Guidance for Industry

Use of Nucleic Acid Tests on Pooled and Individual Samples from Donors of Whole Blood and Blood Components, including Source Plasma, to Reduce the Risk of Transmission of Hepatitis B Virus

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Guidance for Industry

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I. INTRODUCTION

We, FDA, are providing you, blood establishments that collect Whole Blood and blood components for transfusion or for further manufacture, including recovered plasma, Source Plasma and Source Leukocytes, with recommendations concerning the use of FDA-licensed nucleic acid tests (NAT) to screen blood donors for hepatitis B virus (HBV) deoxyribonucleic acid (DNA). We are also providing you with recommendations for product testing and disposition, donor management, methods for donor requalification, and product labeling.

In addition, we are notifying you in this guidance that we consider the use of an FDA-licensed HBV NAT to be necessary to reduce adequately and appropriately the risk of transmission of HBV. FDA-licensed HBV NAT can detect evidence of infection at an earlier stage than is possible using previously approved hepatitis B surface antigen (HBsAg) and antibody to hepatitis B core antigen (anti-HBc) tests. Therefore, we recommend that you use FDA-licensed HBV NAT, in accordance with the requirements under Title 21 Code of Federal Regulations, 610.40(a) and (b) (21 CFR 610.40(a) and (b)).

This guidance finalizes the draft guidance of the same title dated November 2011. This guidance supplements previous memoranda and guidance from FDA to blood establishments concerning the testing of donations for HBsAg and anti-HBc, and the management of donors and units mentioned in those documents (Refs. 1 through 5). Note that when you implement HBV NAT testing, you should continue testing for HBsAg and anti-HBc in Whole Blood and blood components intended for transfusion and Source Leukocytes intended for further manufacture,

and continue testing for HBsAg in Source Plasma.[1] FDA may consider advancements in technology for testing blood donations, as well as data obtained following the implementation of HBV NAT, to make future recommendations on adequate and appropriate testing for HBV.

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

II. DEFINITIONS

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.

Discriminatory NAT: A NAT that uses specific primers for HIV-1 or HBV or HCV to identify the RNA or DNA in the reactive multiplex NAT sample as HIV-1 RNA or HBV DNA or HCV RNA. Performing a discriminatory NAT on a reactive sample is a required step for those establishments using an approved multiplex test. The labeling for licensed multiplex NATs specifies that discriminatory NAT is to be performed. Under § 610.40(b) (21 CFR 610.40(b)), you must use FDA-approved screening tests in accordance with the manufacturer's instructions.

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.

HBV NAT assay with a limited supplemental test indication: Some HBV NAT assays have received a limited supplemental indication for repeatedly reactive HBsAg test results. If a donation tests HBV NAT-positive for HBV DNA using an HBV NAT with such a limited supplemental test indication, and if that donation also tests HBsAg repeatedly reactive in a screening test, the HBsAg test result can be recorded as HBsAg positive. In this case, an HBsAg neutralization test need not be performed. However, if a donation tests HBV NAT-negative for HBV DNA using an HBV NAT with such a limited supplemental test indication, and if that

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[1] FDA does not currently recommend that Source Plasma donors be tested for anti-HBc. If anti-HBc reactive units were excluded from pools used for the manufacture of plasma derivatives, titers of neutralizing antibody to hepatitis B surface antigen (anti-HBs) in those pools would be expected to diminish, as both these antibodies usually occur together. The presence of neutralizing anti-HBs is believed to contribute to the safety of certain plasma products. (Ref. 2). Plasma units that are untested, non-reactive (NR), or repeatedly reactive (RR) for anti-HBc are currently acceptable for the manufacture of plasma derivatives (Ref. 2). Consistent with § 610.40(h)(2)(v), recovered plasma from donations of Whole Blood that test anti-HBc reactive may be used for further manufacture into plasma derivatives.

donation tests HBsAg repeatedly reactive in a screening test, an HBsAg neutralization test should be performed. In this case, the result of the neutralization test serves as the test of record (Ref. 1).

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, HBV DNA, and HCV RNA.

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

III. BACKGROUND

Under § 610.40(a), establishments that collect blood or blood components must test each donation of human blood or blood component intended for use in preparing a product, including donations intended as a component of, or used to prepare, a medical device, for evidence of infection due to certain communicable disease agents, including HBV. In addition, under § 610.40(b), you must perform one or more such tests as necessary to reduce adequately and appropriately the risk of transmission of communicable disease.

Currently, all Whole Blood and blood components intended for transfusion and all Source Leukocytes intended for further manufacture are routinely tested for HBsAg and anti-HBc in order to reduce the risk of transmission of HBV (Refs. 1, 2, 3 and 5). In addition, all Source Plasma collections intended for further manufacture into plasma derivatives are routinely tested for HBsAg in order to reduce the risk of transmission of HBV in manufacturing pools of plasma derivatives.[2]

In the preamble to the final rule entitled "Requirements for Testing Human Blood Donors for Evidence of Infection Due to Communicable Disease Agents," published in the *Federal Register* of June 11, 2001 (66 FR 31146), we discussed the approved donor screening tests that we considered, as of that date, to be necessary to reduce adequately and appropriately the risk of transmission of HBV. We also stated that as technology advances, we intend to issue guidance describing those tests that we consider to reduce adequately and appropriately the risk of transmission of communicable disease agents. Accordingly, in this guidance document, we are notifying you that we consider FDA-licensed HBV NAT to be necessary to reduce adequately and appropriately the risk of transmission of HBV.

[2] See Footnote 1.

We note that the tests referenced in this document have been licensed by FDA for the screening of donors for HBV DNA and have the ability to detect the evidence of infection at an earlier stage than is possible using previously approved HBsAg and anti-HBc tests. Because FDA-licensed HBV NAT are now widely available, we recommend that establishments use these tests, in accordance with § 610.40.

A. Rationale for Donor Screening Using HBV NAT

HBV is a major human pathogen that may cause acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma (Ref. 6). Most primary infections in adults are self-limited, the virus is cleared from blood and liver, and individuals develop a lasting immunity. Fewer than 5% of infected adults develop persistent asymptomatic infections (i.e., a carrier state). However, infants and young children have a much higher likelihood of developing a chronic hepatitis B infection than do older children and adults. According to data obtained in 2004 from the Centers for Disease Control and Prevention, about 1% of adults without other preexisting conditions are estimated to get chronic hepatitis B if infected, but 2% to 10% of children more than 5 years of age get chronic hepatitis B, and 30% to 90% of children less than 5 years of age develop chronic hepatitis B if infected (Ref. 7). In addition, many patients receiving blood are immunocompromised because of their underlying disease and/or because of medications that suppress the immune system making them more susceptible to severe HBV infection than otherwise healthy individuals. About 20% of chronically infected individuals can develop cirrhosis. Chronically infected subjects have 100 times higher risk of developing hepatocellular carcinoma than non-carriers (Ref. 6).

Currently, HBV is transmitted by blood transfusions more frequently than hepatitis C virus (HCV) or human immunodeficiency virus (HIV). The residual risk of post-transfusion HBV infection is estimated to be about 1:357,000 to 1:280,000 per transfusion. In comparison, those risks for HIV and HCV are estimated to be 1:1,467,000 and 1:1,149,000, respectively (Ref. 8). Depending on the sensitivity of the test, implementation of HBV NAT has the potential to reduce risk of infection to levels similar to those for HIV and HCV. HBV can be transmitted by blood from asymptomatic donors with acute HBV infections who have not yet developed HBsAg or anti-HBc (i.e., donors in the seronegative window period), when HBV DNA can be detected in the donor's blood (Refs. 9 and 10). Depending on the relative sensitivities of HBsAg and HBV NAT assays used, HBV DNA can be detected 2 to 5 weeks after infection, and up to 40 days (mean 6 to 15 days) before HBsAg (Ref. 7). HBV DNA levels rise slowly and are present at relatively low levels during the seronegative window period of early infection. HBV DNA can also be detected along with HBsAg and anti-HBc in chronic hepatitis B infections, and sometimes in recovered infections that are negative for HBsAg and positive for antibodies to hepatitis B surface antigen (anti-HBs) and anti-HBc (Refs. 6 and 11). Rarely, HBV DNA can be detected in the absence of HBsAg, anti-HBc and anti-HBs (Ref. 12).

Blood for transfusion in the United States (U.S.) is also tested for anti-HBc. Anti-HBc develops a few days after the appearance of HBsAg and usually remains detectable for

life, irrespective of whether the individual recovers from acute hepatitis B or whether chronic HBV infection develops. Because of the availability and use of tests to detect anti-HBc, HBV NAT's potential utility in further reducing risk of HBV transmission by blood transfusion is mainly restricted to the early HBsAg-negative phase of infection (i.e., a potential reduction of the infectious window period of up to 40 days depending on sensitivity of the HBsAg test).

There are currently three FDA-licensed HBV NAT assays for screening Whole Blood and blood components available in the U.S. Following licensure of the first HBV NAT assay in April 2005 (the Roche COBAS AmpliScreen HBV NAT that uses pools of up to 24 donation samples for testing Whole Blood and blood components for transfusion and pools of up to 96 donation samples for testing Source Plasma), FDA did not recommend use of HBV NAT. At that time, FDA's position on the use of HBV NAT was based, in part, upon discussions by the Blood Products Advisory Committee (BPAC or Committee) at the meeting on July 23, 2004 (Ref. 13), and on a recommendation from the Department of Health and Human Services Secretary's Advisory Committee on Blood Safety and Availability (ACBSA) on August 27, 2004 (Ref. 14). In making its recommendations, the ACBSA considered a number of broad public health issues including cost-effectiveness, feasibility, and overall public health benefit, in addition to scientific data on detection of HBV in donors. FDA's principal reason for not recommending HBV NAT at that time was that the sensitivity of HBV NAT in the available format, when compared to the then-available serologic testing, did not provide sufficient additional safety to the blood supply to warrant recommending its use. FDA's reasoning was based on then current information that most blood establishments would have to test pools of 24 samples (thus diluting the individual samples by 1:24), because it was not feasible for most blood establishments to test single samples from donations or even small pools of samples.

Since licensure of the first HBV NAT in 2005, the following changes have occurred:

1. FDA has licensed two additional HBV NAT assays with indications for blood donor screening: Procleix® ULTRIO®Plus Assay (Gen-Probe, Inc., San Diego, California), which uses up to 16 donation samples in a pool for testing Whole Blood and blood components for transfusion and Source Plasma; and COBAS® TaqScreen MPX Test (Roche Molecular Systems, Inc., Pleasanton, California), which uses up to 6 donation samples in a pool for testing Whole Blood and blood components for transfusion and pools of up to 96 donation samples for testing Source Plasma. These multiplex assay systems can simultaneously detect HIV, HCV and HBV in a single donation, thus improving the feasibility of routine NAT testing for HBV. FDA has also licensed the UltraQual™ HBV PCR Assay (National Genetics Institute, Los Angeles, California), which provides results of HBV NAT of Source Plasma samples or of plasma samples from Source Plasma donors at the time of donation. This assay is an "in-house" test; no kit is sold. This assay tests up to 512 donation samples in a pool.

2. With the recent advance in technology and increased automation enabling the performance of NAT with smaller pools of samples and individual samples, more sensitive HBV NAT testing of blood donations is now possible, resulting in an increase in the number of window period HBV DNA positive/HBsAg negative units that can be detected.

3. There is now more information available on the role of vaccination of donors and recipients against HBV infection that indicates that protection for the long term is not absolute (i.e., breakthrough infections can occur in previously vaccinated individuals who are exposed to the virus) (Refs. 10 and 15). Breakthrough infections are characterized by HBV NAT positivity, the presence of HBV-neutralizing anti-HBs (developed as a result of hepatitis B vaccination), low viral load and lack of symptoms. HBsAg and anti-HBc may not subsequently develop or their appearance may be delayed. The infectivity of units obtained from hepatitis B-vaccinated donors with breakthrough HBV infections is unknown at the present time.

As mentioned above, in breakthrough HBV infections, HBsAg and anti-HBc may not develop or their appearance may be delayed. However, should development occur, it is more likely to be detected by HBV NAT, particularly in the early stages of infection. As younger cohorts in the population, who have received hepatitis B vaccine in a greater proportion than older cohorts (Refs. 16, 17, and 18), become eligible to donate blood, the proportion of vaccinated donors compared to non-vaccinated donors is expected to increase. Therefore, the proportion of donors with HBV breakthrough infections, compared to those with non-breakthrough, wild-type, HBV infections, also is expected to increase. These donors' asymptomatic breakthrough infections are more likely to be detected by HBV NAT than to be detected by HBsAg or anti-HBc assays because HBsAg and anti-HBc development might be delayed or might not occur, even though HBV DNA is present and detectable by HBV NAT in the initial stage of the infection. In addition, HBV mutants appear to be more likely to be detected by HBV NAT than by HBsAg assays (Ref. 10).

Much of the available literature does not support transmission of HBV by HBV NAT positive/anti-HBs positive/HBsAg negative blood, irrespective of anti-HBc test results, (Refs. 11, 12, 19, 20, 21, and 22). However, there are at least two reports of such possible transmissions by hematopoietic stem cells and blood (Refs. 23 and 24), and one report that appears to have confirmed transmission of HBV by HBV NAT positive/anti-HBs positive/HBsAg negative blood (Ref. 25). Therefore, there can be no assumption of non-infectivity of units from donors with breakthrough infections containing HBV DNA and vaccine-induced, HBV-neutralizing anti-HBs when transfused into recipients. Nor can we assume a lack of morbidity and mortality in recipients, especially when many recipients are immunocompromised, as previously mentioned.

At the April 1, 2009 BPAC meeting (Ref. 26), the Committee agreed with FDA's position that there is no assumption of non-infectivity to recipients of units from donors

with breakthrough infections. Therefore, in this guidance, we are recommending that all units of Whole Blood and blood components used for transfusion should be tested by an FDA-licensed HBV NAT. The Committee also advised that FDA should set a sensitivity standard of 200 IU/mL HBV DNA for detection of HBV DNA in an individual donation when HBV NAT assays are used to test blood and blood components intended for transfusion. However, because of technological advances that have occurred since the April 2009 BPAC meeting, we are now recommending a sensitivity standard of 100 IU/mL for HBV DNA detection in an individual donation (see section IV.A). Because of advances in technology and automation, FDA considers a sensitivity standard of 100 IU/mL to be both attainable and practical for blood establishments that collect donations of Whole Blood and blood components intended for transfusion.

With regard to testing Source Plasma units for further manufacture into injectable plasma derivatives for HBV DNA, we believe that such testing adds another layer of safety for plasma derivatives by limiting the viral load in plasma pools for fractionation (in viral inactivation and/or removal steps during their manufacture and the presence of neutralizing anti-HBs in manufacturing pools). During the BPAC meeting held on April 28, 2011 (Ref. 27), the Committee agreed with FDA that the available scientific data supports the concept that testing Source Plasma donations by HBV NAT increases the safety margin of plasma derivatives. Therefore, FDA is recommending that all units of Source Plasma intended for manufacture into injectable plasma derivatives be tested by an FDA-licensed HBV NAT. In consideration of viral inactivation and removal steps in plasma fractionation, FDA also is recommending a sensitivity standard of 500 IU/mL for detection of HBV DNA in an individual collection, rather than 100 IU/mL (see section IV.A). BPAC endorsed this sensitivity standard at the April 28, 2011 meeting (Ref. 27).

Similar to plasma derivatives, the HBV safety of products made from Source Leukocytes depends in large measure on viral removal and inactivation during manufacturing. However, since Source Leukocytes are obtained from Whole Blood donors, consistent with our recommendation for Whole Blood and blood components intended for transfusion, we are also recommending a sensitivity standard of 100 IU/mL for HBV DNA detection in the individual donation of Source Leukocytes.

B. Donor Requalification

Under § 610.41(b), "[a] deferred donor subsequently may be found to be suitable as a donor of blood or blood components by a requalification method or process found acceptable for such purposes by FDA." [3]

At the July 21, 2005 BPAC meeting (Ref. 28), the Committee agreed with FDA's proposed requalification criteria for donors of Whole Blood and blood components for transfusion and Source Plasma for further manufacture, who tested positive by HBV

[3] A deferred donor may serve as an autologous donor in accordance with § 610.40 and § 610.41. Note that a deferred donor who donates for autologous use is not deemed to be reentered and remains deferred, until the criteria for reentry are met.

NAT, when a follow-up sample is tested using HBV NAT and serologic tests. Data presented at the meeting demonstrated that a 6-month follow-up period encompasses the pre-seroconversion window period with sufficient confidence that negative test results for HBsAg, anti-HBc and HBV DNA by NAT, after a 6-month period, rule out HBV infection. For purposes of reentry, we recommend that you use an FDA-licensed HBV NAT labeled as having a sensitivity of $\leq$ 2 IU/mL at 95% detection rate [1 IU = ~5 copies of HBV DNA/mL].[4] Donors with negative results for HBV DNA at this level of sensitivity are highly unlikely to be infected with HBV (Ref. 29). Depending upon the assay and the platform used, this sensitivity may only be achieved when testing individual donor samples. Recommended criteria for donor requalification are presented in section IV.C.

IV. RECOMMENDATIONS

A. Donor Screening Using HBV NAT

Under § 610.40(b), you must use screening tests that FDA has approved for such use, in accordance with the manufacturers' instructions. Under this provision, you also must perform one or more such tests as necessary to reduce adequately and appropriately the risk of transmission of communicable disease, including HBV.

1. In order to meet the requirement under § 610.40(b) for Whole Blood and blood components intended for transfusion and Source Leukocytes intended for further manufacture, we recommend that you use an FDA-licensed donor screening test for HBV DNA by NAT, in addition to testing for HBsAg and anti-HBc. If the FDA-licensed tests for detection of both HBsAg and anti-HBc are negative or non-reactive, we recommend that you test the donation further using an FDA-licensed HBV NAT that has a lower limit of detection of < 100 IU/mL HBV DNA for HBV DNA detection in an individual donation. The FDA-licensed screening HBV NAT that you use may be in a minipool donation-sample testing format or an individual donation testing format, and may include multiplex NAT with testing of other agents, such as HIV and HCV or may be single virus NAT for HBV only. Testing for HBsAg, anti-HBc and HBV DNA by NAT may be performed concurrently.

[4] COBAS AmpliScreen HBV Test (Roche Molecular Systems, Inc., Pleasanton, California): Triplicate testing using the multiprep specimen processing procedure. See package insert.
Procleix® ULTRIO®Plus Assay (Gen-Probe, Inc., San Diego, California): Testing 3 replicates. See package inserts.

2. In order to meet the requirement under § 610.40(b) for testing Source Plasma intended for further manufacture into plasma derivatives, we recommend that you use an FDA-licensed donor screening test for the detection of HBsAg. If the FDA-licensed test for detection of HBsAg is negative or non-reactive, we recommend that you test the donation further using an FDA-licensed HBV NAT that has a lower limit of detection of < 500 IU/mL HBV DNA for HBV DNA detection in an individual donation. The FDA-licensed screening HBV NAT that you use may be in a minipool donation-sample testing format or an individual donation testing format, and may include multiplex NAT with testing of other agents, such as HIV and HCV, or may be single virus NAT for HBV only. Testing for HBsAg and HBV DNA by NAT may be performed concurrently. As explained in footnote 1, FDA does not currently recommend that Source Plasma donors be tested for anti-HBc (Ref. 2).

   As a general matter, under § 610.40(h)(1), if any of the FDA-licensed tests for the detection of either HBsAg or anti-HBc is reactive, the donation must not be shipped or used.[5] In this instance, we believe that you have met the standard for adequate and appropriate screening for HBV and you do not need to test the unit further using an FDA-licensed HBV NAT. However, you may choose to test such a reactive donation by using an FDA-licensed HBV NAT to provide useful information to the donor, or if you wish to reenter the donor as described below in this guidance.

   We note that in regard to HBsAg reactivity, as required by § 610.40(e), you must proceed to supplemental testing for HBsAg to determine whether or not a reactive HBsAg test result can be confirmed positive, and is not a false positive (i.e., test result recorded HBsAg negative), using either an additional, more specific test, such as an HBsAg neutralization test or an HBV NAT assay with a limited supplemental test indication. Some HBV NAT assays have received this limited supplemental indication for repeatedly reactive HBsAg test results. If a donation tests HBV NAT-positive for HBV DNA using an HBV NAT with a limited supplemental test indication, and if that donation also tests HBsAg repeatedly reactive in a screening test, the HBsAg test result can be recorded as HBsAg positive. In this case, an HBsAg neutralization test need not be performed. However, if a donation tests HBV NAT-negative for HBV DNA using an HBV NAT with a limited supplemental test indication, and if that donation tests HBsAg repeatedly reactive in a screening test, an HBsAg neutralization test should be performed. In this case, the result of the neutralization test serves as the test of record (Ref. 1). We further note that there is no licensed supplemental test for anti-HBc at the present time. Donors with anti-HBc reactive results may be

[5] Whole Blood or blood components that are reactive for HBsAg and/or anti-HBc may be shipped or used if they meet the conditions for an exception as described in § 610.40(h)(2).

requalified as described in the FDA guidance entitled, "Guidance for Industry: Requalification Method for Reentry of Blood Donors Deferred Because of Reactive Test Results for Antibody to Hepatitis B Core Antigen (Anti-HBc)," dated May 2010 (Ref. 3).

B. Management of Donors and Units Based on HBV Test Results

1. Donor and Unit Management When the HBV DNA NAT Result is Negative

   a. If a unit tests negative by individual donation NAT (ID-NAT) for HBV DNA or is part of a minipool that tests negative, then the donor and the unit should be managed consistent with FDA guidances and recommendations, as appropriate (Refs. 1 through 5), provided that the donor satisfies all applicable regulatory requirements, including donor eligibility criteria in §§ 640.3 and 640.63, and the unit is otherwise suitable for release.

   b. Units of Whole Blood and blood components and units of Source Leukocytes that test negative for HBV using FDA-licensed HBV NAT and HBsAg and anti-HBc assays may be used for transfusion or further manufacture (as appropriate), provided that the donor satisfies the donor eligibility criteria in § 640.3, and that all other donor screening tests for communicable disease agents required in § 610.40(a) and (i) for Whole Blood and blood components, including Source Leukocytes, are negative, and that the units are otherwise suitable for release.

   c. Units of Source Plasma and recovered plasma that test negative for HBV using FDA-licensed HBV NAT and HBsAg assays may be used for further manufacture, provided that the donor satisfies the donor eligibility criteria in § 640.63 (for Source Plasma) and § 640.3 (for recovered plasma), and that the requirements in § 610.40 are met and that all other screening tests for communicable disease agents required in § 610.40(a) and (i) are negative, and that the units are otherwise suitable for release (see footnote 1).

2. Donor and Unit Management when the HBV DNA NAT Result is Positive

   a. In accordance with § 610.40(h), except for autologous donations under § 610.40(h)(2)(i) or where you have obtained FDA's written approval for the shipment or use in accordance with § 610.40(h)(2)(ii)(A), you must not ship or use a unit of Whole Blood or blood components for transfusion, or a unit of Source Leukocytes for further manufacture, if it tests positive by HBV ID-NAT (either from direct screening by ID-NAT or from deconstruction of a NAT-positive minipool) (Table 1,

Categories 1 through 6).

b. In accordance with § 610.41, you must defer a donor who tests reactive for HBV, and in accordance with 21 CFR Part 630 (Part 630) you must notify the blood donor. You should permanently defer a donor of Whole Blood or blood components, including Source Leukocytes for further manufacture, whose NAT and serologic test results are as follows (the donor is not eligible for reentry):

    i. HBV NAT-positive, HBsAg RR and confirmed positive, either by neutralization or when using a NAT with a limited supplemental claim, regardless of anti-HBc results (Table 1, Categories 1 and 2); or

    ii. HBV NAT-positive when using a NAT that does not have a limited supplemental test indication and HBsAg RR is not confirmed by neutralization, and anti-HBc is RR (Table 1, Category 3).

c. In accordance with § 610.41, you must defer a donor who tests reactive for tests for HBV, and in accordance with Part 630, you must notify the donor. You should indefinitely defer a donor of Whole Blood or blood components, including Source Leukocytes, whose NAT and serologic test results are as follows (note that the donor may be eligible for reentry, as described in section IV.C):

    i. HBV NAT-positive, HBsAg non-reactive (NR), anti-HBc RR (Table 1, Category 4); or

    ii. HBV NAT-positive, and both HBsAg and anti-HBc are NR (Table 1, Category 5); or

    iii. HBV NAT-positive using a NAT that does not have a limited supplemental test indication and HBsAg RR is not confirmed by neutralization, and is anti-HBc NR (Table 1, Category 6).

Table 1. Donor and Unit Management (Whole Blood and Blood Components for Transfusion, and Source Leukocytes for Further Manufacture) when the HBV DNA NAT Result is Positive

Category	HBV NAT Result	HBsAg Result	Anti-HBc Result	Donor and Unit
1	Positive	Repeatedly Reactive / Confirmed Positive*	Non-Reactive	Discard unit; Permanently defer donor; Donor not eligible for reentry
2	Positive	Repeatedly Reactive / Confirmed Positive*	Repeatedly Reactive	
3	Positive	Repeatedly Reactive / Not Confirmed	Repeatedly Reactive	
4	Positive	Non-Reactive	Repeatedly Reactive	Discard unit; Indefinitely defer donor; Donor may be eligible for reentry
5	Positive	Non-Reactive	Non-Reactive	
6	Positive	Repeatedly Reactive / Not Confirmed	Non-Reactive	

*Using either an HBsAg neutralization test or an HBV NAT with a limited supplemental test indication, as described in section IV.A.2.

d. In accordance with § 610.40(h), except where you have obtained FDA's written approval for the shipment or use in accordance with § 610.40(h)(2)(ii)(A), you must discard and not use for further manufacture a unit of Source Plasma that tests positive by HBV ID-NAT (Table 2, Categories 1 through 3).

e. In accordance with § 610.41, you must defer a donor who tests reactive for tests for HBV, and in accordance with Part 630, you must notify the donor. You should permanently defer a donor of Source Plasma whose donation tests HBV NAT-positive and is HBsAg RR, confirmed positive either by neutralization, or when using a NAT with a limited supplemental test indication. The donor is not eligible for reentry (Table 2, Category 1).

f. In accordance with § 610.41, you must defer a donor who tests reactive for tests for HBV, and in accordance with Part 630 you must notify the blood donor. You should indefinitely defer a donor of Source Plasma whose donation tests HBV NAT-positive when using a NAT that does not have a limited supplemental claim, and is either HBsAg NR or is HBsAg RR not confirmed by neutralization (Table 2, Categories 2 and 3). The donor may be eligible for reentry, as described in section IV.C.

Table 2. Donor and Unit Management (Source Plasma for Further Manufacture) when the HBV DNA NAT Result is Positive

Category	HBV NAT Result	HBsAg Result	Donor and Unit
1	Positive	Repeatedly Reactive / Confirmed Positive*	Discard unit; Permanently defer donor; Donor not eligible for reentry
2	Positive	Non-Reactive	Discard unit; Indefinitely defer donor; Donor may be eligible for reentry
3	Positive	Repeatedly Reactive/ Not Confirmed	

* Using either an HBsAg neutralization test or an HBV NAT with a limited supplemental test indication, as described in section IV.A.2.

C. Requalification Methods for Donors on the Basis of HBV NAT and HBV Serologic Test Results on the Follow-up Sample

For purposes of reentry, we recommend that you use an FDA-licensed HBV NAT having a sensitivity of ≤ 2 IU/mL at 95% detection rate.

1. Requalification of a Donor of Whole Blood or Blood Components for Transfusion, and Source Leukocytes for Further Manufacture

To reenter an indefinitely deferred donor of Whole Blood or blood components for transfusion, or Source Leukocytes for further manufacture, you should obtain a follow-up sample from the donor (no donation is made at this time) at least 6 months after the collection of the sample that gave test results described in section IV.B.2.c. You should perform follow-up testing using HBV NAT (having a sensitivity of ≤ 2 IU/mL at 95% detection rate), HBsAg and anti-HBc FDA-licensed assays.

a. If the new follow-up sample tests positive by HBV NAT, regardless of HBsAg and/or anti-HBc test results, we recommend that you permanently defer the donor (Table 3, Category 1).

b. If the new follow-up sample tests negative by HBV NAT and NR by HBsAg and anti-HBc assays, the donor may be reentered (i.e., the donor is eligible to donate in the future), provided the donor meets all donor eligibility criteria in § 640.3 (Table 3, Category 2).

c. If the new follow-up sample tests negative by HBV NAT and RR by HBsAg and/or RR by anti-HBc, we recommend that you evaluate the donor further as described in the FDA guidance documents cited in References 1, 2 and 3 (Table 3, Category 3) in this document.

NOTE: If you wish to perform follow-up testing on a donor of Whole Blood or blood components for transfusion or on a donor of Source Leukocytes for further manufacture who is deferred because of HBV NAT test results, you may do so before the end of the 6-month waiting period for donor notification purposes or for medical reasons. Negative test results on follow-up for HBsAg, anti-HBc and HBV DNA by NAT (sensitivity at 95% detection rate of $\leq$ 2 IU/mL), may be useful in donor counseling. However, only negative results for all three tests (HBsAg, anti-HBc and HBV NAT), obtained at least 6 months after the collection of the sample that gave the test results described in section IV.B.2.c, would qualify the donor for reentry. If you obtain a positive HBV NAT, or RR anti-HBc, or RR HBsAg that is positive by neutralization during this 6-month waiting period, the donor would not be eligible for reentry, and we recommend that you defer the donor permanently.

A donor of Whole Blood or blood components for transfusion, or a donor of Source Leukocytes for further manufacture who has been requalified as described above in section IV.C.1., may on subsequent occasions be indefinitely deferred because of HBV NAT reactive results. You may reenter such a donor into the donor pool by again following all the procedures described in section IV.C.1.

2. Requalification of a Donor of Source Plasma for Further Manufacture

To reenter an indefinitely deferred donor of Source Plasma, you should obtain a follow-up sample from the donor (no donation is made at this time) at least 6 months after the collection of the sample that gave the test results described in section IV.B.2.f. You should perform follow-up testing using HBV NAT (having a sensitivity of $\leq$ 2 IU/mL at 95% detection rate) and HBsAg FDA-licensed assays.

a. If a new follow-up sample tests positive by HBV NAT, regardless of the HBsAg test result, you should permanently defer the donor (Table 3, Category 1).

b. If a new follow-up sample tests negative by HBV NAT and NR by HBsAg, the donor is eligible to donate in the future, provided the donor satisfies all donor eligibility criteria in § 640.63 (Table 3, Category 2).

c. If a new follow-up sample tests negative by HBV NAT and RR HBsAg, you should evaluate the donor further, as described in the FDA document cited in Reference 1 (Table 3, Category 3).

NOTE: If you wish to perform follow-up testing on a donor of Source Plasma who is deferred because of HBV NAT test results, you may do so

before the end of the 6-month waiting period for donor notification purposes or for medical reasons. Negative test results on follow-up for HBsAg and HBV DNA by NAT (sensitivity at 95% detection rate of ≤ 2 IU/mL), may be useful in donor counseling. However, only negative results for both tests (HBsAg and HBV NAT), obtained at least 6 months after the collection of the sample that gave the test results described in section IV.B.2.f, would qualify the donor for reentry. If you obtain a positive HBV NAT, or a RR HBsAg that is positive by neutralization, the donor would not be eligible for reentry, and we recommend that you defer the donor permanently.

A donor of Source Plasma who has been requalified as described above in section IV.C.2, may on subsequent occasions be indefinitely deferred because of HBV NAT positive results. You may reenter such a donor into the donor pool by again following all procedures described in section IV.C.2.

Table 3. Reentry of Donors of Whole Blood and Blood Components for Transfusion or Further Manufacture on the Basis of HBV NAT and HBV Serologic Test Results on the Follow-up Sample

For purposes of reentry, we recommend that you use an FDA-licensed HBV NAT labeled as having a sensitivity of ≤ 2 IU/mL at 95% detection rate.

Category	HBV NAT Result (sensitivity of ≤ 2 IU/mL at 95% detection rate)	HBsAg and/or Anti-HBc Result (Anti-HBc not required for SP)	Donor
1	Positive	Any test result	Permanently defer donor
2	Negative	Non-Reactive	Donor may be reentered if all other eligible criteria are met
3	Negative	Repeatedly Reactive	For further evaluation, see FDA guidance documents that discuss donor testing for HBsAg and anti-HBc (Refs. 1, 2 and 3).

3. Management of Donors and Units with Non-Discriminated Reactive Test Results

If you obtain a reactive Multiplex HIV-1 RNA/HCV RNA/HBV DNA NAT result on an individual donor sample, and if the Discriminatory NATs are non-reactive for HIV-1 RNA, HCV RNA and HBV DNA, the sample is "Non-Discriminated Reactive." The unit must be quarantined and destroyed (§ 610.40(h)) or, if released for research or further

manufacture, be appropriately relabeled as described in section V.C. The donor must be deferred (§ 610.41). Note that the donor should be deferred for 6 months and is eligible for reentry after the 6-month waiting period. If you choose to reenter the donor, you may do so at the time of a donation without prior testing of a follow-up sample.

V. LABELING

A. Circular of Information for Whole Blood and Blood Components Intended for Transfusion

Under § 606.122, your circular of information must include a statement that the product was prepared from blood that was found negative when tested for communicable disease agents, as required under § 610.40, including each test that was performed. When an FDA-licensed NAT for HBV DNA is used to screen donors and the results of testing are negative, we recommend that the circular of information include the following statement:

> "Licensed nucleic acid test (NAT) for HBV DNA has been performed and found to be Non-Reactive."

B. Blood Components Intended for Further Manufacture

Under § 606.121(c)(11), if your product is intended for further manufacturing use, a statement listing the results of all the tests for communicable disease agents required under § 610.40 for which the donation has been tested and found negative, is required on the container label.[6] Upon implementation of an FDA-licensed NAT,[7] we recommend that you include the following statement on the container label for blood components intended for further manufacture into injectable or non-injectable products that test non-reactive:

> "Non-Reactive for HBV DNA."

This statement may be placed at the end of the current statement on the container label which lists the results of the tests for communicable disease agents for which the donation has been tested and found negative. Alternatively, you may incorporate this information into your current statement without making it a separate sentence.

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[6] Note that the container label for Source Plasma is not required to list the negative results of serological syphilis testing under §§ 610.40(i) and 640.65(b).

[7] See the preamble to the final rule entitled "Revisions to Labeling Requirements for Blood and Blood Components, Including Source Plasma," published in the *Federal Register* of January 3, 2012 (77 FR 7), for additional information on labeling Source Plasma with NAT results.

See paragraph C of this section for recommendations for donations that test reactive for HBV.

C. Reactive Units and Product Disposition

NAT reactive units must not be shipped or used, except as provided in § 610.40(h)(2). If released for these uses, the units must be relabeled consistent with the labeling requirements in §§ 606.121 and 610.40. Thus, for example, you must label the reactive unit with the "BIOHAZARD" legend and with the following cautionary statements, as applicable:

> "Reactive for HBV DNA"
>
> and
>
> "Caution: For Further Manufacturing into In Vitro Diagnostic Reagents For Which There Are No Alternative Sources."

In addition, you should label the reactive unit with the following legend, if applicable:

> "Caution: For Laboratory Research Use Only."

VI. REPORTING CHANGES TO AN APPROVED APPLICATION

Under 21 CFR 601.12 (§ 601.12), FDA-licensed blood establishments are required to report changes to an approved biologics license application to FDA. FDA-licensed blood establishments must report the changes in paragraphs A, B, and C.1 and C.2.a of this section, as described below. However, except as specified in paragraph C.2.b of this section, unlicensed blood establishments are not required to report the changes to FDA.

A. Test Implementation

1. If you begin using an FDA-licensed NAT for the detection of HBV DNA in your facility according to the manufacturer's instructions, you must notify FDA of the testing change in your annual report (AR), in accordance with § 601.12(d), indicating the date that the revised standard operating procedures were implemented.

2. If you are already approved to use a registered contract donor testing laboratory to perform infectious disease testing of Whole Blood and blood components, including Source Plasma and Source Leukocytes, and the contract testing laboratory will now perform a NAT for HBV DNA, you must report this change in your AR (§ 601.12(d)).

3. If you will use a new contract testing laboratory to perform a NAT for HBV DNA, report as follows:

a. If the new testing laboratory is registered with FDA and has been performing infectious disease testing for Whole Blood and blood components, including Source Plasma and Source Leukocytes, report this as a "Supplement - Changes Being Effected" (CBE), in accordance with § 601.12(c)(5).

b. If the new testing laboratory has not previously performed infectious disease testing for Whole Blood and blood components, including Source Plasma and Source Leukocytes, you must report this as a "Prior Approval Supplement" (PAS), in accordance with § 601.12(b). The new testing laboratory must register with FDA in accordance with 21 CFR Part 607 and § 610.40(f).

4. Donor screening with a licensed NAT for HBV DNA should be implemented no later than 6 months after publication of this guidance document.

B. Labeling

Labeling refers to the circular of information, required under § 606.122, and the container labels on blood and blood components required under, among other provisions, §§ 606.121 and 610.40.

1. If you revise your labeling to include the statements in this guidance in their entirety and without modification, you must submit the revised labeling in a "Special Labeling Supplement-Changes Being Effected" in accordance with § 601.12(f)(2).

2. If you revise your labeling to include alternative statements, then you must submit the labeling change in a labeling supplement in accordance with § 601.12(f)(1).

C. Procedures for Requalification of Donors

1. We consider the implementation of recommendations in this guidance in their entirety and without modification to be a minor change to an approved license application. Therefore, FDA-licensed establishments are not required to have FDA prior approval and may submit a statement of this change in their ARs under § 601.12(d), indicating the date that the revised standard operating procedures were implemented.

2. Under § 610.41(b), you may reenter a previously deferred donor using a requalification method found acceptable by FDA for such purposes. We consider the requalification methods described in this guidance to be acceptable. If you choose to use an alternative requalification method, you must report this as follows:

a. FDA-licensed blood establishments must submit the alternative requalification method as a PAS (§ 601.12(b)).

b. FDA must find an alternative requalification method proposed by an unlicensed establishment to be acceptable before it is implemented. (§ 610.41(b)).

VII. REFERENCES

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