND and PLA, the rationale for the specific indication and for use of the specific test methodology, and type of nucleic acid target (DNA or RNA) for detection of the infectious agent and for the specific indication. A detailed description of all aspects of the technique including sample preparation, assay optimization, amplification and detection methods should be provided. Quality control procedures that are state-of-the-art should be used to assure manufacturing consistency. The sponsor should provide details of assay optimization and establish the format of the final product during the preclinical stage of development. Changes in assay format may necessitate new studies. The rationale for assay design should address the following aspects:

1. Selection of target sequence including the degree of gene sequence conservation, guanine:cytosine (GC) ratio, and length);
2. Selection of single or multiple target gene sequences (multiplex based detection);
3. Assay format (e.g., sample type, conjugate, detector, etc.);
4. Selection of primer and probe sequences (e.g., degree of gene sequence conservation); and
5. Design and nature of the quantitation standards for a quantitative assay.

#### **B. Assay Optimization**

This phase is critical to product development and has significant impact on product performance. The sponsor should address the various aspects of optimization for nucleic acid extraction, target sequence, amplification, detection, quantitation, and instrumentation for these processes, and set specifications for performance. The IND, PLA, or both, should contain information on the details of:

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1. The length, region, specificity, and efficiency of primer or capture sequence;
2. Methods of extraction, amplification, hybridization, detection, and quantitation;
3. Percent recovery of nucleic acid for the total assay and for each significant step in the process;
4. Optimization of reaction conditions and kinetics of amplification with multiple primers or hybridization with multiple probes or both (e.g., for a multiplex format);
5. Internal and external assay calibrators/controls; and
6. Procedures to prevent cross-contamination.

During this phase of assay optimization, the sponsor should determine and define the optimal assay conditions, reaction kinetics and lower limit of detection (LOD) of the assay. This limit should be well-defined in terms of copy numbers and the unit of sampling. This limit should be validated by an independent method, e.g., another amplification technique or another type of assay. The cut-off point can be established by in-house testing and should be further defined or verified based on the data from clinical trials. If the assay is quantitative, the following aspects should be addressed:

7. Linearity in the readable range in order to ensure accurate interpolation of unknown specimens. This range should also be clinically meaningful to demonstrate the clinical utility of quantitation; and
8. The assay should be capable of accurate and reproducible quantitation of analytical specimens on a standard curve (analytical sensitivity) and have a defined LOD and lower limit of quantitation.

### **C. Sample Preparation**

The sponsor should specify the type of specimen (e.g., cells, plasma, whole blood, dried blood spots, etc.) and the template for amplification (DNA and RNA) and hybridization, as appropriate. The composition of the buffers, reagents, and detergent or chaotropic agents used for nucleic acid extraction should be clearly specified. The effect of anticoagulants and any potential inhibitors present in the sample or extraction buffers on assay performance should be evaluated.

Controls that monitor the efficiency of the extraction and reverse transcription (when the template is RNA) procedures should be included and whenever possible, these controls should simulate the actual sample type. Spiked controls are also acceptable.

The reproducibility of the sample preparation method should be determined under the specimen processing conditions including sample handling, storage, and shipping conditions. The sponsor should also verify possible interference of specimen processing reagents with reverse transcription, amplification, hybridization, detection, and quantitation.

#### **D. Primers and Probes**

The primers and probes are the main components of a nucleic acid based detection system and the performance of the assay is highly dependent on the quality of these reagents. The sponsor should provide the:

1. Rationale for selection of primers and probes including specific sequences used;
2. Justifications for alignments made to generate consensus sequences or best-fit modifications made to existent sequences, e.g., to permit maximum homology to several strains; and
3. Information on size, GC content, melting temperatures, hairpin or other secondary structures if any, and the nucleotide position on the genome map of the primers and probes.

If synthetic oligonucleotides are used as primers and probes, details of the manufacture and purification should be provided. The following additional information should be included:

4. The yield and composition for the first 5 lots by absorbance and DNA fingerprinting or restriction endonuclease mapping;
5. A description of the chemical nature of the modification, for modified oligonucleotides and procedure(s) to ensure lot to lot consistency of ligand content;
6. Nucleotide sequence analysis to establish the fidelity of the procedure for oligonucleotide synthesis;
7. The purity of the final product should be analyzed by reverse phase high performance liquid chromatography, electrophoresis, or other appropriate state-of-the art analytical techniques; and
8. Potency of primers and probes, e.g., by dilutional analysis.

#### **E. Reaction Buffers**

The sponsor should demonstrate the identity and purity of reagents used in the preparation of reaction buffers that may be employed in amplification, hybridization and detection reactions. The potency and stability of the reagents on storage and under cycling conditions should be verified. If reagents are obtained from vendors, a certificate of analysis should be provided for purposes of verification and acceptance testing defined. The sponsor should also be aware that the current good manufacturing practice regulations require a sponsor to sample and test components if deviations from component specifications could result in the product being unsuitable for use.

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**F. Enzymes**

The source and function of all enzymes used in the assay should be identified and clearly defined. The identity, purity, potency, and specific activity should be demonstrated and criteria for acceptance established.

For rDNA-derived enzymes manufactured by the sponsor, the master and working cell banks should be characterized for cell and genetic stability, and freedom from adventitious agents. Plasmid stability should be monitored by assays that include restriction mapping. The amino acid composition and sequence should be established by peptide mapping and amino acid sequencing.

Enzyme preparations should be tested for other enzymatic activities, e.g., exonucleases and DNA and RNA dependent polymerase activities and specifications should be established. For enzymes obtained from vendors, the certificate of analysis should be provided. In addition, functional testing designed to assure that the component is suitable for its intended use, should be performed as part of establishing the acceptance criteria.

**G. Controls/Calibrators**

Controls ensure that the assay has performed within accepted specifications and are, therefore, a vital component of any test kit. Controls should be separate from, and in addition to, reagents used for calibration.

In nucleic acid analysis, there are several steps in the testing process, as outlined above, each of which should be monitored and verified. It is therefore necessary to include multiple controls or controls that serve multiple purposes in the final kit. These should include controls for extraction, amplification, quantitation, contamination, etc. These controls should be similar to the specimen type whenever feasible although spiked controls may be acceptable, particularly for labile analytes. It is highly recommended that sponsors include one or more positive controls, including one close to the LOD and quantitation of the linear range of assay.

Multiple negative controls should be included such as non-target sequences and nucleic acid free controls, to monitor false positivity due to contamination. Due to the high sensitivity of amplification assays, it is highly recommended that sponsors include control measures for prevention of contamination events.

Specifications should be defined for both positive and negative controls, validation data for derivation of the cut-off value of the assay, and for the specifications of controls should be provided. The sponsor should define the source of the controls and calibrators, and have a plan for their continued renewal. Controls should be non-infectious, and validation for viral inactivation should be provided.



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For quantitative assays, validation data should be provided for all quantitation standards and calibrators. Specifications and acceptance criteria should be established for each control/calibrator and for the collective set of controls. Quantitation should be based on co-amplification of heterologous internal control and/or a competitive RNA template or other compatible methods. For RNA assays, the efficiency of reverse transcription should be determined for the specific assay format.

**H. Other Test Kit Components**

The sponsor should provide a description of the anchoring solid phase component (e.g., plates, beads, filters, etc.), concentration of antigen or oligonucleotide on the component method of conjugation, or binding to the component, and a demonstration of lot to lot consistency of manufacture of bound component.

If more than one component is used for coating (e.g., two oligonucleotides) a description of the validation of coating methods including ratios used and acceptance criteria for the coating process should be provided. If the sponsor purchases a solid phase component (e.g., beads, plates, chips) a description of the source, quality assurance methods, and acceptance criteria should be included.

**I. Detection and Quantitation of Amplicons**

A detailed description of the chemical/biochemical nature of capture probes, conjugates, detectors, etc., which are part of the assay system should be provided. This should include:

1. The chemistry and limits of detection of system of choice (e.g., chemiluminescence, fluorescence, etc.);
2. Chemical and biochemical characterization of the ligand, chromophore, fluorochrome, including stability under reaction conditions;
3. Quality control and assurance of conjugation to detector or capture oligo- or polynucleotide sequence, including functional testing; and
4. Nature and copy numbers of the quantitation controls and standards.

Validation of controls and specifications should be provided for the individual and collective set of controls used for quantitative nucleic acid assays.

**J. Instrumentation**

Any dedicated equipment used in the amplification, detection and quantitation of the amplified product should be validated for its use. These would include devices such as thermal cyclers,

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waterbaths, and cycling ovens, etc.

Validation of thermal cyclers should include demonstration of the accuracy of temperatures of individual wells during the cycling process, specify limits for well-to-well variation, if any, as well as any impact there may be on test results. If software is utilized for amplification, detection, and calculation of quantitative or qualitative results, validation of such software for the intended function should be provided.

For non-dedicated instruments, the premarket notification (510k) submission number should be cited for review. If previously approved under PMA, a supplement for use with the product under review should be submitted.

If special specimen collection, storage and/or transport devices are used, specifications should be provided for conditions of collection, storage and transport. Criteria should be established for suitability and adequacy of the specimen for the test.

**K. Sterility/Bioburden**

Refer to the "Points to Consider in the Manufacture and Clinical Evaluation of *In Vitro* Tests to Detect Antibodies to Human Immunodeficiency Virus Type 1" (1989) for guidance in this area.

**L. Kit and Component Stability**

The stability of the final kit and individual components should be tested using a panel of specimens, including weak reactives (e.g., near cut-off, middle, and upper end of the readable range). The real time stability at storage and shipping temperatures should be evaluated using specimens with varied reactivities in the readable range.

**IV. CLINICAL VALIDATION OF ASSAY PERFORMANCE**

**A. Preclinical Studies**

Preclinical studies are performed as a preliminary evaluation of assay performance while clinical validation through testing of statistically significant appropriate clinical specimens will provide final specific performance characteristics of the test kit. In general, preclinical testing should be performed prior to a large scale clinical trial for all clinical indications. Preclinical studies can be performed in-house or at field sites. Studies should be aimed at obtaining a preliminary assessment of the sensitivity, specificity, and reproducibility of the test kit, as well as defining the lower limit of reproducible detection, quantitation, and the cut-off for the assay.

**1. Specificity and Sensitivity Studies for Preclinical Testing**

- a. Specificity should be established by testing a minimum of 500 random blood or plasma donors; and
- b. Sensitivity should be established by testing at least 300 seropositive repository specimens, including 100 known to be positive for HIV-1 antigen.

Testing should be performed with licensed comparator assays including antibody, antigen, and other nucleic acid detection technologies, whenever feasible.

**2. Analytical Sensitivity**

Analytical sensitivity should be evaluated by testing dilutional series of at least 10 HIV positive samples. The highest dilution reproducibly and consistently detected and/or quantitated by the investigational assay should be defined in copies per unit sampling. Comparator assays should include antibody, antigen, and other state-of-the-art amplification/probe technologies.

**B. Clinical Trials: General Issues**

Clinical trials designed to assess clinical sensitivity, specificity, and reproducibility should be performed at clinical trial sites by qualified independent investigators. Refer to Section VI. of the "Points to Consider in the Manufacture and Clinical Evaluation of *In Vitro* Tests to Detect Antibodies to the Human Immunodeficiency Virus Type 1" (1989) for additional general guidance on clinical trial design issues.

Common elements of a clinical trial for any specific intended use are studies designed to assess the precision (reproducibility), and nonspecificity of the investigational assay.

**1. Precision studies**

These studies are designed to assess the coefficient of variation for the test results for each sample and for the various lots tested.

- a. Reproducibility Testing

A panel of 10 or more samples including low reactives should be tested at all clinical trial sites. This could be a series of samples spiked with the analyte or human specimens with known reactivity. Samples should

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be tested in duplicate or triplicate. Testing should be performed on multiple days and lots by at least two operators. This type of a study should be designed to assess the intra and inter-site, intra and inter-assay variability, intra and inter-lot variability, and total variability for both qualitative and quantitative assays.

b. Proficiency Testing

A panel of samples similar to that described above should be tested by a number of operators on multiple days and using different kit lots, at all clinical or testing sites. This study is designed to assess operator proficiency.

**2. Non-specificity studies**

Most assays have some inherent non-specific reactivity resulting in false positive reactions or interference resulting in false negative reactions which may be due to specific assay components or the nature of the sample being tested. Such reactivity should be assessed using a variety of specimens that may be expected to cause interference in the assay or to be present in test samples. These samples can be obtained by spiking the agent into known HIV positive and negative samples, in addition to testing original specimens. Some conditions that may be expected to cause cross-reactivity or interference include:

- a. Other infections including HIV-2, Human T-cell Lymphotropic Virus Type I/II (HTLV-I/II), Cytomegalovirus (CMV), Epstein-Barr Virus (EBV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), yeast infections, and pneumocystis;
- b. Samples collected in various anticoagulants, each type, 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), and Rheumatoid Arthritis;
- h. Nucleic acid based drugs and metabolites and binding substances; and

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- i. Drugs and biologicals increasing circulating nucleic acid.

**C. Specificity and Sensitivity Studies for Test Kits with a Proposed Labeling for Screening of Blood and Plasma Donors**

FDA does not currently envision discontinuation of antibody testing for donor screening. However, nucleic acid testing may be the most sensitive method currently available for early detection of a virus during the pre-seroconversion phase of infection and may, therefore, have added value in blood safety. Clinical specificity should be established by testing a large number of specimens from random U.S. blood and plasma donors. Testing should be performed at multiple distinct clinical sites (at least 2) and clinical sensitivity should include samples from at least 10,000 individuals. The trial sites should represent areas of both high and low prevalence. Ideally, studies in areas of high prevalence should be performed in a linked fashion to permit clinical follow-up. If positive results are encountered, the donor should be deferred temporarily and units held in quarantine until test results are confirmed by repeat testing or by clinical follow-up and testing subsequent to a 3-6 month period. The basis for reinstatement of donors with false-positive investigational test results should be defined in the study protocol.

Clinical sensitivity may be established by testing a total of at least 1,000 specimens from seropositive individuals in various risk groups and from different stages of the disease. It is recommended that the study should include a gender based analysis with at least 20-30% derived from females. Geographically diverse specimens (minimum 20-30) representing various known viral subtypes should be included in this data set to evaluate the performance and establish the sensitivity of the kit for detection of variant HIV-1 strains. Testing should include specimens from individuals infected with the various subtypes of HIV-1. At least 300 of the seropositive specimens should be derived from persons with a clinical diagnosis of AIDS. A comparison should also be made between freshly drawn and frozen specimens, paired serum and plasma specimens or other specimens (whole blood) in a subset of this seropositive population to demonstrate the equivalence of the two storage conditions and the two types of specimens, respectively.

Prospectively collected, freshly drawn specimens from high risk individuals should be tested in a linked study so that a minimum of 50 positive cases are identified to estimate sensitivity in a high risk population. The study should include methods to objectively resolve discordances between investigational and comparator assays that include antibody and antigen assays. This may require follow-up testing of the study subjects. A subset of these samples (100) should be tested by another state-of-the-art test (amplification/probe test).

The limited availability of specimens in the antibody negative, pre-seroconversion phase, necessitates the use of specimens from the following categories for clinical validation of assay performance: ongoing cohort studies and lookback investigations; and a large number of

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seroconversion panels and specimens from high risk individuals enrolled in prospective studies being conducted in areas of high prevalence. These data should be analyzed on the basis of mathematical models for the window period. Testing should include a head-to-head comparison with a licensed assay for HIV p24 antigen.

If testing will be done on pooled specimens, additional issues will need to be addressed, to include:

1. Demonstration of enhanced sensitivity or equivalence of testing pools to currently licensed methods for donor screening by laboratory and field testing;
2. The impact, if any, of possible matrix effects, generated during pooling, on test performance;
3. Procedures for logging and tracking of inventory of specimens in a given pool;
4. Specimen retrieval procedures to identify a positive specimen in a positive pool;
5. Quality assurance in computing and reporting test results;
6. Validation of instrumentation and automation; and
7. Validation of software that may be used in conjunction with any of the procedures listed above.

Testing of specimens from appropriate primate models may also be useful towards establishing utility for detection in the pre-seroconversion phase.

**D. Studies to Validate Intended Use as Additional, More Specific Tests**

These tests are used to further evaluate the accuracy of the positive test results of a screening assay. Gene based tests may be developed as an alternative to Western Blot (WB), Strip Immunoblot Assay (SIA), or Immunofluorescence Assays (IFA) currently in use for this purpose. In some instances, the test may be used to resolve the indeterminate patterns seen on additional, more specific tests including those referred to above.

In specificity and sensitivity testing for confirmatory tests, random donors should be tested at two or more sites. At least 500 samples should be tested to assess specificity. Approximately 300 specimens from random donors that are repeatedly reactive (RR) by licensed screening assays should be evaluated along with other additional, more specific tests to establish clinical sensitivity. In addition, a minimum of 300 known positive specimens should be tested to determine clinical sensitivity.

For tests that are used to resolve indeterminate results of other confirmatory assays, a minimum of 300 samples from persons with such indeterminate test results using licensed tests should be tested. Testing should be performed on a combination of WB, SIA, and IFA

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indeterminate specimens from random donors. Sponsors should demonstrate that the sensitivity of the second, more specific test is equivalent to or better than the screening assay. In all cases, a plan for resolving discordant/discrepant results should be included. In addition, a minimum of 500 random donor specimens and 300 known positive specimens should be tested to establish clinical specificity and sensitivity.

**E. Clinical Prognosis and Management of Patients on Therapy**

**1. Clinical Specificity**

Clinical specificity should be established by testing at least 500 specimens from healthy, random donors.

**2. Clinical Sensitivity**

Testing should be conducted head-to-head with a licensed or approved reference test. Clinical sensitivity should be established by testing samples from seropositive individuals and from high risk groups. Performance should be evaluated in cross-sectional studies involving patients stratified by CD4 counts with known clinical histories. Since viral nucleic acid in HIV disease is less frequently detected in the asymptomatic phase of infection, it is recommended that a large number of individuals in this disease phase be tested.

<u>Category</u>	<u>No. of subjects</u>
CD4 < 200	200
CD4 200-500	300
CD4 > 500	300

Other studies designed to assess the background variation of viral nucleic acid in an individual over a given length of time should also be performed. At least 40 patients should be followed on a weekly basis over a period of 12-16 weeks. In addition, the effect of diurnal variation on viral nucleic acid levels should be evaluated in at least 20-30 patients, monitored at frequent intervals in the first 24 hour interval and less frequently in the next 24-hour interval. This study should include individuals from different risk groups at different disease stages, and should attempt to determine gender based effects on viral nucleic acid detection and quantitation, if any. In addition, testing of a subgroup of patients harboring a drug resistant virus should be evaluated. Information from these studies will be important in establishing the significance and validity of changes observed after initiation of a specific therapy and the utility of these tests in patient management.

### **3. Clinical Prognosis**

The prognostic value of the assay may be established by conducting a prospective study involving patients at different stages of disease and monitoring their disease free time interval. Data may also be derived from well-characterized, retrospectively collected samples. A statistical analysis of this data should be performed to evaluate the impact of nucleic acid levels on disease progression. The utility in determining patient prognosis should be based on a combination of longitudinal and cross-sectional studies and should include information on clinical outcome. A cross-sectional study should include a study similar to that described above for clinical sensitivity. For the longitudinal study, a total of approximately 500 patients from different disease stages based on the Centers for Disease Control and Prevention classification should be followed. Clinical outcome may be defined as time to the first AIDS defining event or duration of disease free survival.

### **4. Performance in Patients Undergoing Therapy**

Studies to validate the utility of nucleic acid assays in patient management may be performed using well-characterized, repository specimens from cohorts for which data on clinical outcome is known. Specimens should fulfill specimen adequacy requirements so that meaningful results may be obtained and discordant results resolved. At least 300 patients undergoing therapy should be evaluated and their viral load monitored frequently to determine the utility of the assay in this population. Studies should also include a subset of patients whose therapy had been changed as a result of viral load measurements (switching studies) and the development of drug resistance. Prospective clinical trials that measure virus burden and its impact on clinical outcome should be designed similarly and conducted at clinical trial sites. These trials should be randomized, placebo controlled, double-blinded, and designed to factor in the intent-to-treat and use in patient management.

## **F. Perinatal Diagnosis**

Nucleic acid tests may be useful for early diagnosis of infection in infants born to seropositive mothers. The specificity of tests intended for this purpose should be established by testing at least 300 healthy donors. In addition, approximately 100-200 samples from infants born to healthy, seronegative mothers should be tested to assess specificity in the target population. Clinical sensitivity for early diagnosis should be evaluated by testing at least 200 infants born to seropositive mothers. Testing should include the first 0-6 weeks after birth and the results should be compared with licensed antibody and antigen tests. Long term follow-up may be



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needed to resolve test results in some cases.

**V. CONCLUSIONS**

Gene based tests for viral etiologic agents are regulated by FDA either as biologics or devices. Although this document outlines some of the major regulatory and scientific issues concerning gene based tests for HIV-1, these criteria also apply to tests for other transfusion transmitted viruses including HIV-2, HCV, HBV, and HTLV-I and II. Sponsors are advised to consult with the Agency during the product development phase so that major issues in manufacturing and clinical trial design may be addressed early on in the validation phase.

**VI. REFERENCES**

1. "Points to Consider in the Manufacture and Clinical Evaluation of *In Vitro* Tests to Detect Antibodies to the Human Immunodeficiency Virus Type 1" (1989).
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