appropriate clinical specimens to provide final specifications for test kit performance characteristics.

1. Specificity and Sensitivity Studies for Preclinical Testing (statistical determination of false positive and false negative rates)

   a. For donor screening assays or diagnostics, specificity should be established by testing samples from a minimum of 500 random blood or plasma donors. For quantitative assays, a minimum of 100 samples should be tested in the preclinical development phase; and
   b. For both assay types, sensitivity should be established by testing at least 300 seropositive repository specimens.

Testing should be performed in parallel with appropriate licensed comparator assays (i.e., antigen or antibody assays for blood screening or diagnosis and a nucleic acid based test for quantitation).

2. Analytical Sensitivity

   Analytical sensitivity should be evaluated by testing a dilution series of at least 10 distinct HIV positive samples obtained from different individuals (clinical specimens). Prior to initiation of these studies, the starting concentration of viral RNA in each sample should be determined by an appropriate independent technology (see section III.B, above). The evaluation of patient isolates should be run in parallel with samples from a recognized reference panel (e.g., reference HIV panels produced by the WHO, CBER or the ACTG VQA).

   For quantitative assays the dilution series should cover the full range of accurate quantitation for the assay under development. For pool testing, the lowest concentration in the dilution series must be at or below the minimum sensitivity level required for these assays (i.e., 100 copies/ml in a plasma pool or 5,000 copies/ml in an individual sample, based on a 95% detection rate). The highest dilution reproducibly and consistently detected and/or quantitated by the investigational assay should be defined in copies per unit sampling until recognized international standards for viral quantitation are available for the target virus, at which time, analytical sensitivity can be expressed in international units/ml. Comparator assays should include an antibody, antigen, or other state-of-the-art amplification/probe

technology. The selection of an appropriate comparator assay will be based on the technology under development and the proposed indication.

For products seeking a labeling claim for quantitation of HIV that includes non-clade B viruses, analytical sensitivity should be demonstrated for each subtype or variant proposed for inclusion in the label. A minimum of 10 distinct HIV positive specimens obtained from different individuals would be advisable for each subtype/variant. In the event that 10 distinct clinical specimens of a specific viral subtype are not available, culture derived specimens may be used to supplement clinical samples.

**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. in 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 components of a clinical development program for any specific intended use include studies designed to assess the precision, reproducibility, and non-specificity 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. Proficiency Testing

      A panel of samples similar to that described in section IV.B.1.b (below) should be tested by a number of operators on multiple days, at all clinical testing sites. This study is designed to assess operator proficiency.

   b. Reproducibility and Precision

      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 be tested in duplicate or triplicate. Testing should be performed on a minimum of three different lots, on multiple days and by at least two operators. The

operators chosen to conduct these studies should have demonstrated a high degree of proficiency with the assay. Reproducibility studies should be designed to assess variability intra- and inter-site, intra- and inter-assay and intra- and inter-lot, as well as total variability for both qualitative and quantitative assays. Assay precision may be established by performing multiple tests using multiple operators and multiple kit lots on a panel of specimens. Testing may be performed in-house and at least one clinical site.

c. Instrumentation

Instrumentation effects on product performance should be evaluated using the sample panel employed for reproducibility testing and a minimum of three different machines.

2. Non-specificity studies

Most assays are subject to some inherent non-specific reactivity resulting in false positive reactions or interference resulting in false negative reactions or reduced accuracy of quantitation. This effect may be due to specific assay components or the nature of the sample being tested. The presence of non-specific reactivity and the impact of potential interfering factors on assay performance should be assessed. Appropriate samples for these studies can be obtained by spiking the agent or factor into known HIV positive and/or negative samples, in addition to testing original specimens or reference panels. Examples of conditions, factors or sample characteristics that should be considered in the evaluation of cross-reactivity or interference include the following:

a. Other infections including 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, and pneumocystis;
b. The impact of different anticoagulants (i.e., heparin, ACD, or EDTA) or other suitable collection tube/media (e.g., plasma preparation tube);
c. Hemolyzed, icteric, lipemic, and bacterially contaminated samples;
d. Samples treated with chemicals, drugs, heat or detergents;
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. The impact of subject age, gender, race, or ethnic group;
i. The impact of drug resistance mutations. This evaluation should include a cross section of viruses that demonstrate phenotypic and genotypic resistance to each of the currently approved drug classes. Multiple drug resistant viruses should also be evaluated;
j. The presence of nucleic acid based drugs and metabolites and binding substances; and
k. The presence of drugs or biologicals that increase 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 (NAT) may be more sensitive than other methods currently available for early detection of a virus during the pre-seroconversion phase of infection and may, therefore, have added value in blood safety. For NAT, clinical specificity should be established by testing a large number of specimens from random U.S. blood and plasma donors. The evaluation of clinical specificity should include samples from at least 10,000 individuals or plasma pools, based on the intended use of the product. Testing should be performed at a minimum of 3 distinct clinical sites, including areas of both high and low prevalence. Studies should be performed in a manner that allows for donor identification (i.e., "linked") 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 NAT testing and/or by clinical follow-up and antigen or antibody-based testing over the subsequent 3-6 month period. The basis for reinstatement of donors with false-positive investigational test results should be defined in the study protocol.

1. Additional Studies to Establish the Clinical Sensitivity of Tests Intended for Screening of Individual Blood Donations

   A minimum of 1,000 specimens from seropositive individuals including samples from various risk groups and different stages of HIV-1 disease should be tested at 3 distinct sites. At least 200 of these seropositive specimens should be derived from persons with a clinical diagnosis of AIDS. It is recommended that the study also include a gender-

based analysis with at least 20-30% of the samples derived from females. Geographically diverse specimens representing all known viral subtypes (a minimum of 20 of each) 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. All of these samples should be actual clinical specimens as opposed to culture derived virus stocks or cloned species. However, if 20 distinct clinical specimens of a specific viral subtype are not available, culture derived specimens may be used to supplement clinical samples.

A minimum of 200 samples known to be positive for HIV-2 should also be tested in the evaluation of clinical sensitivity for single donation screening tests, including a subset that contain both HIV-1 and -2. These samples may be obtained from a repository.

Prospectively collected, freshly drawn specimens from individuals at high risk for HIV-1 should be tested in a linked study so that a minimum of 50 positive cases are identified (by serology, p24 antigen or NAT) to estimate sensitivity. A similar prospective trial in a population at high risk for HIV-2 should also be conducted, with sufficient enrollment to identify a minimum of 30 seropositive cases. These studies should include methods to objectively resolve discordances between investigational and comparator assays that include both antibody and antigen-based tests. 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).

A comparison should be made between freshly drawn and frozen specimens, as well as paired serum and plasma or other specimens (e.g., Whole Blood). The purpose of this study is to establish the comparability of the two storage conditions and the two specimen types, respectively.

2. Additional Studies to Establish the Clinical Sensitivity of Assays Intended for Pool Testing

Clinical sensitivity testing for pool tests should include the evaluation of 1,000 known seropositive specimens in a minimum of 100 separate pools. Of these, at least 25 pools should contain weakly reactive seropositive specimens introduced singly into the pool. All pools should contain at least several known negative specimens. The positions of the seropositive specimens should vary from one pool to another, in a random fashion. These studies should be performed at 3 sites, one of which could be in-house. In addition, the ability to detect known HIV subtypes/variants and HIV-2 should be determined using a minimum of 20 samples for each variant. These samples may be

plasma pools spiked with the appropriate subtype/variant or a virus preparation diluted in seronegative plasma. Additional HIV-2 samples may be required to support a label indication for detection of HIV-2. These studies should demonstrate that the NAT is capable of detecting a minimum of 100 RNA copies/ml in the pooled sample or 5,000 copies/ml of virus in the original donation, with a ≥95% detection rate.

3. Additional Issues for NAT Intended for Screening of Plasma Pools

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

a. Demonstration of equivalent or superior sensitivity of the assay for testing donor pools compared to currently licensed methods for donor screening by laboratory and field testing;
b. Rationale for the proposed pool size;
c. The impact, if any, of possible interference or matrix effects generated during pooling, on test performance;
d. Sample stability during collection, storage, pooling and transport , including a comparison of assay performance and sample stability with freshly drawn versus frozen specimens;
e. Procedures for logging and tracking of specimens in a given pool, including validation of the pooling process (i.e., procedures included to verify that appropriate test samples are obtained for all donations included in a pool and that all donations intended for a specific pool are actually included;
f. Specimen retrieval procedures to identify a positive specimen in a positive pool;
g. Quality assurance in computing and reporting test results;
h. Validation of instrumentation and automation; and
i. 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 of infection in the pre-seroconversion phase.

4. Clinical Validation of Assay Performance for Blood Screening Tests

Because of the limited availability of specimens in the antibody negative, pre-seroconversion phase, the agency urges the use of specimens from the following categories for clinical validation of assay performance for all blood screening tests: ongoing cohort studies and retrospective investigations; as well as a large number of 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 that estimate the timing and duration of the window period. Testing should include a head-to-head comparison with a licensed assay for HIV p24 antigen. These studies are particularly important for NAT, where the value added may be in the ability to reliably detect early infections.

### 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 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. This test series is distinct from the preclinical evaluation of specificity and should be conducted at an appropriate clinical trial site.

2. Clinical Sensitivity

Clinical sensitivity should be established by testing samples from seropositive individuals and from high risk groups in a head-to-head comparison with a licensed or approved nucleic acid detection test. Performance should be evaluated in cross-sectional studies involving HIV positive individuals stratified by CD4 counts and clinical history.

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

Studies designed to assess variability in consecutive viral nucleic acid measurements in an individual over time should also be performed. A minimum of 40 adult and 20 pediatric (age ≤ 12 years) patients with stable viral loads in the ranges listed above should be followed on a bi-weekly basis for a period of 16 weeks or weekly for a minimum of 8 weeks. The data should be evaluated with respect to short-term variability (1-2 weeks) and at 8 week intervals (typical monitoring frequency for routine patient care). The distribution of male and female participants in each adult study cohort should be sufficient to allow for the evaluation of gender based differences in assay variability.

The information obtained from the studies listed above 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. Performance in Patients Undergoing Therapy

The clinical utility of an assay in treated individuals should be demonstrated in prospectively conducted drug efficacy trials by direct comparison to an approved nucleic acid quantitation test. Randomized, concurrently controlled clinical trials that compare different drug combinations or investigate the utility of new drugs as adjuncts to existing therapies based on their effects on plasma virus RNA are acceptable for assay comparator studies.

A possible alternative approach to testing in the context of a prospectively conducted clinical study is the use of well-characterized, repository specimens from cohorts for which clinical outcome is known. However, if a retrospective analysis is planned, the study employed should be consistent with current treatment regimens and practices. In addition, it is essential that specimen adequacy requirements be confirmed prior to initiation of the study so that meaningful results may be evaluated and discordant results resolved. Prior to the conduct of a retrospective analysis of clinical utility, sponsors are strongly encouraged to consult with the division about the suitability of the study(s) selected.

Regardless of the approach taken to demonstrate assay utility in patient monitoring a minimum of 300 patients who underwent therapy with regular monitoring of virus load should be evaluated. This study population should include a subset of patients whose therapy was modified as a result of clinically defined disease progression, changes in virus load and/or the development of drug resistance. In the context of these studies, an effort should be made to address gender and age-related (i.e., pediatric versus adult) differences in kit performance.

Sponsors interested in pursuing a claim for patient monitoring are encouraged to consult the FDA Draft Guidance for Industry: Clinical Considerations for Accelerated and Traditional Approval of Antiretroviral Drugs Using Plasma HIV RNA Measurements (Ref. 4) for additional information on clinical testing strategies and accepted use of plasma RNA measurements in clinical therapeutic trials.

4. Clinical Prognosis

A number of different approaches may be taken to establish the prognostic value of a nucleic acid quantitation assay. A prospective study may be conducted involving

patients at different stages of disease with monitoring of their disease free time interval as the primary endpoint. Data may also be derived from well-characterized, retrospectively collected samples obtained from appropriate clinical endpoint trials. In some instances the prognostic utility of an assay may be demonstrated by direct comparison to another nucleic acid technology that is currently approved for prognosis.

When prognostic utility is defined by evaluation of clinical trial samples with clinical outcome data, whether prospectively or retrospectively identified, a statistical analysis should be performed to determine the relative predictive value of nucleic acid levels as it relates to disease progression. This analysis should be based on a combination of longitudinal and cross-sectional studies that includes information on clinical outcome. The cross-sectional study should employ a design similar to that described above for clinical sensitivity. For the longitudinal study, a total of approximately 500 patients at different disease stages based on the Centers for Disease Control and Prevention classification scheme should be followed. Clinical outcome may be defined as the time to first AIDS defining event or duration of disease free survival. The studies outlined above should include a sufficient number of female participants to allow for the evaluation of gender based differences in prognostic utility. An independent evaluation of pediatric patients is also strongly encouraged.

A comparative approach to demonstrate equivalent or superior performance may be acceptable in combination with supporting preclinical and clinical data for modified versions of currently approved assays, or for new tests that approximate, in a clearly definable manner, the RNA values generated with a nucleic acid technology approved for prognosis. A minimum of 150 clinical samples spanning the full linear range of both the experimental and reference assays should be evaluated. In addition, a set of sequential clinical specimens from 30 individuals with demonstrable changes in viral load (a minimum 1 $\log_{10}$ change), over time should be evaluated to permit a comparison of the assays based on individual readings and on sequential changes in those readings. A comparative approach is not sufficient for assays that do not demonstrate an acceptable degree of correlation with an approved assay based on both the individual readings and on sequential changes in those readings.

### 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 (adult) donors and approximately 100-200 samples from infants born to healthy, seronegative mothers. 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 needed to resolve test results in some cases.

## V. CONCLUSIONS

Gene based tests for viral 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 and HIV-2, these considerations may also be applicable to tests for other transfusion transmitted viruses including HCV, HBV, and HTLV-I and II. Sponsors are advised to consult with the agency (CBER or CDRH, based on the assay and indication for use) during the development phase so that product specific issues in manufacturing and clinical trial design may be addressed early 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).

2. "Review Criteria for Nucleic Acid Amplification-Based In Vitro Diagnostic Devices for Direct Detection of Infectious Microorganisms" (1993).

3. "Guidance for Industry: Content and Format of Chemistry, Manufacturing and Controls Information and Establishment Description Information for a Biological *In Vitro* Diagnostic Product" (1999).

4. Draft "Guidance for Industry: Clinical Considerations for Accelerated and Traditional Approval of Antiretroviral Drugs Using Plasma HIV RNA Measurements" (1999).

# CONTROL AUTHORITY BATCH RELEASE OF BLOOD PRODUCTS

# 2001

## Validation Of Nucleic Acid Amplification Technology (NAT) For The Detection Of Hepatitis C Virus (HCV) RNA In Plasma Pools

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|---|---|
| **Guideline title** | **Validation Of Nucleic Acid Amplification Technology (NAT) For The Detection Of Hepatitis C Virus (HCV) RNA In Plasma Pools** |
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| **Previous working title** | **The present document was elaborated by the EDQM in the OMCL network and finalised under PA/PH/OMCL (98) 22, DEF** |

# VALIDATION OF NUCLEIC ACID AMPLIFICATION TECHNOLOGY (NAT) FOR THE DETECTION OF HEPATITIS C VIRUS (HCV) RNA IN PLASMA POOLS

## Introduction

The majority of NAT analytical procedures are qualitative (quantal) tests for the presence of nucleic acid with some quantitative tests (either in-house or commercial) being available. For the detection of HCV RNA contamination of plasma pools, qualitative tests are adequate and may be considered to be a limit test for the control of impurities as described in the guideline "Validation of analytical procedures: definitions and terminology", published in "The rules governing medicinal products in the European Union", Volume 3A, 1998, pg. 119-125, ref. 3AQ14a. Therefore the two characteristics regarded as the most important for validation of the analytical procedure are the specificity and the detection limit. In addition, the robustness of the analytical procedure should be evaluated. In accordance with these guidelines, the validation characteristics are described as:

1. Specificity is the ability to unequivocally assess nucleic acid in the presence of components, which may be expected to be present.

2. The detection limit of an individual analytical procedure is the lowest amount of nucleic acid in a sample which can be detected but not necessarily quantitated as an exact value.

3. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

## Validation Guidelines

### 1 Scope

This document describes methods to validate only qualitative NAT analytical procedures for assessing HCV RNA contamination of plasma pools based on the ICH guidelines. However, this document may also be used as a basis for the validation of NAT in general. For the purpose of this document, an analytical procedure is defined as the complete procedure from extraction of nucleic acid to detection of the amplified products.

Where commercial kits are used for part of or the complete analytical procedure, documented validation points already covered by the kit manufacturer can substitute for the validation by the user. Nevertheless, the performance of the kit with respect to its intended use has to be demonstrated by the user (e.g. detection limit, robustness, cross contamination).

## 2 Specificity

The specificity of NAT analytical procedures is dependent on the choice of primers, the choice of probe (for analysis of the final product) and the stringency of the test conditions (for both the amplification and detection steps).

When designing primers and probes, the specificity of the primers and probes to detect only HCV RNA should be investigated by comparing the chosen sequences with sequences in published data banks. For HCV, primers (and probes) will normally be chosen from areas of the 5' non-coding region of the HCV genome which are highly conserved for all genotypes.

The amplified product should be unequivocally identified by using one of a number of methods such as amplification with nested primers, restriction enzyme analysis, sequencing or hybridisation with a specific probe.

In order to validate the specificity of the analytical procedure, at least 100 HCV RNA-negative plasma pools should be tested and shown to be non-reactive.

The ability of the analytical procedure to detect all HCV genotypes will again depend on the choice of primers, probes and reaction parameters. This ability should be demonstrated using characterised reference panels. However, in view of the difficulty in obtaining samples of some genotypes (e.g. genotype 6), the most prevalent genotypes (e.g. genotype 1 and 3 in Europe) should be detected at a suitable level.

## 3 Detection limit

The NAT analytical procedure used for the detection of HCV RNA in plasma pools usually yields qualitative results. The number of possible results is limited to two, either positive or negative. Although the ICH guidelines recommend the determination of the detection limit, for practical purposes, a positive cut-off point should be determined for the NAT analytical procedure. The positive cut-off point (as defined in the Ph Eur General Method *2. 6. 21*) is the minimum number of target sequences per volume sample which can be detected in 95% of test runs. This positive cut-off point is influenced by the distribution of viral genomes in the

individual samples being tested and by factors such as enzyme efficiency and can result in different 95% cut-off values for individual analytical test runs.

In order to determine the positive cut-off point, a dilution series of a working reagent or reference material, which has been calibrated against the WHO HCV International Standard (96/790), should be tested on different days to examine variation between test runs. At least 3 independent dilution series should be tested with a sufficient number of replicates at each dilution to give a total number of 24 test results for each dilution to enable a statistical analysis of the results.

For example, a laboratory could test 3 dilution series on different days with 8 replicates for each dilution, 4 dilution series on different days with 6 replicates for each dilution, or 6 dilution series on different days with 4 replicates for each dilution. In order to keep the number of dilutions at a manageable level, a preliminary test (using, for example, log dilutions of the plasma pool sample) should be done in order to obtain a preliminary value for the positive cut-off point (i.e. the highest dilution giving a positive signal). The range of dilutions can then be chosen around the pre-determined preliminary cut-off point (using, for example, a dilution factor of 0.5 log or less and a negative plasma pool for the dilution matrix). The concentration of HCV RNA which can be detected in 95% of test runs can then be calculated using an appropriate statistical evaluation.

These results may also serve to demonstrate the intra-assay variation and the day to day variation of the analytical procedure.

## 4 Robustness

The evaluation of robustness should be considered during the development phase. It should show the reliability of the analytical procedure with respect to deliberate variations in method parameters. For NAT, small variations in the method parameters can be crucial. However, the robustness of NAT can be demonstrated during the development of the method when small variations in the concentrations of reagents, e.g. $MgCl_2$, primers or dNTP, are tested. To demonstrate robustness, at least 20 HCV RNA negative plasma pools (selected at random) and spiked with HCV RNA to a final concentration of 3 times the previously determined 95% cut-off value should be tested and found positive.

Problems with robustness may also arise with methods which use an initial ultracentrifugation step prior to extraction of the viral RNA. Therefore, to test the robustness of such methods, at least 20 plasma pools containing varying levels of HCV RNA, but lacking HCV specific antibodies, should be tested and found positive.

Cross-contamination prevention should be demonstrated by the accurate detection of a panel of at least 20 samples consisting of alternate samples of negative plasma pools and negative plasma pools spiked with high concentrations of HCV (at least $10^2$ x the 95% cut-off value or at least $10^4$ IU/ml).

## 5 Quality Assurance

For biological tests such as NAT, specific problems may arise which may influence both the validation and interpretation of results. The test procedures must be described precisely in the form of standard operating procedures (SOPs). These should cover:

- the mode of sampling (type of container, etc.)
- the preparation of mini-pools (where appropriate)
- the conditions of storage before analysis
- the exact description of the test conditions including precautions taken to prevent cross contamination or destruction of the viral RNA, reagents and reference preparations used
- the exact description of the apparatus used
- the detailed formulae for calculation of results, including statistical evaluation.

The use of a suitable run control (for example, plasma spiked with an HCV sample calibrated against the WHO HCV International Standard 96/790) can be considered a satisfactory system suitability check and ensures that the reliability of the analytical procedure is maintained whenever used.

Technical qualification: An appropriate installation and operation qualification programme should be implemented for each critical piece of the equipment used. For confirmation of analytical procedure performance after change of critical equipment (e.g. thermocyclers) should be documented by conducting parallel test on 8 replicate samples of a plasma pool spiked with HCV RNA to a final concentration of 3 times the previously determined 95 % cut-off value. All results should be positive.

Training: An appropriate qualification programme should be implemented for each operator involved in the testing. To confirm successful training each operator should test at least 8 replicate samples of a plasma pool spiked with HCV RNA to a final concentration of 3 times the previously determined 95 % cut-off value. This test (8 replicate samples) should be repeated twice on two separate days, i.e. a total of 24 tests performed on three different days. All results should be positive.

山口資料 3

# Guidance for Industry

## Use of Nucleic Acid Tests on Pooled Samples from Source Plasma Donors to Adequately and Appropriately Reduce the Risk of Transmission of HIV-1 and HCV

### DRAFT GUIDANCE

**This guidance document is being distributed for comment purposes only.**

Submit comments and suggestions regarding this draft document by the date provided in the Federal Register notice announcing the availability of the draft guidance. Submit comments to Dockets Management Branch (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that published in the Federal Register.

Additional copies of this draft guidance are available from the Office of Communication, Training and Manufacturers Assistance (HFM-40), 1401 Rockville Pike, 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 draft guidance contact Indira Hewlett, Ph.D., (301) 827-0795.

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

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