**BLOOD PRODUCTS ADVISORY COMMITTEE**
**100th Meeting, April 28, 2011**
**Gaithersburg, MD**

**Issue Summary**

## Topic I: Testing Source Plasma for Hepatitis B Virus by Nucleic Acid Testing

**Issue: FDA seeks advice from the Committee on whether scientific data support the concept that testing Source Plasma for hepatitis B virus (HBV) DNA by nucleic acid testing (NAT) increases the margin of safety of plasma derivatives and whether such detection in donors adds to public health.**

### Introduction:

To reduce the risk of HBV-contaminated collections of Source Plasma from entering plasma pools that are manufactured into plasma derivatives such as albumin, immunoglobulins and clotting factors, all Source Plasma collections in the U.S. are tested for hepatitis B surface antigen (HBsAg). Safety of plasma derivatives is also enhanced by the presence of HBV-neutralizing antibody to hepatitis B surface antigen (anti-HBs) in the manufacturing pools and by validated viral inactivation and removal steps in the manufacture of plasma derivatives. Most (possibly all) Source Plasma is tested voluntarily for HBV DNA by NAT, although there is no FDA recommendation to do so at the present time. FDA is considering the added benefit of routine screening by HBV NAT taking into account a) recent data on the "yield" of HBV DNA positive/HBsAg negative donations in routine Source Plasma screening and b) evidence supporting an increased margin of safety for Source Plasma pools for fractionation.

There are currently three FDA-licensed HBV NAT assays available in the U.S. for screening of Source Plasma using minipools: 1) COBAS AmpliScreen HBV Test; 2) Procleix Ultrio Assay; and 3) COBAS TaqScreen MPX Test. Two additional HBV NAT assays, specifically designed for testing Source Plasma, are under development: 4) UltraQual HBV PCR Assay; and 5) HBV DNA PCR Test (HIQ-PCR).

1) In April, 2005 FDA licensed the COBAS AmpliScreen HBV Test (Roche Molecular Systems, Inc.) for detection of HBV DNA in human plasma. For Source Plasma, this assay is intended for use in screening individual donor samples or pools comprised of not more than 96 individual donations.

2) In October, 2006 FDA licensed the Procleix Ultrio Assay (Gen-Probe, Inc.) for detection of Human Immunodeficiency Virus Type 1 (HIV-1) RNA, Hepatitis C Virus (HCV) RNA and HBV DNA in plasma and serum specimens. For Source Plasma, this assay is intended for use in screening individual donor samples or pools comprised of not more than 16 individual donations.

3) In December, 2008 FDA licensed the COBAS TaqScreen MPX Test (Roche Molecular Systems, Inc.) for detection of HIV-1 Group M RNA, HIV-1 Group O RNA, HIV-2 RNA, HCV RNA and HBV DNA in human plasma. For Source Plasma, this assay is intended for use in screening individual donor samples or pools comprised of not more than 96 individual donations.

4) UltraQual HBV PCR Assay (National Genetics Institute) is under development (Biologics License Application submitted to FDA) with a proposed intended use for the qualitative detection of HBV DNA in individual donor plasma samples or in pools comprised of not more than 512 individual donations.

5) HBV DNA PCR Test (HIQ PCR) (BioLife Plasma Services) is under development (under IND) with a proposed intended use for the qualitative detection of HBV DNA in pools of Source Plasma comprised of equal aliquots of not more than 512 individual plasma samples.

Testing using HBV NAT for detection of HBV DNA is intended to be in conjunction with testing for HBsAg.

In April, 2009 FDA sought the advice of the Committee on issues related to blood donor screening for HBV DNA using HBV NAT in addition to testing for HBsAg and anti-HBc to prevent transfusion transmission of HBV by blood and blood components. The Committee supported routine screening of blood donors by HBV NAT and agreed that units from vaccinated donors with "breakthrough infections" (HBV NAT positive/anti-HBs positive) should be presumed infectious.

At this meeting FDA is seeking the advice of the Committee regarding testing Source Plasma for HBV DNA by NAT in addition to testing for HBsAg to further enhance the margin of safety of plasma derivatives.

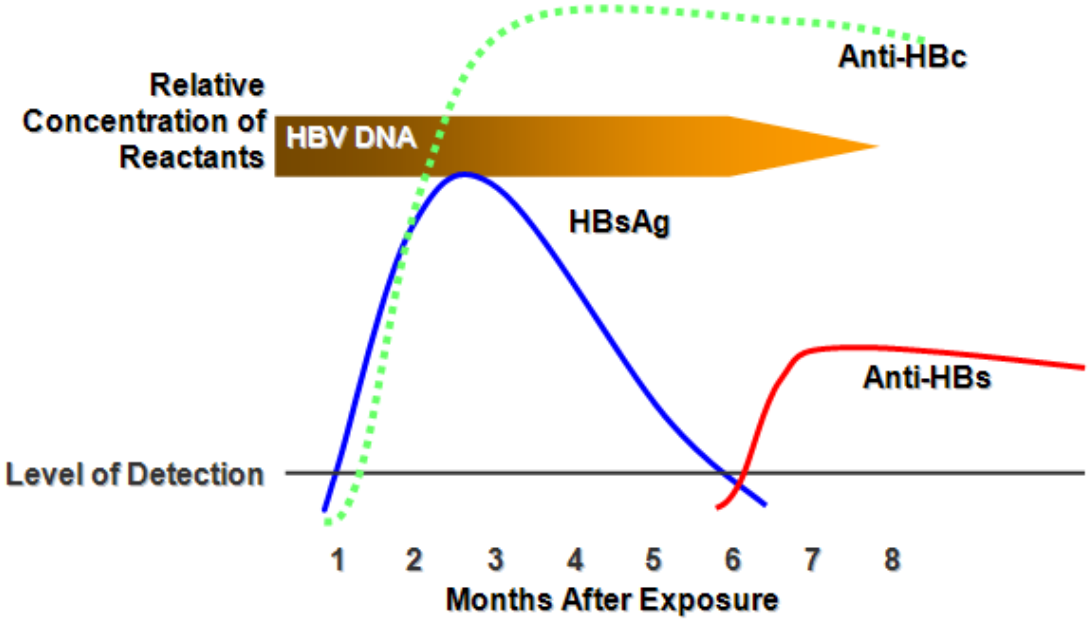
**Background:**

HBV is an enveloped DNA virus that is transmitted through percutaneous or parenteral contact with infected blood or body fluids, including sexual contact. HBV transmission can lead to morbidity and mortality, particularly in immuno-compromised individuals or neonates. There were a total of 4,033 acute, symptomatic cases of hepatitis B infection reported in the U.S. in 2008 with an overall incidence rate of 1.3 cases per 100,000 person-years. The total number of individuals living with chronic hepatitis B infection in the U.S. is estimated at 800,000 - 1.4 million, resulting in approximately 3,000 chronic liver disease deaths per year associated with viral hepatitis B.[1]

HBV DNA is the first marker to appear in plasma during acute HBV infection. HBV DNA levels rise slowly and are present at relatively low levels during the seronegative, HBsAg negative period of early infection (see Figure 1). HBsAg appears an average of 6-8 weeks after exposure to HBV and reaches a peak during the acute stage of the

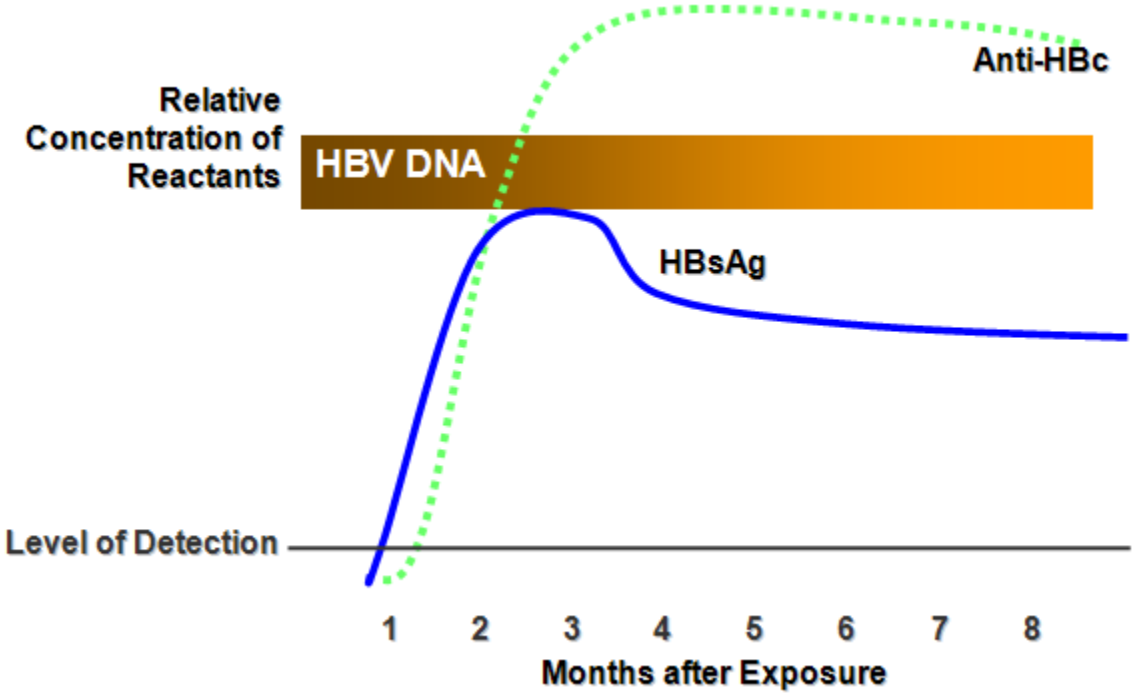
infection. HBsAg declines to undetectable levels within 4-6 months in persons who resolve the infection (94% - 98% of individuals age 5 years or more who become infected with HBV), but anti-HBc persists following recovery. Anti-HBs develops during recovery from infection and ultimately may become undetectable in up to 20% of HBV patients. This neutralizing antibody is also detected after immunization and becomes undetectable in 40% of individuals by 5 years after vaccination, but protection continues.[2]

## Figure 1: Acute HBV Infection with Recovery



In chronic HBV infections HBV DNA and HBsAg remain detectable along with anti-HBc (see Figure 2).

## Figure 2: Chronic HBV Infection




In accordance with 21 CFR 610.40, establishments that collect blood and blood components (e.g., Whole Blood and blood components including Source Plasma) are required to 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 specific communicable disease agents. This rule also requires these establishments to use one or more approved screening tests as necessary to reduce adequately and appropriately the risk of transmission of communicable disease, including HBV infection.

For plasma-derived products, FDA requires that manufacturers validate their fractionation process to show that it has the capacity to achieve 10 or more $\log_{10}$ of clearance for all enveloped viruses such as HBV. This level of virus inactivation and removal should be sufficient to clear HBV contamination from a unit from an infected donor. If we consider that the peak viremia in acute hepatitis B infection can be as high as $10^{10}$ genome equivalents per mL (geq/mL)[7], in a worst case scenario of a unit from such an infected individual entering the manufacturing pool (despite a positive test for HBsAg), we would expect the current fractionation methods to clear HBV in the manufacture of plasma derivatives (viral titer in the pool would be about $10^6$ geq/mL after dilution, and clearance would be $10^{10}$ geq/mL providing a 4 $\log_{10}$ margin of safety.)

Since 1988 there have been no reports of transmission of HBV by plasma derivatives. In that year, there were two reports in the literature that two Factor VIII concentrates, made by different manufacturers, transmitted HBV to recipients, despite being subjected to heating at 60 $^{O}$C for 10 hours.[3,4] FDA has progressively strengthened the overlapping safeguards that protect recipients of plasma derivatives. Source Plasma is an FDA-licensed product collected by plasmapheresis and intended for further manufacture into both injectable and non-injectable plasma-derived products. Source Plasma for further manufacture is currently tested for antibodies to HIV-1 and -2 and HCV, hepatitis B surface antigen (HBsAg), and HIV-1 RNA and HCV RNA using NAT. Additionally, the donors are tested periodically for antibodies to syphilis. Parvovirus B19 NAT and hepatitis A virus (HAV) NAT are also performed as "in-process testing" of plasma pools for manufacture of plasma derivatives, i.e., testing reviewed under the final product license, is performed as a part of the manufacture of particular plasma derivatives. Serology testing (HBsAg) is the current required method for screening Source Plasma for HBV, but HBV NAT testing to detect HBV DNA is already being voluntarily performed on most (possibly all) Source Plasma donations.

FDA does not currently recommend that Source Plasma donors be tested for antibody to hepatitis B core antigen (anti-HBc). If anti-HBc reactive units were excluded from pools used for the manufacture of plasma derivatives, titers of anti-HBs in those pools would be expected to diminish, as both of these antibodies usually occur together. The presence of neutralizing anti-HBs is believed to contribute to the safety of certain plasma products such as immunoglobulins. Plasma units that are untested, non-reactive or repeatedly reactive for anti-HBc are currently acceptable for the manufacture of plasma derivatives. Consistent with 21 CFR 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.

## Discussion:

### 1. Effectiveness of HBV NAT in interdicting potentially infectious Source Plasma units

The possible role of HBV NAT in preventing HBV-contaminated Source Plasma collections from entering manufacturing pools centers around the number of HBV DNA positive/HBsAg negative units a particular HBV NAT assay detects compared to a particular HBsAg assay. This number, referred to as "yield," depends on the relative sensitivities of the HBV NAT and HBsAg assays used.

Actual yield numbers of HBV DNA positive/HBsAg negative Source Plasma units that various HBV NAT tests have detected compared to various HBsAg assays may be obtained from studies performed by collecting establishments, including data from clinical trials prior to licensure and additional data gathered during donor screening post-licensure.

National Genetics Institute (NGI) tested approximately 1.4 million Source Plasma donations in a prospective study of their investigational HBV NAT on pooled samples from 1999 to 2000.[5] NGI originally identified 103 potential yield cases (HBV NAT positive/HBsAg negative) that had follow-up test results, and confirmed 93 of these yield cases (a rate of 1:15,053) by: 1) seroconversion from HBsAg negative to HBsAg positive on follow-up; or 2) presence of anti-HBc on follow-up; or 3) presence of HBV DNA by HBV NAT on follow-up; or 4) presence of HBV DNA by alternate HBV NAT on the index donation; or 5) presence of anti-HBc on the index donation (testing performed retrospectively). The vast majority (90.3%) of the potential yield cases were confirmed, and therefore are considered to be true yield cases.

During the two-year period from September, 2008 to September, 2010, of the 25 million total donations tested by NGI, 734 donations tested positive for HBV DNA by HBV NAT (1:34,107) but were negative by a sensitive HBsAg assay.[5] It is noteworthy that the frequency of detection of HBV NAT positive/HBsAg negative donations appears to have decreased from the time of the 1999-2000 study to the more recent study. This decrease in the frequency of HBV NAT positive donations is presumably due to the more sensitive HBsAg tests that have been in use since 2008; according to NGI, the NAT assay has not been changed between the 1999-2000 study and the recent study. Although there is no follow-up testing information on the donors of these 734 donations, the confirmed data from the 1999-2000 study indicating a high seroconversion rate among potential yield cases support the conclusion that the vast majority of these 734 donations represent true yield cases that are potentially infectious units that were interdicted by the addition of HBV NAT testing of Source Plasma.

Introduction of HBV NAT testing has been shown to reduce the number of HBV DNA contaminated plasma pools for fractionation in a study comparing plasma pools from U.S. and European donors from 1996 and from 2006.[6] Routine HBV NAT testing had not been implemented for testing of the 1996 pools while there was routine testing for the 2006 pools. A rate of 0.5% HBV DNA positive plasma pools (4/873) was determined for the pools from 1996. None of the pools from 2006 tested positive for the presence of HBV DNA (0/331).

HBV NAT testing of donations may reduce the number of contaminated pools used for further manufacture into plasma derivatives. However, it should be noted that, when a 60-day inventory hold for Source Plasma collections is in effect, some units that may be HBV NAT positive in a series of donations from an infected donor prior to seroconversion to HBsAg would not be pooled.

**2. Effectiveness of HBV NAT in reducing the HBsAg negative Window Period and in enhancing public health**

Although effective viral clearance methods are in place for plasma derivatives, performing HBV NAT can be expected to reduce the pooling of Window Period donations (which in this case may be defined as the period when the donor is infected with the virus but prior to appearance of detectable HBsAg), and therefore to enhance the

safety of plasma products by further reducing the residual risk of HBV infectious units entering plasma pools.

In the 1999-2000 NGI study using an investigational HBV NAT and earlier, less sensitive HBsAg assays, the company reported for 20 donations that were HBV NAT positive/HBsAg negative from 12 donors who subsequently seroconverted to HBsAg positivity, HBV NAT closed the HBsAg-negative Window Period by 5 to 63 days.[5] In another study, the Window Period closure by research use HBV NAT assays compared to newer, more sensitive HBsAg assays was estimated to be 0 to 9 days, depending on the assays.[8] In studies that also used less sensitive HBsAg assays, HBV DNA was shown to appear from 2 to 5 weeks after infection, and up to 40 days before HBsAg.[2] The interdiction by HBV NAT of potentially infectious HBsAg negative Window Period units might enhance the safety of Source Plasma by removing units contaminated with HBV DNA from further manufacture into plasma derivatives, thereby lowering the viral titer of fractionation pools.

Donor safety is increased by detecting HBV infection earlier using HBV NAT to allow for appropriate donor notification, counseling and medical follow-up, especially for individuals who may appear healthy and have no symptoms of disease, but yet might be highly infectious. Infected individuals are deferred from donating and properly counseled about their infectious status based on their HBV NAT test results, in addition to their HBsAg test results. Earlier detection of acute HBV infection in donors has public health value as it enables prevention of secondary transmission of HBV infection to the donors' contacts. Appropriate donor counseling also enables the donor to seek medical treatment. Effective medical treatment protocols are becoming increasingly available, particularly for chronic HBV infection.

**3. Effectiveness of HBV NAT in detecting HBsAg negative collections during the post-antigenemia recovery phase of acute and chronic HBV infections**

HBV NAT will also have potential utility in the recovery phase of an acute HBV infection, when HBsAg may no longer be detectable, but virus is still present. In these cases anti-HBc would be present, but Source Plasma is not tested for anti-HBc.

Additionally, individuals with chronic HBV infection are almost always HBsAg positive. However, it is also possible that HBV NAT might detect HBV DNA in a very small number of individuals with asymptomatic chronic hepatitis B infection with undetectable HBsAg. In these cases too, anti-HBc would remain undetected, as Source Plasma is not tested for this marker.

In the studies that NGI performed, 12 out of 93 (12.9%) of the yield cases that were HBV NAT positive and HBsAg negative were found by additional testing of the index donation to also contain anti-HBc.

**4. Effectiveness of HBV NAT in detecting International Units (IU)/mL of HBV compared to current sensitive HBsAg assays**

The limit of detection (LOD) at the assay cutoff (50% detection rate) for one of the more sensitive HBsAg assays, the Abbott PRISM HBsAg assay, has been estimated to be 1664 genome copies/mL.[9] Applying a conversion factor of 3.44 copies/IU for the NGI HBV NAT, 1664 copies/mL is taken to be equivalent to 484 IU/mL. A comparison of this estimated LOD for HBsAg to the LOD at 50% detection rate for the NGI UltraQual HBV PCR Assay shows a five-fold improvement in the analytical sensitivity from approximately 484 IU/mL for the PRISM HBsAg assay to 98 IU/mL for the HBV NAT assay.[5] Therefore, testing of Source Plasma with highly sensitive HBV NAT would be expected to lower the viral titer from a single contaminated unit in a plasma pool for fractionation by a factor of five, or approximately 0.7 $\log_{10}$.

Table 1 shows the LOD at 95% detection rate in IU/mL for the three currently licensed HBV NAT assays with indications for screening of Source Plasma and for two tests under development. The data available to the FDA at this time on yield of HBV NAT obtained by testing of Source Plasma are also included. All donors in these studies were paid donors.

**Table 1**

| Assay | LOD* of Assay (analytical sensitivity) | Minipool Size | LOD* for Maximum Size Minipools (IU/mL) | Yield Cases†/Total Number Tested | Yield Rate |
|---|---|---|---|---|---|
| COBAS AmpliScreen HBV Test | 4.41 | ≤ 96 donations | 423.36 | 3/103,680 | 1:34,560 |
| Procleix Ultrio Assay | 10.4 | ≤ 16 donations | 166.40 | 0/19,168 | N/A |
| COBAS TaqScreen MPX Test | 3.8 | ≤ 96 donations | 364.80 | 12/107,170 | 1:8,931 |
| UltraQual HBV PCR Assay^ | 0.9 | ≤ 512 donations | 460.80 | 93/1.4 million | 1:15,054 |
| HBV HIQ PCR Assay | 11 | ≤ 512 donations | 5632 | 1/1.7 million | 1:1,700,000 |

* LOD at 95% detection rate from Package Inserts or with the permission of the sponsors
^ using older, less sensitive HBsAg assays
† HBV NAT positive/HBsAg negative

The data for LOD in a Source Plasma pool in Table 1 above indicate that detection of HBV DNA at a sensitivity of ≤500 IU/mL for the individual donation in a Source Plasma minipool may represent a reasonable and attainable standard for HBV NAT assays.

**Summary:**

HBV NAT testing of Source Plasma units for further manufacture into plasma derivatives may further enhance the margin of safety by interdicting potentially infectious Source Plasma units and by reducing the viral titer from a single contaminating unit in a plasma pool for fractionation by 0.7 $\log_{10}$. This is in addition to existing validated viral inactivation and removal steps during the manufacture of plasma derivatives and the presence of neutralizing anti-HBs in manufacturing pools.

Detecting HBV DNA positive/HBsAg negative Source Plasma collections that could not be previously identified using HBsAg alone also increases donor safety by reducing the HBsAg negative Window Period and allowing earlier donor deferral, notification and counseling. Early detection of HBV infection in donors also may enhance public health by enabling prevention of secondary infection in the donors' close contacts.

The sensitivity of the currently licensed HBV NAT assays for Source Plasma testing in minipools ranges from 166.40 to 423.36 IU/mL HBV DNA. These assays have been shown to detect HBV NAT positive Source Plasma collections that are HBsAg negative, thereby preventing HBsAg negative collections that contain HBV virus from entering manufacturing pools, and thus increasing the margin of safety of plasma derivatives. In addition, data on the limit of detection of available HBV NAT assays suggest that an LOD of $\leq$500 IU/mL HBV DNA for detection of HBV DNA in a Source Plasma pool is attainable and practical because of advances in technology and automation using HBV NAT assays, including assays designed to test large minipools.

**Questions for the Committee:**

1. Do the available scientific data support the concept that testing of Source Plasma donations by HBV NAT increases the safety margin of plasma derivatives?

2. If so, is a sensitivity of at least 500 IU/mL for the individual Source Plasma collection suitable for HBV NAT when testing minipools of Source Plasma?

3. Please comment whether detection of HBV infection in Source Plasma donors by HBV NAT adds benefit to public health compared with testing only for HBsAg.

**References:**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