Contains Nonbinding Recommendations

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Guidance for Industry

Nucleic Acid Testing (NAT) for Human Immunodeficiency Virus Type 1 (HIV-1) and Hepatitis C Virus (HCV): Testing, Product Disposition, and Donor Deferral and Reentry

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternate approach if the approach satisfies the requirements of the applicable statutes or regulations. If you want to discuss an alternate approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

We, the Food and Drug Administration (FDA), are providing recommendations to you, blood and plasma establishments, manufacturers, and testing laboratories that are implementing a licensed method for Human Immunodeficiency Virus Type 1 (HIV-1) Nucleic Acid Test (NAT) and Hepatitis C Virus (HCV) NAT, for testing individual samples or pooled samples from donors of human blood and blood components for HIV-1 ribonucleic acid (RNA) and HCV RNA. This guidance also contains recommendations regarding product disposition and donor management based on the results of NAT testing for markers of HIV-1 and HCV infection on samples, collected at the time of donation, from donors of human blood and blood components.

This guidance finalizes the draft guidance of the same title, dated July 2005. This guidance also supersedes the recommendations for reentry of donors deferred because of anti-HIV-1 test results, HIV-1 p24 antigen test results, and anti-HCV test results that were provided in the FDA memoranda entitled, "Revised Recommendations for the Prevention of Human Immunodeficiency Virus (HIV-1) Transmission by Blood and Blood Products," April 23, 1992; "Revised Recommendations for Testing Whole Blood, Blood Components, Source Plasma and Source Leukocytes for Antibody to Hepatitis C Virus Encoded Antigen (Anti-HCV)," August 5, 1993; "Recommendations for Donor Screening with a Licensed Test for HIV-1 Antigen," August 8, 1995 (Refs. 1 through 3).

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA guidances means that something is suggested or recommended, but not required.

II. DEFINITIONS

Anti-HIV-1 or -2 Test or Anti-HIV-1/2 Test or Anti-HCV Test: A screening test such as an enzyme immunoassay (EIA) performed on donations of blood and blood components.

Deconstruction: Resolution of the reactivity of a minipool by testing subpools (original or freshly made) or samples from individual donors that formed the minipool. Deconstruction of a reactive minipool to individual units is a required step for all approved tests.[1]

Discriminatory NAT: A NAT that uses specific primers for HIV-1 or HCV to identify the RNA in the reactive multiplex NAT sample as HIV-1 RNA or HCV RNA. Performing a Discriminatory NAT on a reactive sample is a required step for those establishments using an approved multiplex test.[2]

Donor Reentry: A procedure that qualifies a deferred donor as eligible to donate again. Donor reentry procedures may be used following a false positive test result and typically require the passage of time to allow for possible seroconversion prior to the performance of additional serologic testing and NAT (see sections V.A. and V.B.).

Lookback: A series of actions taken by a blood establishment based on donor test results indicating infection with HIV-1 or HCV. These actions relate to the donor's prior donations that possibly were donated during the window period when HIV-1 or HCV RNA and antibody were not detectable by screening tests but the infectious agent might be present in the donor's blood. These actions include: quarantining the remaining inventory of prior collections from that donor, notifying consignees to quarantine prior collections, further testing of the donor, destroying or re-labeling potentially infectious prior collections, and when appropriate, notifying transfusion recipients who received human blood or blood components from that donor.

Minipool: A pool of donor samples on which NAT (minipool NAT or MP-NAT) is performed as a screening test. A minipool is formed by pooling of samples from subpools or by directly pooling samples from individual donors.

Multiplex NAT: A NAT that simultaneously detects HIV-1 RNA and HCV RNA.

Single Virus NAT: A NAT that separately detects either HIV-1 RNA or HCV RNA.

Subpool: A pool of donor samples that was used with other (sub)pools to form the minipool or that was formed as a result of "deconstruction" of the minipool.

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[1] The labeling for all approved screening tests specifies that deconstruction is to be performed. Under Title 21 Code of Federal Regulations 610.40(b) (21 CFR 610.40(b)), you must use FDA-approved screening tests "in accordance with the manufacturer's instructions."

[2] The labeling for licensed multiplex NATs specifies that discriminatory NAT is to be performed. Under 21 CFR 610.40(b), you must use FDA-approved screening tests in accordance with the manufacturer's instructions.

III. BACKGROUND AND DISCUSSION

FDA has progressively strengthened the overlapping safeguards that protect patients from unsuitable blood and blood products. Blood donors are now asked specific and very direct questions about risk factors that could indicate possible infection with a transmissible disease. This "up-front" screening eliminates approximately 90 percent of unsuitable donors. In addition, FDA requires blood centers to maintain lists of deferred donors to prevent the use of collections from them.

During the past decade blood establishments have implemented new donor screening tests, including sensitive tests for viral antibody, antigen (for HIV-1), and nucleic acids, and there has been a dramatic reduction in the transmission of HIV-1 and HCV by human blood and blood components. Sources of remaining risk for HIV-1 and HCV transmission by human blood products include: 1) marker-negative "window period" donations (made during the period that the donor is infected with a virus, but neither the virus nor antibodies to the virus are detectable by current tests); 2) donors infected with genetic and immunovariant viral strains; 3) persistent antibody-negative (immunosilent) carriers and 4) laboratory errors. According to a recent report, donations during the window period constitute most of the risk of HIV-1 and HCV transmission (Ref. 4). Therefore, measures to reduce the window period might further reduce the residual risk of HIV-1 and HCV transmission by human blood and blood components.

Studies performed using seroconversion panels indicate the value of NAT in reducing the window period for HIV-1 and HCV. The estimated mean window-period reduction for HIV-1 RNA by pooled sample NAT is approximately 11 to 15 days relative to antibody testing and 5 to 9 days relative to HIV-1 p24 antigen testing (Refs. 5 through 7). NAT for detection of HCV has been estimated to reduce the window period by 50 to 60 days relative to that for HCV antibody. In large-scale studies performed nationwide, NAT for HIV-1 detected 4 antigen-negative/antibody-negative window period donations and NAT for HCV detected 42 additional antibody-negative window period donations. As a result, subsequent to implementation of NAT, the residual risk of HIV-1 and HCV in screened human blood and blood component donations is estimated to be approximately 1 in 2,135,000 donations for HIV-1 and 1 in 1,935,000 donations for HCV (Ref. 6).

In September 1994, we held a workshop to discuss the potential application of nucleic acid based methods to donor screening for HIV-1. We concluded at the time that these methods clearly were sensitive, but they were not ready for implementation on a large scale.

The industry actively pursued the development of NAT for screening donors of human blood and blood components. There was much interest in testing pools of plasma donor samples (minipools) by NAT because of the cost and labor intensiveness of testing individual donor samples. By 1997, some manufacturers in Europe had voluntarily instituted NAT on minipools. At about that time, the European Union issued a directive

that by July 1, 1999, HCV RNA testing would be required in Europe for all plasma for fractionation and that the requirement for HIV-1 RNA testing would follow at a later date.

Large-scale clinical studies were needed to demonstrate the efficacy of NAT because of the expected low frequency of window period donations. Test kit manufacturers and testing laboratories submitted Investigational New Drug applications (INDs) describing their test method and in-house validation of that method. Blood organizations and establishments intending to use the assay for donor screening also filed INDs to describe their clinical trial protocol for validation of pooled-donor sample NAT (MP-NAT) and individual donor sample NAT (ID-NAT).

In December 1999, we issued a guidance for industry on the validation of NAT methods to screen plasma donors (Ref. 8). This document provided guidance on test standards, manufacturing requirements, and clinical trial requirements for licensure of the test method for use in donor screening for transfusion-transmitted viruses.

In September 2001, we licensed the first NAT system, the National Genetics Institute (NGI) UltraQual HIV-1 and HCV Reverse Transcription Polymerase Chain Reaction (RT-PCR) assays. Under that license, NGI performs RT-PCR assays on pooled samples from donors of Source Plasma.

In February 2002, we licensed the Procleix HIV-1/HCV Assay, a qualitative NAT for detection of HIV-1 RNA and/or HCV RNA in plasma from donors of human blood and blood components for transfusion. This assay was approved for use with individual donor samples or pooled donor samples.

In December 2002, we licensed the COBAS AmpliScreen HCV Test, v 2.0 and the COBAS AmpliScreen HIV-1 Test, v 1.5. These tests are qualitative *in vitro* tests for the direct detection of HCV RNA and HIV-1 RNA in plasma samples from human donors, including donors of Whole Blood, blood components, and Source Plasma, and from other living donors. These assays were approved for use with individual donor samples or pooled donor samples.

In October 2006, we licensed the Procleix Ultrio Assay, a qualitative *in vitro* assay system to screen for HIV-1 RNA and HCV RNA in plasma and serum specimens from human donors, including donors of Whole Blood, blood components, and Source Plasma, and from other living donors. This assay was also approved for use with individual donor samples or pooled donor samples.

In February 2007, we licensed the BioLife Plasma Services HIQ-PCR HIV-1 RT-PCR assay and the HIQ-PCR HCV RT-PCR assay for the qualitative detection of HIV-1 RNA and HCV RNA, respectively, in pools of human Source Plasma.

In December 2008, we licensed the cobas TaqScreen MPX Test, a qualitative in vitro screening test for individual human donors, including donors of Whole Blood and blood components, and other living donors, for the presence of HIV-1 Group M RNA, HCV

RNA, and HBV DNA. Plasma from all donors may be screened as individual specimens. For donations of Whole Blood and blood components, plasma specimens may be tested individually or in pools.

In October 2004, we issued a final guidance, "Use of Nucleic Acid Tests on Pooled and Individual Samples from Donors of Whole Blood and Blood Components (including Source Plasma and Source Leukocytes) to Adequately and Appropriately Reduce the Risk of Transmission of HIV-1 and HCV." That guidance combined and finalized the draft guidance "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" dated December 2001, and the draft guidance "Use of Nucleic Acid Tests on Pooled and Individual Samples from Donors of Whole Blood and Blood Components for Transfusion to Adequately and Appropriately Reduce the Risk of Transmission of HIV-1 and HCV" dated March 2002.

The October 2004 guidance informed establishments collecting blood and blood components that we have licensed NAT to screen blood donors for HIV-1 RNA and HCV RNA and that these licensed tests could detect evidence of infection at a significantly earlier stage than is possible under previously approved tests using antibody or antigen detection technology, including the HIV-1 p24 antigen test. We also informed those establishments that we believe these newly licensed tests are now widely available and meet the criteria in Title 21 Code of Federal Regulations 610.40(b) (21 CFR 610.40(b)) for screening tests that are necessary to reduce adequately and appropriately the risk of transmission of communicable disease through blood products.

In that guidance, we recommend the use of HIV-1 NAT and HCV NAT on units that are not reactive on a donor-screening test for the detection of antibodies to HIV-1 or HCV, respectively. However, for donations that are reactive on a test for the detection of antibodies to HIV-1 and are to be discarded or used in the manufacture of non-injectable products, we do not consider HIV-1 NAT and HCV NAT to be necessary as part of the adequate and appropriate testing required under § 610.40(b). We informed the establishments that even though testing for these products is not required under § 610.40(b), they may decide to perform HIV-1 NAT and HCV NAT for these donations in order to obtain useful information regarding the donor's infection status. This information might be useful as part of donor notification.

This guidance is intended to assist you with testing, product disposition, donor deferral, donor notification, donor reentry, and lookback. We have written this document in general form because other NAT may be approved in the future. However, where appropriate, we will identify sections that apply to NAT that are already approved. You must follow manufacturers' instructions regarding testing under § 610.40(b). Note that screening of donors of human blood and blood components for HIV-1 p24 antigen may be replaced by a NAT that has been validated by the manufacturer as a replacement for the HIV-1 p24 antigen EIA.

A. NAT Algorithms

Under § 610.40(b), you must use FDA approved screening tests "in accordance with the manufacturer's instructions." If you perform NAT on pooled samples and obtain a reactive NAT result on a minipool, the manufacturer's instructions instruct you to perform subsequent testing to identify the individual unit(s) that contains the RNA identified in the minipool test. Once you have identified a positive unit, either by subsequent testing of a minipool or by initial individual donor sample testing, you must not use the donation for transfusion or for manufacturing into injectable products (§ 610.40(h)(1)) unless an exception applies (§ 610.40(h)(2)). You must defer the donor (§610.41(a)), and you must inform the donor of the deferral and the basis for the deferral, including test results (§ 630.6). A reactive NAT result may indicate ongoing infection of the donor. Thus prior donations from that donor, although NAT-non-reactive, may pose a risk to transfusion recipients.

In the FEDERAL REGISTER of August 24, 2007 (72 FR 48765), FDA published the final rule entitled "Current Good Manufacturing Practice for Blood and Blood Components; Notification of Consignees and Transfusion Recipients Receiving Blood and Blood Components at Increased Risk of Transmitting Hepatitis C Virus Infection ("Lookback")" (Lookback rule) (Ref. 9). The Lookback rule established lookback requirements related to HCV infection and revised the requirements related to HIV infection. It requires that you perform lookback for HIV-1 and for HCV when donor samples test reactive using HIV-1 NAT or HCV NAT. Donors infected with HIV-1 or HCV may experience intermittent viremias for a variable period of time prior to a persistently detectable viremia or an antibody response. Since these episodes of transient viremia may extend over a longer window period than previously estimated, the Lookback rule requires you to review all records for a period of 12 months before the donor's reactive NAT. A 12-month time frame is necessary to encompass with sufficient confidence the window period for HIV-1 or for HCV prior to the detection of antibody. We have not established an alternative (possibly shorter than 12 months) lookback period based on the last non-reactive NAT, in order to minimize operational complexity and because the appropriate period has not been well established scientifically.

At the meeting of the Blood Products Advisory Committee (BPAC) in March 2001, FDA requested advice on appropriate algorithms for management of donations of human blood and blood components tested by pooled donor sample NAT for both HIV-1 RNA and HCV RNA. In particular, FDA sought comment on actions to be taken in the event of discrepant testing results, such as when the minipool is reactive but individual donor samples test non-reactive. Data generated using NAT under IND that was presented in the BPAC meeting showed that in each discrepant case it was the minipool that was falsely reactive, due to contamination either during specimen handling or during the assay run. In response to FDA questions the BPAC voted to consider the NAT result on samples from individual donors as the definitive test result, and recommended

release from quarantine for donations from those donors, on the basis of non-reactive individual test results.

This guidance document contains four recommended algorithms for use when NAT-reactive results are obtained on individual samples or pooled samples from donors of human blood and blood components. Note that these algorithms apply when only the HIV-1 NAT or HCV NAT result is reactive (i.e., serologic tests for HIV-1/2 and HCV are negative). This guidance also contains recommendations on product disposition, donor deferral criteria, follow-up testing of the donor, donor notification, and lookback (product retrieval and recipient notification). This guidance is not intended to replace manufacturers' instructions for testing using approved tests.

The first and second algorithms (see sections IV.A. and IV.B, Figures 1 and 2, and Tables 1 and 2) recommend actions to deconstruct a reactive minipool by testing archived or freshly pooled subpools or by testing individual donor samples. The third and fourth algorithms (see sections IV.C. and IV.D, Figures 3 and 4, and Tables 3 and 4) recommend actions to be taken when a NAT-reactive result is obtained on an individual sample from a donor of human blood or blood components.

B. Donor Reentry

Each year many donors are deferred from donating blood for an indefinite period because of a false positive test result on a serologic test, followed by a negative or indeterminate supplemental test for antibodies to HIV-1 or HCV. In addition to these deferrals the implementation of NAT for HIV-1 RNA and HCV RNA has resulted in deferrals of many donors each year due to potentially false reactive NAT results.

These deferred donors may be eligible to be considered for reentry to donate blood or blood components. Under § 610.41(b), a deferred donor subsequently may be found to be suitable as a donor by a re-qualification method or process found acceptable for such purposes by FDA. However, some establishments are not attempting to reenter donors because of the complexity of the current reentry algorithms and concerns about inappropriately reentering a donor. Although we do not require reentry of donors deferred because of false positive test results, we issued guidance in April 1992 on reentry of donors deferred because of a repeatedly reactive (RR) test for antibodies to HIV-1 or HIV-2, and in August, 1993 on reentry of donors deferred because of a RR test for antibodies to HCV (Refs. 1 and 3).

This guidance contains recommendations for reentry of donors deferred because of reactive HIV-1 NAT or HCV NAT or certain other test results in accordance with § 610.41(a). We find these reentry methods to be acceptable within the meaning of § 610.41(b). These recommendations include two new reentry algorithms based on the combined use of NAT and serologic testing for

antibodies: one for donors deferred because of HIV-1 test results, and a second for donors deferred because of HCV test results. Note that the reentry of a donor permits prospective donations from a reentered donor who meets donor suitability criteria. It does not affect the status of previous collections from that donor, including donations subject to lookback.

Reentry of Donors (HIV Test Results)

In this guidance we recommend that you consider for reentry three groups of donors deferred because of reactive HIV-1 NAT or RR anti-HIV-1 or -2 test or RR anti-HIV-1/2 test or RR HIV-1 p24 antigen test results (see section V.A., Figure 5, and Table 5). These three groups of donors include:

1. Donors with a reactive HIV-1 NAT result but who were seronegative for antibodies to HIV-1 and HIV-2;

2. Donors with non-reactive HIV-1 NAT (or NAT was not performed) who had a RR anti-HIV-1/2 test and an HIV-1 Western Blot (WB) or immunofluorescence assay (IFA) that was indeterminate (viral bands may be present), unreadable, negative, or was not performed; and

3. Donors with a positive or indeterminate result on the HIV-1 p24 Neutralization test (Ref. 2), even on more than one occasion. This last group of donors may be eligible for reentry because there are many donors who had (false) positive Neutralization test results who are currently non-reactive by HIV-1 NAT and negative by an anti-HIV-1 or anti-HIV-1/2 test.

FDA no longer recommends that blood and plasma establishments using certain approved NAT methods perform screening for HIV-1 p24 antigen. If antigen testing continues to be performed concurrent with NAT and antibody testing, donors deferred because of HIV-1 p24 test results would continue to be eligible for reentry.

Data presented at the June 2001 BPAC meeting demonstrated that an 8-week waiting period encompasses the pre-seroconversion window period for HIV-1 with sufficient confidence that negative tests, after at least 8 weeks have passed, rule out HIV-1 infection (Ref. 10). Absent evidence for seroconversion, a negative NAT on follow-up testing would be evidence that any prior reactive (but unconfirmed) NAT result was an error.

Accordingly, for all three groups of donors, after a minimum time period of 8 weeks we recommend that you take a follow-up sample from the donor for testing by both HIV-1 ID-NAT and an anti-HIV-1/2 test. Performing follow-up testing first on a new sample from the donor, collected before another donation, may prevent a potentially contaminated unit from being collected and placed in inventory at the blood establishment. If the ID-NAT is non-reactive and the anti-HIV-1/2 test is negative on the follow-up sample, the donor may be reentered.

The donor would then be tested again at the time of his/her next donation using the battery of screening tests required under § 610.40. That donation may be tested by NAT as part of a minipool (i.e., MP-NAT) or as an individual donation (i.e., ID-NAT). Thus, two HIV-1 NAT tests would be performed and must be non-reactive and two anti-HIV-1/2 tests would be performed and must be negative before a unit from that donor could be used. For purposes of donor counseling, you may choose to test the deferred donor with an HIV-1 NAT and an anti-HIV-1/2 test at any time prior to the end of this 8-week waiting period after the original donation. However, if an HIV-1 NAT is reactive prior to the end of this 8-week waiting period, the donor would not be eligible for reentry and we recommend that you defer the donor permanently. If an anti-HIV-1/2 test is RR prior to the end of this 8-week waiting period, and a licensed supplemental test for antibodies to HIV-1 (i.e. WB or IFA), if performed, is not positive, you may take another follow-up sample from the donor for testing by both HIV-1 ID-NAT and an anti-HIV-1/2 test after another 8-week waiting period has passed.

Reentry of Donors (HCV Test Results)

In this guidance we recommend that you consider for reentry two groups of donors deferred because of reactive HCV NAT or RR anti-HCV test results (see section V.B., Figure 6, and Table 6). These two groups of donors include:

1. Donors with a reactive HCV NAT result but who were seronegative for antibodies to HCV; and

2. Donors with non-reactive HCV NAT (or NAT was not performed) who had a RR anti-HCV test and radioimmunoblot assay (RIBA) results that were indeterminate or negative, or a RIBA was not performed.

Data presented at the June 2001 BPAC meeting demonstrated that a 6-month follow-up period encompasses the pre-seroconversion window period with sufficient confidence that negative tests after at least 6 months have passed rule out HCV infection (Ref. 10).

For purposes of reentering both of these groups of deferred donors we recommend that you take a follow-up sample from the donor after a minimum time period of 6 months for testing by both HCV ID-NAT and an anti-HCV test. Current research indicates that detectable viremia may be intermittent or may be resolved in about 15-25% of cases of HCV infection (Refs. 11 and 12). If the ID-NAT is non-reactive and the anti-HCV test is negative on the follow-up sample, the donor may be reentered. The donor would then be tested again at the time of his/her next donation using the battery of screening tests required under § 610.40(b). That donation may be tested by NAT as part of a minipool (i.e., MP-NAT) or as an individual donation (i.e., ID-NAT). Thus, two HCV NAT tests would be performed and must be non-reactive and two anti-HCV tests would be performed and must be negative before a unit from that donor could be used. For purposes of donor counseling and to detect possible HCV viremia, you may also choose to

test the deferred donor with an HCV NAT and an anti-HCV test at any time prior to the completion of this 6-month period after the original donation. However, if an HCV NAT is reactive prior to the end of this 6-month period, the donor would not be eligible for reentry and we recommend that you defer the donor permanently. If an anti-HCV test is RR prior to the end of this 6-month waiting period and you conducted a licensed supplemental test for antibodies to HCV (e.g. RIBA) in which the result was not positive, you may take another follow-up sample from the donor for testing by both HCV ID-NAT and an anti-HCV test after another 6-month waiting period has passed.

IV. RECOMMENDATIONS FOR NAT ALGORITHMS

As discussed in sections A and B below, currently approved tests on minipools of donor samples for HIV-1 RNA and HCV RNA may be either Multiplex NAT for the simultaneous detection of HIV-1 RNA and HCV RNA or Single Virus NATs conducted separately for the RNA of the two viruses.

A. Testing, Product Disposition, Donor Management, and Lookback for a Minipool that is Reactive on a Multiplex NAT (MP-NAT): Resolution by Testing Subpools or by Testing Individual Donor Samples

If you obtain a reactive Multiplex HIV-1 RNA/HCV RNA NAT result for a minipool, the test instructions for use instruct you to perform subsequent testing to identify the donor sample(s) that are NAT-reactive as the basis for the NAT-reactive result on the pool. In general, the manufacturer's instructions for a licensed NAT describe two methods for resolving a minipool that is reactive on a Multiplex NAT, and you must follow the instructions in the package insert that provide specific testing algorithms (§ 610.40(b)).

METHOD 1: Deconstruction of the NAT-reactive minipool may be performed by testing the subpools (original or freshly made) that formed the minipool. This deconstruction of the minipool to identify the donor sample(s) that are NAT-reactive as the basis for the NAT-reactive result on the pool may involve several layers of testing using original or freshly pooled subpools, followed by testing of individual donor samples in the reactive subpool(s) (see Figure 1 and Table 1).

1. If you test subpools that were used to construct a Multiplex NAT-reactive minipool, in accordance with the manufacturer's instructions you must test the original subpools or freshly pooled subpools using the same Multiplex NAT method that was used in the original NAT on the minipool (§ 610.40(b)).

NOTE: In some cases the manufacturer's instructions provide for a different sample preparation procedure. However, the primers and

probes would be the same as those used in the original NAT on the minipool.

a. If all subpools are non-reactive, we recommend that you release from quarantine all individual donations that comprise the non-reactive subpools, provided that serologic tests on those donor samples are negative and the donations are otherwise suitable for release.

NOTE: Laboratory control procedures must make adequate provisions for monitoring the reliability, accuracy, precision, and performance of laboratory test procedures and instruments (§ 606.140(b)). This includes monitoring the frequency of reactive minipools that, upon deconstruction, remain unresolved. Laboratory control procedures must also include adequate identification and handling of all test samples (§ 606.140(c)). Use of supplies and reagents must be in a manner consistent with the instructions provided by the manufacturer (§§ 606.65(e), 610.40(b)). You must conduct and record a thorough investigation of any unexplained discrepancy (§ 606.100(c)), such as when the frequency of unresolved reactive minipools exceeds the threshold defined in your laboratory control procedures, or when there are indications of possible laboratory contamination of negative donor samples with positive samples. This investigation must include a determination of the cause of the initial reactivity of the unresolved minipool.

b. If one or more of the subpools are reactive, we recommend that you release from quarantine the individual donations that comprise the non-reactive subpools, provided that serologic tests on those donor samples are negative and the donations are otherwise suitable for release. Consistent with the manufacturer's instructions, you must test the individual donor samples that comprise the reactive subpool(s) using the same Multiplex NAT method that was used in the original NAT on the minipool (§ 610.40(b)).

(1) If all individual donor samples are non-reactive, we recommend that you release from quarantine all individual donations (if serologic tests on those donor samples are negative and the donations are otherwise suitable for release). See the NOTE in section IV.A.1.a. on laboratory control procedures and the possible need to conduct additional analyses to determine the cause of the initial reactivity of the unresolved minipool.

(2) If one or more individual donor sample(s) are reactive, perform the steps in section IV.C.1. (including testing using Discriminatory NAT, product disposition, donor management, and lookback).

We recommend that you release from quarantine all non-reactive individual donations provided that serologic tests on those donor samples are negative and the donations are otherwise suitable for release.

METHOD 2: For comparatively small minipools, for example, minipools that consist of up to 24 individual samples, deconstruction of the NAT-reactive minipool may be performed by directly testing the individual donor samples that formed the minipool (see Figure 1 and Table 1).

2. If you directly test the samples from individual donors that constituted the Multiplex NAT-reactive minipool, in accordance with the manufacturer's instructions you must test the individual donor samples using the same Multiplex NAT method that was used in the original NAT on the minipool (§ 610.40(b)).

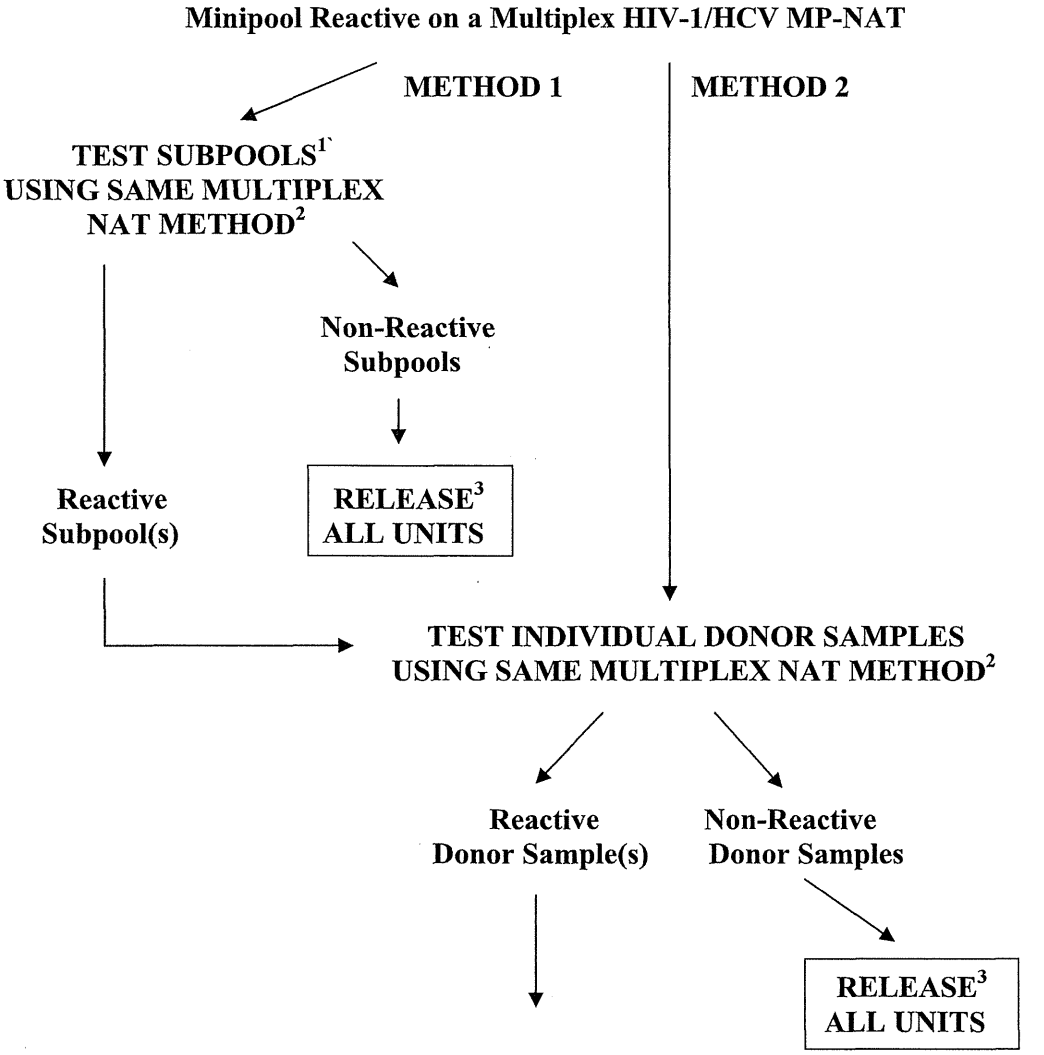
NOTE: In some cases the manufacturer's instructions provide for a different sample preparation procedure. However, the primers and probes would be the same as those used in the original NAT on the minipool.

a. If all individual donor samples are non-reactive, we recommend that you perform the steps in section IV.A.1.b.(1).

b. If one or more individual donor sample(s) are reactive, perform the steps in section IV.C.1. (including testing using Discriminatory NAT, product disposition, donor management, and lookback).

We recommend that you release from quarantine all non-reactive individual donations provided that serologic tests on those donor samples are negative and the donations are otherwise suitable for release.

FIGURE 1. Testing, Product Disposition, Donor Management, and Lookback for a Minipool that is Reactive on a Multiplex NAT (MP-NAT): Resolution by Testing Subpools or by Testing Individual Donor Samples



PERFORM THE STEPS IN FIGURE 3 FOR TESTING USING DISCRIMINATORY NAT, PRODUCT DISPOSITION, DONOR MANAGEMENT, AND LOOKBACK

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[1] Several layers of deconstruction using original or freshly pooled subpools, may be needed.

[2] In some cases a different sample preparation procedure may be used per manufacturer's instructions. However, primers and probes should be same as those used in the NAT on the minipool.

[3] Units may be released only if serologic tests for HIV-1 and HCV are negative and the units are otherwise suitable for release.

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TABLE 1. Testing, Product Disposition, Donor Management, and Lookback for a Minipool that is Reactive on a Multiplex NAT (MP-NAT): Resolution by Testing Subpools or by Testing Individual Donor Samples

If:	*Then:*	*After that if:*	*Then:*	*After that if:*	*Then:*
Minipool Reactive on a Multiplex HIV-1/HCV MP-NAT	METHOD 1: Test subpools[1] using same Multiplex NAT method[2]	Reactive subpool(s)	Test the individual donor samples using same Multiplex NAT method[2]	Reactive donor sample(s)	Perform the steps in Table 3 for testing (discriminatory NAT), product disposition, donor management, lookback
				Non-reactive donor samples	Release[3] all units
		Non-reactive subpool(s)	Release[3] all units		
	OR METHOD 2: Test the individual donor samples using same Multiplex NAT method[2]	Reactive donor sample(s)	Perform the steps in Table 3 for testing (discriminatory NAT), product disposition, donor management, lookback		
		Non-reactive donor samples	Release[3] all units		

[1] Several layers of deconstruction using original or freshly pooled subpools may be needed.

[2] In some cases a different sample preparation procedure may be used per manufacturer's instructions. However, primers and probes should be same as those used in the NAT on the minipool.

[3] Units may be released only if serologic tests for HIV-1 and HCV are negative and the units are otherwise suitable for release.

B. Testing, Product Disposition, Donor Management, and Lookback for a Minipool that is Reactive on a Single Virus NAT (MP-NAT): Resolution by Testing Subpools or by Testing Individual Donor Samples

If you obtain a reactive result for a NAT for HIV-1 RNA and/or HCV RNA performed separately on a minipool, the test instructions for use instruct you to perform subsequent testing to identify the donor sample(s) that are NAT-reactive as the basis for the NAT-reactive result on the pool. In general, the manufacturer's instructions for a licensed NAT describe two methods for resolving a minipool that is reactive on a Single Virus NAT, and you must follow the instructions in the package insert that provide specific testing algorithms (§ 610.40(b)).

METHOD 1: Deconstruction of the NAT-reactive minipool may be performed by testing the subpools (original or freshly made) that formed the minipool. This deconstruction of the minipool to identify the donor sample(s) that are NAT-reactive as the basis for the NAT-reactive result on the pool, may involve several layers of testing using original or freshly pooled subpools, followed by testing of individual donor samples in the reactive subpool(s) (see Figure 2 and Table 2).

1. If you test subpools that were used to construct a NAT-reactive minipool, in accordance with the manufacturer's instructions you must test the original subpools or freshly pooled subpools using the same Single Virus NAT method that was used in the original NAT on the minipool (§ 610.40(b)).

   NOTE: In some cases the manufacturer's instructions provide for a different sample preparation procedure. However, the primers and probes would be the same as those used in the original NAT on the minipool.

   a. If all subpools are non-reactive, we recommend that you release from quarantine all individual donations that comprise the non-reactive subpools provided that serologic tests on those donor samples are negative and the donations are otherwise suitable for release. See the NOTE in section IV.A.1.a. on laboratory control procedures and the possible need to conduct additional analyses to determine the cause of the initial reactivity of the unresolved minipool.

   b. If one or more of the subpools are reactive, we recommend that you release from quarantine the individual donations that comprise the non-reactive subpools provided that serologic tests on those donor samples are negative and the donations are otherwise suitable for release. Consistent with the manufacturer's instructions, you must test the individual

donations that comprise the reactive subpool(s) using the same Single Virus NAT method that was used in the original NAT on the minipool (§ 610.40(b)).

(1) If all individual donor samples are non-reactive, we recommend that you release from quarantine all individual donations provided that serologic tests on those donor samples are negative and the donations are otherwise suitable for release. See the NOTE in section IV.A.1.a. on laboratory control procedures and the possible need to conduct additional analyses to determine the cause of the initial reactivity of the unresolved minipool.

(2) If one or more individual donor sample(s) are reactive, perform steps 1 through 4 in section IV.D. (including product disposition, donor management, and lookback).

We recommend that you release from quarantine all non-reactive individual donations provided that serologic tests on those donor samples are negative and the donations are otherwise suitable for release.

METHOD 2: For comparatively small minipools, for example, minipools that consist of up to 24 individual samples, deconstruction of the NAT-reactive minipool may be performed by directly testing the individual donor samples that formed the minipool (see Figure 2 and Table 2).

2. If you directly test the samples from individual donors that constituted the NAT-reactive minipool, in accordance with the manufacturer's instructions you must test the individual donor samples using the same Single Virus NAT method that was used in the original NAT on the minipool (§ 610.40(b)).

NOTE: In some cases the manufacturer's instructions provide for a different sample preparation procedure. However, the primers and probes would be the same as those used in the original NAT on the minipool.

a. If all individual donor samples are non-reactive, we recommend that you perform the steps in section IV.B.1.b.(1).

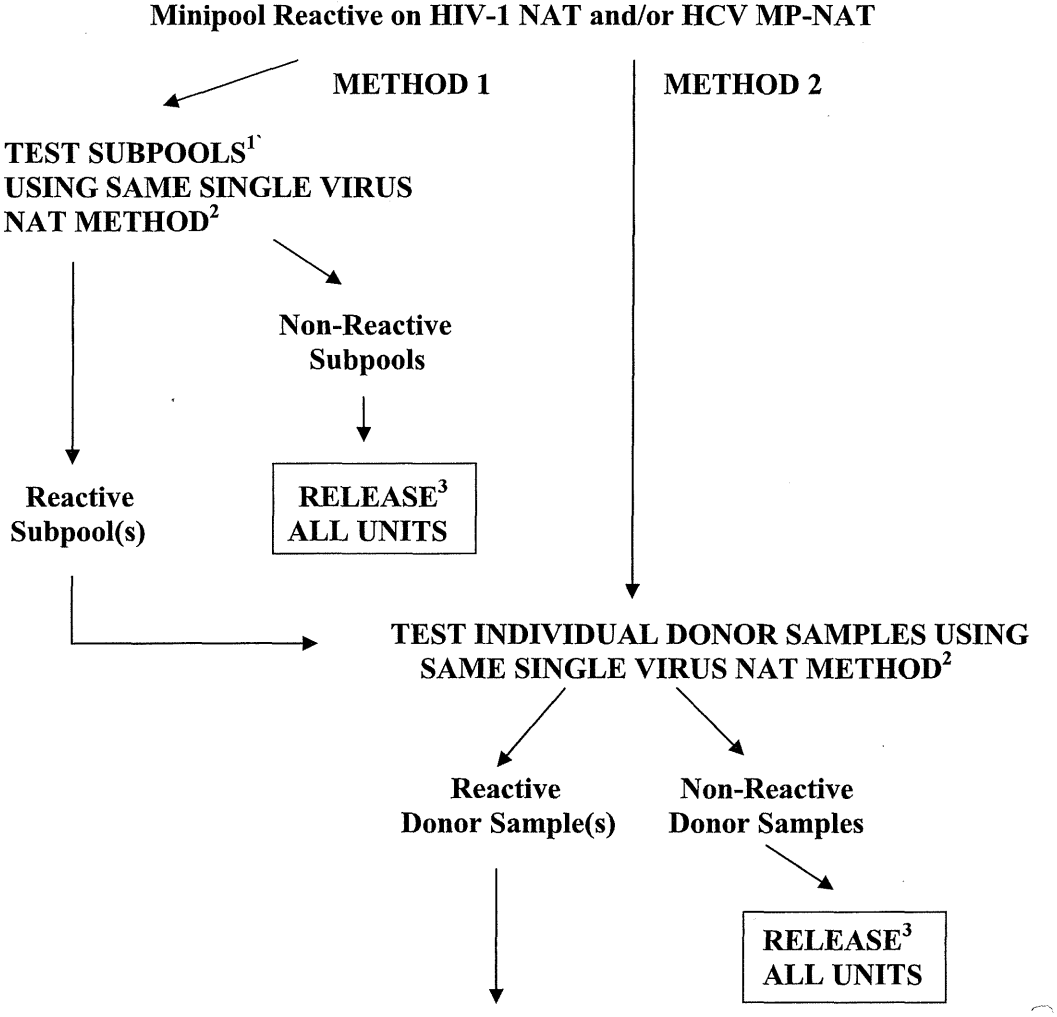
b. If one or more individual donor sample(s) are reactive, perform steps 1 through 4 in section IV.D. (including product disposition, donor management, and lookback).

Contains Nonbinding Recommendations

We recommend that you release from quarantine all non-reactive individual donations provided that serologic tests on those donor samples are negative and the donations are otherwise suitable for release.

Contains Nonbinding Recommendations

FIGURE 2. Testing, Product Disposition, Donor Management, and Lookback for a Minipool that is Reactive on a Single Virus NAT (MP-NAT): Resolution by Testing Subpools or by Testing Individual Donor Samples



PERFORM THE STEPS IN FIGURE 4 FOR PRODUCT DISPOSITION, DONOR MANAGEMENT, AND LOOKBACK

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[1] Several layers of deconstruction using original or freshly pooled Subpools, may be needed.

[2] In some cases a different sample preparation procedure may be used per manufacturer's instructions. However, primers and probes should be same as those used in the NAT on the minipool.

[3] Units may be released only if serologic tests for HIV-1 and HCV are negative and the units are otherwise suitable for release.

Contains Nonbinding Recommendations

TABLE 2. Testing, Product Disposition, Donor Management, and Lookback for a Minipool that is Reactive on a Single Virus NAT (MP-NAT): Resolution by Testing Subpools or by Testing Individual Donor Samples

<table>
<tr><th>If:</th><th>Then:</th><th>After that if:</th><th>Then:</th><th>After that if:</th><th>Then:</th></tr>
<tr><td rowspan="4">Minipool Reactive on HIV-1 MP-NAT and/or HCV MP-NAT</td><td rowspan="2">METHOD 1: Test subpools[1] using same Single Virus NAT method[2]</td><td rowspan="2">Reactive subpool(s)</td><td rowspan="2">Test the individual donor samples using same Single Virus NAT method[2]</td><td>Reactive donor sample(s)</td><td>Perform the steps in Table 4 for product disposition, donor management, and lookback</td></tr>
<tr><td>Non-reactive donor samples</td><td>Release[3] all units</td></tr>
<tr><td>Non-reactive subpool(s)</td><td>Release[3] all units</td><td></td><td></td></tr>
<tr><td>OR

METHOD 2: Test the individual donor samples using same Single Virus NAT method[2]</td><td>Reactive donor sample(s)</td><td>Perform the steps in Table 4 for product disposition, donor management, and lookback</td><td rowspan="2"></td><td rowspan="2"></td></tr>
<tr><td>Non-reactive donor samples</td><td>Release[3] all units</td></tr>
</table>

[1] Several layers of deconstruction using original or freshly pooled subpools may be needed.

[2] In some cases a different sample preparation procedure may be used per manufacturer's instructions. However, primers and probes should be same as those used in the NAT on the minipool.

[3] Units may be released only if serologic tests for HIV-1 and HCV are negative and the units are otherwise suitable for release.