health benefit, in addition to scientific data on detection of HBV in donors. One of FDA's reasons for not recommending HBV NAT at that time was the sensitivity of HBV NAT in the available format, when compared to the available serologic testing, did not provide sufficient additional safety to the blood supply to warrant recommending its use. FDA's reasoning was based on 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® Assay (Gen-Probe, Inc., San Diego, California), which uses up to 16 donation samples in a pool and COBAS TaqScreen MPX Test (Roche Molecular Systems, Inc., Pleasanton, California), which uses up to 6 donation samples in a pool. 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 UltraQualTM 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. The assay is an "in-house" test; no kit is sold. The assay uses 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 could 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 development may be delayed or might not occur. Development 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, would also be 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 seem to indicate that HBV NAT positive/anti-HBs positive/HBsAg negative blood, irrespective of anti-HBc test results, does not transmit HBV (Refs. 11, 12, 19, 20, 21, and 22). However, there are at least two reports of such possible transmissions (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 blood used for transfusion should be tested by an FDA-licensed HBV NAT. The Committee also supported FDA setting 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 time of the BPAC meeting in 2009, we are recommending a sensitivity standard of 100 IU/mL for HBV DNA detection in an individual donation (see section IV.A). Due to advances in technology and automation, FDA considers a sensitivity standard of 100 IU/mL to be 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 addition to 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 in plasma fractionation, FDA 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). This sensitivity standard was endorsed by BPAC 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, for consistency, 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 reactive by HBV 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. You must perform one or more such

---

[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.

[4] COBAS AmpliScreen HBV Test (Roche Molecular Systems, Inc., Pleasanton, California): Triplicate testing using the multiprep specimen processing procedure. See package insert.
Procleix® ULTRIO® Assay (Gen-Probe, Inc., San Diego, California): Testing 6 replicates. See package inserts.

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 the detection of 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.

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. (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 be 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

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

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, more specific, test for anti-HBc at the present time. Donors with anti-HBc reactive results may be requalified as described in Ref 3.

B. Management of Donors and Units Based on Hepatitis B 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 donor eligibility criteria and the unit is otherwise suitable for release.

   b. Units of Whole Blood and blood components may be used for transfusion and Source Leukocytes may be used for further manufacture that test negative for HBV using FDA-licensed HBV NAT, HBsAg, and anti-HBc assays, provided that the donor satisfies the donor eligibility criteria in § 640.3 (21 CFR 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 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

all other screening tests for communicable disease agents required in § 610.40(a) and (i) are negative and 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 that 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, or Source Leukocytes, 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 claim 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 blood 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 (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 non-reactive (Table 1, Category 5); or

iii. HBV NAT-positive using a NAT that does not have a limited supplemental claim 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	Repeat Reactive / Confirmed Positive*	Non-Reactive	Discard unit; Permanently defer donor Donor not eligible for reentry
2	Positive	Repeat Reactive / Confirmed Positive*	Repeat Reactive	
3	Positive	Repeat Reactive / Not Confirmed	Repeat Reactive	
4	Positive	Non-Reactive	Repeat Reactive	Discard unit; Indefinitely defer donor; Donor may be eligible for reentry
5	Positive	Non-Reactive	Non-Reactive	
6	Positive	Repeat Reactive / Not Confirmed	Non-Reactive	

† Using a screening test, as described in section IV.A.1.
*Using either an HBsAg neutralization test or an HBV NAT with a limited supplemental test indication, as described in section III and section IV.A.3.

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 blood 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 claim. 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 21 CFR 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 is 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	Repeat 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	Repeat Reactive / Not Confirmed	

† Using a screening test, as described in section IV.A.2.
* Using either an HBsAg neutralization test or an HBV NAT with a limited supplemental test indication, as described in section III, and section IV.A.3.

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, a new sample should be obtained from the donor at least 6 months after the collection of the sample that gave test results described in section IV. B.2.c. (no donation is made at this time). You should perform follow-up testing using HBV NAT (having a sensitivity of $\leq$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 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 Refs. 1, 2 and 3 (Table 3, Category 3).

NOTE: If you wish to perform follow-up testing on a donor of Whole Blood or blood components for transfusion or 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 test results described in section IV.B.2.c, would qualify the donor for reentry. If you obtain a reactive HBV NAT, or repeatedly reactive anti-HBc, or repeatedly reactive 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 documents cited in Ref. 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 reactive HBV NAT, or repeatedly reactive 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 eligible for reentry
3	Negative	Repeat 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 (ID-NAT), 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 IV.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

Consistent with other donor screening tests, the instruction circular, also known as the "Circular of Information", must be updated to state that an FDA-licensed NAT for HBV DNA was used to screen donors and that the results of testing were negative (§ 606.122(h)). We recommend that you use the following statement on the labeling for donations that test Non-Reactive:

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

B. Blood Components Intended for Further Manufacture

Upon implementation of an FDA-licensed NAT, we recommend that you use the following statement on the labeling for blood components intended for further manufacture into injectable or non-injectable products that test Non-Reactive:

> "Non-Reactive for HBV DNA."

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, 610.40 and 640.70. 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 Changes Being Effected (CBE) Supplement, in accordance with § 601.12(c)(5).

    b. If the new testing laboratory has not previously performed infectious disease testing for blood products, 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).

B. Labeling

Labeling refers to the instruction circular (e.g., Circular of Information) required under § 606.122 and the container labels on blood or blood components required under, among other provisions, §§ 606.121, 610.40 and 640.70.

1. If you revise your labeling to include the statements in this guidance in their entirety and without modification, you must report this change as a CBE labeling supplement in accordance with § 601.12(f)(2)).

2. If you revise your labeling to include alternative statements, you must report this change as a PAS 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 AR under § 601.12(d), indicating the date that the revised standard operating procedures were implemented.

2. Under § 610.41(b), you may only re-enter 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. Unlicensed blood establishments must submit the alternative requalification method to FDA before it is implemented so that we may determine whether it is acceptable.

VII. REFERENCES

1. FDA Memorandum to All Registered Blood Establishments: Recommendations for the Management of Donor and Units that are Initially Reactive for Hepatitis B Surface Antigen (HBsAg), December 2, 1987. http://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/otherrecommendationsformanufacturers/memorandumtobloodestablishments/default.htm.

2. FDA Memorandum to All Registered Blood Establishments: Recommendations Concerning Testing for Antibody to Hepatitis B Core Antigen (Anti-HBc), September 10, 1991. http://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/otherrecommendationsformanufacturers/memorandumtobloodestablishments/default.htm.

3. FDA 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), May 2010. http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/default.htm.

4. FDA Memorandum to All Registered Blood Establishments: Recommendations for the Quarantine and Disposition of Units from Prior Collections from Donors with Repeatedly Reactive Screening Tests for Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and Human T-Lymphotropic Virus Type I (HTLV-1), July 19, 1996. http://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/otherrecommendationsformanufacturers/memorandumtobloodestablishments/default.htm.

5. FDA Memorandum to All Registered Blood Collecting Facilities: Collection of Human Leukocytes for Further Manufacturing (Source Leukocytes), January 28, 1981. http://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/otherrecommendationsformanufacturers/memorandumtobloodestablishments/default.htm.

6. Ganem, D, Prince, AM., Hepatitis B infection — Natural History and Clinical Consequences. New England Journal of Medicine, 350, 1118-1129 (2004).

7. Alter, MJ, Centers for Disease Control and Prevention, Epidemiology of HBV Infection and Prevention Programs. Presentation to the Advisory Committee on Blood Safety and Availability, August 27, 2004.

8. Epstein, J.S., Holmberg, J.A., Progress in monitoring blood safety. Transfusion, 50, 1408-1412 (2010).

9. Roth, W.K., Weber, et al., NAT for HBV and Anti-HBc Testing Increase Blood Safety. Transfusion, 42, 869-875 (2002).

10. Kleinman SH, Lelie, N, et al., Infectivity of Human Immunodeficiency Virus-1, Hepatitis C Virus and Hepatitis B Virus and Risk of Transmission by Transfusion. Transfusion, 49, 2454-2489 (2009).

11. Prince, AM, Lee, DH, Brotman, B., Infectivity of blood from PCR-positive, HBsAg-negative, anti-HBs-positive cases of resolved hepatitis B infection. Transfusion, 41, 329-332 (2001).

12. Hollinger, FB., Hepatitis B infection and Transfusion Medicine: Science and the Occult. Transfusion, 48,1001-1026 (2008).

13. Blood Products Advisory Committee Meeting, July 23, 2004, http://www.fda.gov/ohrms/dockets/ac/04/transcripts/2004-4057t2%20.htm.

14. Advisory Committee on Blood Safety and Availability 24th Meeting; Transcript August 27, 2004. http://www.hhs.gov/ophs/bloodsafety/pastmeetings/pastmeetings.html.

15. Stramer, SL, Wend, U, Candotti, D, et al., Nucleic Acid Testing to Detect HBV Infection in Blood Donors. New England Journal of Medicine, 364, 236-247 (2011).

16. National Center for Health Statistics and Centers for Disease Control and Prevention. National Health Interview Survey, 2006 (machine readable data file and documentation). 2007. Hyattsville, Maryland.

17. Royston, P. and W. Sauerbrei, Multivariable Modeling With Cubic Regression Splines: A Principled Approach. The Stata Journal 7.1 (2007): 45-70.7.

18. StataCorp. Stata Statistical Software: Release 10. 2007. College Station, TX, StataCorp LP.

19. Dreier, J, Kröger, M, et al., Low-Level Viremia of Hepatitis B Virus in an Anti-HBc- and Anti-HBs- Positive Blood Donor. Transfusion Medicine, 14, 97-103(2004).

20. Gerlich, WH., Breakthrough of Hepatitis B Virus Escape Mutants After Vaccination and Virus Reactivation. Journal of Clinical Virology, 36 (Suppl. 1):S18-S22 (2006).

21. Mosley JW, Stevens, CE, et al., Donor Screening for Antibody to Hepatitis B Core Antigen and Hepatitis B Virus Infection in Transfusion Recipients. Transfusion, 35, 5-12 (1975).

22. Satake, Tairo, R, et al., Infectivity of Blood Components with Low Hepatitis B Virus DNA Levels Identified in a Lookback Program. Transfusion, 47, 1197-1205 (2007).

23. Hui, CK, Sun, J, et al., Occult Hepatitis B Virus Infection in Hematopoietic Stem Cell Donors in a Hepatitis B Virus Endemic Area. Journal of Hepatology, 42, 813-819 (2005).

24. Gerlich, WH, Wagner, FF, et al., HBsAg Non-Reactive HBV Infection in Blood Donors:

Transmission and Pathogenicity. Journal of Medical Virology, 2007, 79 (Suppl. 1), S32-S36 (2007).

25. Levicnick-Stezinar, S, Rahne-Potocar, Urska, et al., Anti-HBs Positive Occult Hepatitis B Virus Carrier Blood Infectious in Two Transfusion Recipients. Journal of Hepatology, 48, 1022-1025 (2008).

26. Blood Donor Screening for Hepatitis B Virus (HBV) Infection by Nucleic Acid Testing (NAT): Blood Products Advisory Committee 94th Meeting; Transcript April 1, 2009. http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/BloodProductsAdvisoryCommittee/ucm121612.htm.

27. Blood Products Advisory Committee Meeting, April 28, 2011, http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/BloodProductsAdvisoryCommittee/ucm247665.htm.

28. Blood Products Advisory Committee Meeting, July 21, 2005, http://www.fda.gov/ohrms/dockets/ac/05/transcripts/2005-4164t1.htm.

29. Reesink, H.W., Engelfriet, CP, et al., Occult Hepatitis B Infection in Blood Donors. Vox Sanguinis, 94, 153-166 (2008).

平成24年度 厚生科学研究費補助金（レギュラトリーサイエンス研究事業）
分担研究報告書

血液製剤のNATガイドラインの評価技術の開発と国際動向の研究

分担研究者　山口照英（国立医薬品食品衛生研究所生物薬品）

研究要旨：

本年度は、2004年に策定されたNATガイドラインの見直しのための作業グループ（WG）を組織した。WGでは昨年度実施した血液製剤のNAT試験に関する海外動向等の調査結果に基づいてNATガイドライン改定に必要な要素の抽出と課題解決のための議論を行った。また、NAT試験のバリデーションに必要な要素としてのウイルスパネルについての検討も実施した。

我が国のNATガイドラインについて改定すべき点について専門家を含めた班会議を開催し、検討を進めた。その結果、1）国際標準品の整備が進んでいることから、全体記載のコピー数表示からIU表示への変更、2）最終製品でのNAT検査の要否ついての記載の削除、3）適用するウイルスの範囲、4）NATに用いられる機器等の自動化が進んでいることから施設･設備要件について記載事項の整備、5）定量的PCRやMultiplex PCRなどの最新技術の取り込みなどの必要性が指摘された。また、NATガイドラインに関連する事項として、国内標準品や参照パネルの整備についての検討を行った。NATガイドラインでは主としてHBV、HCV、HIVを対象とするNAT検査を想定して書かれていたが、パルボウイルスB-19や北海道で限定的に試行されているHEVのNAT検査やウエストナイルウイルス（WNV）のアウトブレイクを想定した対応なども検討する必要があるとの意見が出された。これを受けて、パルボウイルスB-19のパネル候補品作製を行い、次年度に共同検定によりその評価を行うこととした。

一方、2005年に厚生労働科学研究費で作製されたHBV、HCV、HIV参照パネルについて、作製後、長期にわたって保存されてきたことから、安定性を評価するとともに広く活用するための検討として各パネル候補品の力価の再測定を行った。その結果、選択したHBV、HCV、HIVパネル候補品は、は総体的に-70℃で安定に保存されていることが明らかになった。しかし一部の極めて低濃度のパネル検体については力価の低下が認められた。

パルボウイルスB-19のパネル候補品について、樹立したEPO依存性の細胞株を用いて感染能の違いを解析した。その結果、ジェノタイプ1とジェノタイプ3との感染価の差異を容易に検出できた。本細胞系は今後ジェノタイプごとの感染価の比較や中和抗体の影響評価等に有用であることが明らかになった。

研究協力者
松倉 晴道　日本赤十字社

A. 目的

血液製剤のウイルス安全性は長年にわたる検出手法の開発、改良により大きく向上してきている。特に、1990 年代後半より、原料血漿のウイルススクリーニングとして核酸増幅試験（NAT）が実施されるようになり、その安全性は飛躍的に増してきている。しかしながら、NAT 検査においても現在の技術では検出できないウインドウ期が存在し、きわめて低頻度であるが検査をすり抜けたウイルス陽性血液製剤により感染が起こることが報告されてきている。また、輸入感染症とも言われる海外のみで見られるサブタイプ、ジェノタイプへの対応も指摘されてきている。

一方で、NAT の技術開発にも大きな努力が払われており、試験に用いる検体量や抽出効率の改良、さらには輸入感染症への対応などに多くの努力が払われている。わが国でも、欧米と同様に血液製剤のウイルス安全性指針の下位指針として NAT のウイルス検査についてのガイドラインを発出しているが、FDA 等では既に、このような技術進歩や社会的要因を含めた対応のためにガイドラインの策定や改定が行われている。

そこで本研究では、平成 16 年に発出された血液製剤の NAT ガイドラインについて、NAT を取り巻く技術進歩や血液製剤のウイルス感染症に関する情報の更なる蓄積や疫学的な変化等を調査、研究し、時代に即応した血液製剤の NAT ガイドラインの改定を目指す。さらに、NAT のバリデーションのためのウイルス標準パネル等についての必要性を明らかにし、その策定を目指す。

昨年度は NAT ガイドラインの改訂に資するため、血液製剤のウイルス検出のための NAT 関連技術の進歩や欧米の規制当局の最新のガイドラインについて検討を行ったが、本年度は昨年度の調査結果を基礎に、我が国の NAT ガイドラインについて改定すべき点について専門家を含めた班会議を開催し、検討を進めた。

B. 方法

本年度は NAT ガイドライン改定に向けて作業グループ（WG）を立ち上げ、海外の規制動向も参考にしながら改定すべき事項について議論を行った。特に最新の NAT 関連技術についての調査結果から市販キットの利用や自動化システムの採用など、従来の用手法とは異なる視点が必要な点や NAT の高感度化が進み、ランコントロール等で用いる標準品や標準物質の作製での考慮点等を議論の中心とした。

また、ウイルスの NAT 試験におけるバリデーションに用いられる参照パネルについて、再調査や感染価の評価などに用いる技術的要件についての検討を行った。

C. 結果

C-1. NAT ガイドライン改定に向けた検討

NAT ガイドライン改定に向け、研究分担者のみならず日本赤十字社をはじめ各血液製剤メーカーの専門家の協力を得るために作業グループ（WG）を立ち上げ、現行の NAT ガイダンスの問題点とその対応案の検討を行った。

1) NAT 試験全体について

国内の献血血液の NAT 検査は、日本赤十字社で HCV、HBV、HIV を対象として 1997 年（輸血用血液製剤は 1999 年から）から開始され、当初のスクリーニングにおけるＮＡＴプール本数は、500 本であったが 2000 年から 50 本に、そして、2004 年からは 20 本に縮小することで高感度化が図られている。また直近では、採取する検体量も増量されており、これまで様々な方策により感度の向上が図られている。また、国内 NAT スクリーニングは、当初より対象とする HCV、HBV、HIV を同時に検出する Multiplex PCR として開発された。さらに、NAT の検査手法として増幅産物をリアルタイムで検出する方法（real-time PCR）が採用されている。また、検査対象としては、HCV、HBV、HIV のみならず、北海道で HEV の輸血後感染が増加したことを受け、HEV の NAT 検査が試験的に実施されている。一方、血漿分画メーカーなどでは Parbovirus B19 などのウイルスを社内受け入れスクリーニングとして実施しているとされる。

さらに現在米国等、海外ではウエストナイルウイルス（WNV）の NAT 試験が行われており、万が一国内で WNV が発症した場合を想定して、WNV の NAT 試験の準備が行われている。また、バイレミアを起こすような新型インフルエンザウイルスの発症時の対応としても NAT 試験が利用される可能性がある。したがって、対象とするウイルスは初期の 3 ウイルス（HCV、HBV、HIV）以外にも適用できるような記載が望ましいと考えられる。

2) NAT のウイルス除去工程評価への利用

また、対象とするウイルスの問題だけではないが、血漿分画製剤を含めバイオ医薬品製造におけるウイルスクリアランス試験として、特にウイルス除去能の評価に NAT の利用が広がっている。NAT の迅速性や real-time PCR 等による高い定量性を利用することが有用とされるようになってきている。この場合には定量が重要である点と、ウイルスゲノムだけを対象にした試験で本当に評価すべきウイルス粒子の除去能を正確に反映しているかという問題が指摘されている。ウイルス感染価とウイルスコピー数に乖離がある場合には、ウイルス除去能を過大評価あるいは過小評価する可能性がある。

このような NAT 試験をウイルスクリアランス評価への適用が増加している背景としては real-time PCR 装置の目覚ましい進歩もあり、定量性については非常に正確度が上がってきていると考えられる。一方で、ウイルス粒子の除去能との相関性について様々な取り組みが行われている。ウイルス除去能を評価するために当該工程にスパイクするウイルスのウイルス感染価とそのゲノムコピー数の相関性を評価することが必要である。一方、スパイクするウイルス粒子のウイルスタンパク質を定量することによりウイルス粒子数を推定したり、電子顕微鏡でウイルス粒子をカウントし、ゲノムコピー数との相関性を調べたりすることが必要とされている。さらに実際にウイルスをスパイクした工程評価で、工程の前後でのウイルス感染価の減少とウイルスコピー数の減少について

の相関性を確認することにより試験の妥当性を示す試みも行われている。特にRNA ウイルスについては、ウイルス粒子外に RNA が存在する場合には RNase による速やかな分解を受けやすいことから、測定における過大評価や過小評価は受けにくいとする意見も多いが、感染価と粒子数とが必ずしも相関するわけではないために、調製したウイルスロットごとに評価を行う必要もある。ただし、ウイルスクリアランス能の評価に NAT を用いる場合には定性的な NAT と異なる要件の記載が必要と考えられることから、この点についてはさらに検討を続ける必要がある。

3) 各種 NAT 試験法

ウイルス遺伝子の増幅法としては PCR（ポリメラーゼ連鎖反応）法や RT-PCR（逆転写ポリメラーゼ連鎖反応）が主として用いられているが、PCR 法以外にも様々な NAT 法が採用されている。例えばＴ７ＲＮＡポリメラーゼを用いて等温で RNA ウイルスゲノムを増幅する Transcription Mediated Amplification（TMA）法が PCR についで利用されており、逆転写酵素と併用することにより DNA ウイルスも対象として用いられている。他にも多数の NAT 試験法が開発されている。

WG でだされたガイドライン全般にわたる意見の一つは海外の規制当局との調和であった。FDA や EMA から血液製剤の NAT 試験によるスクリーニングや検査に関していくつかのウイルスガイドラインや考慮事項（Point-to-consider）が発出されており、またその改定も行われている。2004 年に国内指針が策定された時点では海外規制当局の動向も考慮した上で策定されており、調和は取れていたと考えられる。一方 FDA からは、その後に HIV、HCV に関する追加のガイドラインや HBV の NAT 検出を対象としたガイドライン案が出されている。HIV 及び HCV に関する FDA の追加ガイドラインでは、いったん陽性と判定された供血者のリエントリーの方法やそのためのアルゴリズムについて書かれており、我が国おける血液製剤の NAT ガイダンスの目的とは異なった点にまで言及されている。同様に HBV の NAT ガイドライン案でもリエントリーにおける条件などの記載がされている。一方で、ミニプールで陽性にもかかわらず個別 NAT で陰性となった場合の最終判定のあり方や判定基準などについては我が国のガイドラインでは言及していない点であり、参考になると考えられる。また、ミニプールで陽性判定が出た場合に、個別 NAT を実施する際の同定方法についての記載もされている。NAT ガイドラインの改定に当たっては、最新の規制当局の動向も取り入れることが望ましいと考えられる。

4) NAT 試験の市販の ready-to-use キットの利用と操作の自動化

NAT 試験の大きな課題のひとつは、増幅産物による汚染で偽陽性が起こることであり、NAT が高感度になればなるほどその対策が重要とされてきた。一方、スクリーニングでは膨大な検体を対象として試験を実施するために、各試験機関で試薬の調製をするよりもキット化試薬を購入