

Summary Basis for Regulatory Action

Date: September 1, 2011

From: Abdur Razzaque, Ph.D., Scientific Lead, BLA Review Committee

BLA/ STN#: 125193

Applicant Name: National Genetics Institute, Los Angeles, CA 90064-1748

Date of Submission: May 8, 2006

PDUFA Goal Date: September 2, 2011

Proprietary Name/ Established Name: UltraQual™ HBV PCR Assay

Indication: The NGI UltraQual™ HBV PCR Assay, when used in combination with FDA approved pooling and resolution algorithms, is indicated for the qualitative detection of hepatitis B virus (HBV) deoxyribonucleic acid (DNA) in individual or pooled samples of human Source Plasma (or plasma samples obtained from Source Plasma donors at the time of donation). Pooled samples shall be comprised of equal aliquots of not more than 512 individual plasma samples.

Recommended Action: Approval

Signatory Authorities Action: Office's Signatory Authority: Jay S. Epstein, M.D., Director, OBRR

- I concur with the summary review.
- I concur with the summary review and include a separate review to add further analysis.
- I do not concur with the summary review and include a separate review.

Material Reviewed/ Consulted - List of Specific documentation used in developing the SBRA

Clinical Review	Pawan Jain, Guang Gao and Abdur Razzaque
Preclinical Review	Guang Gao and Abdur Razzaque
Statistical Review	Chinying Wang
CMC Review/ Facilities	Guang Gao and Martha O'Lone
Biomonitoring Review	Bhanu Kannan
Establishment Inspection Report	Martha O'Lone
Lot Release Review	Stephen Kerby
Software and Instrumentation	Hilary Hoffman
Advisory Committee Transcript	http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/BloodProductsAdvisoryCommittee/UCM263093.pdf

1. Introduction

National Genetics Institute (NGI) submitted IND -(b)(4)- for the UltraQual™ HBV PCR Assay to CBER, and CBER notified NGI on October 28, 1999 that the proposed clinical trials may proceed. On May 8, 2006, NGI submitted to CBER a BLA for the UltraQual™ HBV Polymerase Chain Reaction (PCR) Assay for the qualitative detection of HBV DNA in pools of human Source Plasma comprised of equal aliquots of not more than 512 individual plasma samples. The UltraQual™ HBV PCR Assay is an “in-house” test performed only by NGI and no kit is sold. The test is intended to be used in conjunction with Hepatitis B Surface Antigen (HBsAg) testing on individual samples using an FDA licensed test.

2. Background

All Source Plasma collections in the United States are tested for hepatitis B surface antigen (HBsAg) to reduce the risk of hepatitis B virus (HBV) contamination in plasma derivatives. Testing Source Plasma for HBV DNA by nucleic acid testing (NAT) may increase the margin of safety of plasma derivatives by detecting HBV in donations that test negative by an HBsAg assay, as discussed at a meeting of the Blood Products Advisory Committee on April 28, 2011. Most Source Plasma collections in the U.S. are voluntarily tested for HBV DNA by NAT, although there is no FDA recommendation to do this test at present.

NGI’s UltraQual™ HBV PCR Assay is a polymerase chain reaction assay for the qualitative detection of the HBV genome in pools of human plasma or individual human plasma samples. The assay process is composed of two discrete functional areas: a) sample collection and pooling, and b) PCR to amplify and detect HBV DNA sequences that may be present.

3. Chemistry, Manufacturing and Controls (CMC)

a. Sample Collection and Processing

Sample collection and pooling are conducted according to an FDA approved algorithm. Samples are from individual Source Plasma donations collected by automated or manual plasmapheresis in 4% sodium citrate anticoagulant. Samples may also be collected by thawing and sampling of the frozen plasma donation, if required. Plasma samples may also be collected in tubes directly from individual donors using citrate or EDTA as the anticoagulant. NGI prepares pools of not more than 512 samples using -----(b)(4)-----, a Class I FDA 510(k) cleared device.

b. Amplification and Detection

The second component of the assay process is NGI’s proprietary UltraQual™ HBV PCR Assay test of the pools or individual samples of Source Plasma. -----(b)(4)-----

The sample is positive if one or more of the reactions produces a detectable HBV-specific PCR product, irrespective of the internal DNA control result. A positive unit in the pool is identified by deconstructing the pool according to an approved algorithm. The sample is negative if none of the reactions produces a detectable HBV-specific product and the internal control DNA is detected.

c. *In Vitro Substances: Primers and Probes*

The NGI UltraQual™ HBV PCR Assay uses synthetic oligonucleotide primers for the amplification of the HBV target DNA, the amplification of the ---(b)(4)--- internal control DNA, and for the production of the corresponding hybridization probes. NGI uses -(b)(4)- sets of primers and all primers are specific deoxynucleotide sequences. Primers were selected to use conserved regions of the HBV genome to assure detection of all published HBV genotypes. Primers are characterized by nucleic acid sequencing, by purity, and by binding to specific regions of the HBV or -----(b)(4)-----. Primers are obtained from outside vendors and all incoming quality control specifications must be met.

With regard to specificity of the primers, a BLASTn homology search did not show any cross-reactivity between the HBV amplification primer pairs and any other known genomic material.

The NGI UltraQual™ HBV PCR Assay uses synthetic hybridization probes containing -----(b)(4)----- for the detection of the specific HBV or ---(b)(4)--- amplification products.

d. Assay Controls

In addition to the internal control included in every sample, each run is conducted with negative, positive and step run controls to assure the validity of the assay. The mandatory positive controls consist of a pooled HBV positive human plasma control (generated by NGI) with -----(b)(4)----- of the current National Institute for Biological Standards and Control (NIBSC) working control (1000 IU/mL). The positive controls must show the appropriate bands for a valid test. The mandatory negative controls are Negative Human Plasma and Reagent Blank (only reagents), which contain no HBV and which must not produce target-specific bands. The -----(b)(4)-----

e. Manufacturing Quality Control

A complete review of the manufacturing process, quality control of the HBV PCR assay, and stability data was done by the CMC reviewers. Their review concluded that the overall manufacturing of the test was performed according to validated and approved procedures. Their initial review identified some minor issues in method validation, e.g., production of hybridization solution, development solution, etc., and manufacturing details for different controls. These issues were communicated to the sponsor by fax on August 3, 2006 for clarification. The issues were addressed adequately in Amendments 4 and 7 in NGI's responses to CBER's first Complete Review (CR) letter of December 20, 2006. Acceptance criteria and specifications have been established for all reagents and controls. Several reagents have been defined as critical and must undergo functional testing prior to release. These include primers, probes, deoxynucleotide triphosphates and Taq polymerase. NGI purchases critical reagents from approved vendors. Each lot of these reagents must also meet the specifications for functional testing with in-house HBV DNA panels and the fully configured assay must meet the performance requirements of the FDA/CBER HBV NAT lot release panel.

f. CBER Lot Release

Each new lot of a donor screening test typically is subject to lot release by testing at CBER using panels developed for that purpose. For testing services, however, CBER conducts lot release testing of each "product" lot, which in this case is defined as a set of independent lots of matched critical reagents (primers, probes, Taq polymerase, and nucleotides) and other components that form a functional in-house assay system. Each time any critical reagent is changed, this results in a new lot that is subject to the lot release process. -(b)(4)- sets of blinded/randomized panel are sent to NGI at one time to be used on their next -(b)(4)- lots. Thus, NGI does not know which vial belongs to which

member and reactivity for a particular vial changes with each set. NGI tests a panel set and submits results in the Lot Release Protocol. The results are returned to CBER where they are unblinded and, if all test results meet the established specifications for the lot release panel, satisfy the lot release testing requirement. The criteria for lot release for NGI are identical to all other manufacturers with specifications for certain members as reactive, non-reactive, or either.

NGI performed testing of 3 different lots of product on the blinded CBER HBV Lot Release Panel and submitted data in a draft Lot Release Protocol. Lot Release Protocols were reviewed and approved by CBER.

g. Stability Studies

NGI conducted stability studies for critical reagents, controls, other reagents and reagent mixes. Based on the results of real-time stability studies, NGI assigns each lot of primers and probes an expiration date of -----(b)(4)----- . If NGI has not performed a stability study for a specific reagent, NGI uses the manufacturer control or expiration date for each raw material. Since the assay is an in-house service, there is no assembled kit and it is not necessary to perform a stability study of the kit as a whole.

The review identified some minor issues regarding storage temperature and shelf-life claim for primers and probes, and NGI has addressed these issues satisfactorily.

h. Facilities Review/Inspection

NGI will manufacture the UltraQual™ HBV PCR Assay in their currently licensed multiproduct facilities at the following Los Angeles, California locations: 2440 South Sepulveda Boulevard (pooling and pre-PCR) and -----(b)(4)----- (PCR and post-PCR). For both facilities, the UltraQual™ HBV PCR Assay components will be manufactured in unclassified areas and only used on site to assay human Source Plasma samples for HBV DNA. The UltraQual™ HBV PCR Assay will be manufactured on a campaign basis with the two currently licensed UltraQual™ assays, the HIV-1 RT-PCR assay (BLA 103902) and the HCV RT-PCR assay (BLA 103868).

The review of facility and equipment-related issues conducted by an OCBQ/DMPQ reviewer identified concerns about the lack of information and validation of manufacturing processes, equipment, and cleaning procedures. These issues were noted in CBER CR letters. The sponsor responded adequately to each of CBER's comments.

A pre-license inspection of the NGI Los Angeles facilities was not required because of the following:

- NGI is already approved to manufacture two UltraQual PCR Assays;
- The facility was inspected within the past two years;
- There are no outstanding cGMP issues from the previous inspections.

i. Environmental Assessment

A request for a categorical exclusion from an Environmental Assessment under 21 CFR §25.31(c) was submitted to the BLA. The sponsor believes this application meets the categorical exclusion criteria and to their knowledge no extraordinary circumstances exist. The Agency agrees with this conclusion and an environmental assessment is not warranted.

j. Software and Instrumentation Issues

The submission initially did not contain any information about software and instruments to be used in this assay. In the first CR letter, NGI was asked to provide documentation for software and instruments, a list of requirements and functions performed by the system, risk assessment that identifies critical control points, validation of the entire test system, and validation protocols and SOPs for operation and maintenance. NGI addressed each issue adequately in their response to the CR letter in Amendment 4.

4. Non-clinical / Analytical Studies

a. Analytical Sensitivity

The analytical sensitivity of the NGI UltraQual™ HBV PCR test was established by an analytical methods validation study. The estimated 95% detection point is the concentration of HBV that is estimated to be detected 95% of the time by the test method.

The -----(b)(4)-----, was used as the positive starting material. This material was diluted with three different lots of pooled normal human plasma.

Table 1 summarizes the analytical sensitivity of the 8-reaction and 4-reaction assay formats. Values are expressed in HBV IU/mL. The analytical sensitivity for the 8-reaction and 4-reaction formats is 0.90, and 1.55 IU/mL (for 1 mL) and 15.5 IU/mL (for 100 µL), respectively.

Table 1. Analytical Sensitivity of the NGI UltraQual™ HBV PCR Assay Configurations with -----(b)(4)-----

Assay Configuration*	Limit of Detection (LOD) at an Estimated 95% Detection Rate (HBV DNA IU/mL)**
2 mL, 8 reaction	0.90
1 mL, 4 reaction	1.55
100 µL, 4 reaction	15.5

Table 3. HBV Genotype Sample Identification

[--(b)(4)--]

----- (b)(4) -----

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c. Precision Study

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous material under the prescribed conditions. Precision may be performed at three levels: repeatability, intermediate precision, and reproducibility.

Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Repeatability of the NGI UltraQual™ HBV PCR assay was determined by comparing independent Spearman-Kärber analyses of the mean sensitivities (at a 50% detection rate) achieved for each of three spiked plasma pool diluents using the Master Pool (i.e. 8-reaction) assay. ----- (b)(4) -----

----- The results are shown in Table 4.

Table 4. Summary of Repeatability Study Results

[
--(b)(4)--
]

The 95% confidence intervals of the mean sensitivities obtained for each of the three spiked plasma pools overlap, thus demonstrating that the mean sensitivities achieved using the three lots of diluent were sufficiently similar and that the assay performs with an acceptable level of repeatability.

Intermediate precision

Intermediate precision expresses within laboratory variations: different days, different analysts, different critical reagents, etc. Intermediate precision of the NGI UltraQual™ HBV PCR assay was determined by analyzing identical dilution panels on different days, by different analysts, and using different equipment. The results are shown in Table 5.

Table 5. Summary of Intermediate Precision Study Results

[
--(b)(4)--
]

The 95% confidence intervals for the mean sensitivities achieved by each analyst group overlap and are therefore statistically similar.

Reproducibility

Reproducibility expresses the precision between laboratories. Reproducibility is not applicable since this assay is performed only at NGI.

d. Assay Specificity

Assay specificity measures the ability of the assay to only detect the sequence of interest when it is present.

The assay specificity of the NGI UltraQual™ HBV PCR test is based on the specificity of the primers and probes used. The identity of the amplified product is confirmed by the ability of sequence-specific probes to bind to the amplified material and by the electrophoretic mobility of the amplicon detected. Assay specificity was evaluated by analyzing plasma pool samples containing -----(b)(4)-----

or -----(b)(4)-----, in the NGI UltraQual™ HBV PCR assay at least -(b)(4)- times utilizing the 4-reaction test.

The results for all assay specificity samples were negative for HBV and positive for the added virus by the corresponding PCR test.

e. Analytical Specificity

Analytical specificity was evaluated by analyzing -(b)(4)- HBV-negative plasma pools utilizing the 8-reaction test. All analytical specificity samples tested negative for HBV DNA.

f. Interference

An interference study was conducted to evaluate whether substances likely to be present in plasma samples might affect the detection of HBV DNA with this assay. The study involved testing of contrived pools of 512 samples containing up to --(b)(4)-- of samples that were hemolyzed, lipemic, or icteric, and were spiked with HBV DNA to a level of 3X LOD at 95% detection rate (-(b)(4)-) HBV DNA. The other potential interferents were tested in samples containing up to the levels indicated. All samples were tested using the 1 mL, 4-reaction assay configuration; at least -(b)(4)- assays were performed for each category of matrix.

------(b)(4)-----

Matrices consisting of -(b)(4)- lipemic samples (e.g. -(b)(4)- of 512 pooled samples) resulted in invalid results (i.e. no detection of either HBV or internal control nucleic acid sequences). When the number of lipemic samples was reduced to -(b)(4)- (e.g. -(b)(4)- pooled samples) all results were positive. Caution should therefore be taken to avoid the introduction of highly lipemic samples into pools to be tested using the UltraQual™ HBV PCR assay, as doing so could result in invalid results. Matrices containing alternate anticoagulants, nucleoside analogues, or up to -(b)(4)- hemolyzed, icteric or bacteremic samples were found positive in all assays, indicating that these substances do not significantly interfere with the ability of the UltraQual™ HBV PCR assay to detect HBV DNA.

5. Clinical/ Statistical Studies

a. Clinical Studies

NGI conducted three Phase III clinical studies under IND -(b)(4)- in which a total of 1,407,679 Source Plasma donations were screened in pools of up to 512 samples to evaluate the safety and effectiveness of the NGI UltraQual™ HBV PCR Assay.

Clinical sensitivity measures the degree to which a test correctly identified samples from a donor with confirmed infection. The NGI UltraQual™ HBV PCR test had a clinical sensitivity of 96.25% for all donations screened in plasma pools. See Table 6.

Clinical specificity is a measure of the degree to which a test correctly identified samples that are true negative samples with no confirmed infection. The NGI UltraQual™ HBV PCR test had a clinical specificity of 99.999% calculated using all donations screened. In other words, of the greater than 1.4 million donations screened, 14 were falsely identified as HBV PCR-positive. See Table 6.

Table 6. Clinical Sensitivity and Clinical Specificity for the UltraQual™ HBV PCR Assay on All Donations Screened During the Clinical Trials

UltraQual™ HBV PCR Result	HBV Status by Either UltraQual™ HBV PCR or HBsAg	
	Positive	Negative
Positive	154*	14
Negative	6	1, 407, 665
Total	160	1, 407, 679
Clinical Sensitivity of UltraQual™ HBV PCR = $154/160 = 96.25\%$		
Clinical Specificity of UltraQual™ HBV PCR = $1,407,665/1,407,679 = 99.999\%$		

* 61 were detected by both PCR and HBsAg; 93 were detected by PCR alone.

The primary objective of the clinical studies was to demonstrate that the NGI UltraQual™ HBV PCR Assay can detect HBV in Source Plasma donations having negative test results for HBsAg by licensed tests. The three Phase III studies are ATC 00-1, NGI 99-01 Baxter and NGI 99-01 Bayer, conducted in 1999, 2000 and 2001, respectively. NGI compared the results of donor screening with the UltraQual™ HBV PCR Assay with the results of HBsAg testing of individual samples alone to determine the “yield” of HBV DNA positive/HBsAg negative donations; that is, to demonstrate clinical benefit of the UltraQual™ HBV PCR Assay over current required HBV testing for Source Plasma donations. Any donor who was reactive for any of the viral markers during the course of testing in the clinical trials was deferred from future donations.

The study design included testing of index donations in pools of 512 Source Plasma samples, which constitute a Master Pool. The Master Pool was comprised of Primary Pools. A Master Pool that was positive using the investigational UltraQual™ HBV PCR Assay resulted in testing of the Primary Pools that made up that Master Pool. In turn, for the Primary Pool that was positive, samples from individual donations that made up the

Primary Pool were tested to identify the donation that gave the original investigational test positive result. Confirmation of reactive UltraQual™ HBV PCR Assay results included weekly testing of the donor for six months or for eight weeks after an HBsAg, anti-HBs or anti-HBc positive sample was identified and one additional positive sample was obtained.

From the clinical studies to determine if the UltraQual™ HBV PCR Assay could detect yield specimens, NGI submitted data for 107 HBsAg negative/HBV DNA positive cases. Fourteen (14) of the 107 were PCR positive due to possible laboratory contamination of the samples; HBV infection could not be confirmed for these donors, thus reducing the number of yield cases claimed by NGI to 93. In the review of these data, the review committee found that some information was missing (identification numbers for donors, master pools, and primary pools) and that some of the data did not agree with line listings previously submitted in prior amendments and in the original BLA. These discrepancies were most likely attributed to the fact that the clinical studies were conducted 10 years earlier and the original regulatory staff responsible for conducting the study were no longer at NGI. Thus, it was the responsibility of new NGI staff to organize the original data.

The evidence for “yield” from the 93 claimed cases came from several sources, as indicated in Table 7 below.

Table 7.

EVIDENCE FOR YIELD CASES	NUMBER
Serology (presence of anti-HBc antibodies, or seroconversion for anti-HBc or HBsAg on a subsequent donation)	59
Multiple investigational NAT-reactive or alternate NAT-reactive	16
Data from independent testing by Alpha Therapeutics (source of specimens) (HBsAg, anti-HBc, HBV NAT)	12
Reactive alternate primer NAT	6
TOTAL YIELDS CLAIMED	93

In considering the data in support of yield case detection by the UltraQual™ HBV PCR Assay, two members of the review committee had concerns about incomplete line listings associated with many of the putative yield cases, as well as data that appeared to differ from that in prior Amendments to the BLA submitted in response to information requests by CBER. Their conclusion was that there were sufficient data to support the finding of 10 yield cases, but that they had concerns about the accuracy of the overall dataset. Their recommendation was for NGI to conduct an additional clinical trial in which a minimum of 500,000 donations would be screened, with follow-up testing of the donors, to generate a complete, verified dataset that would provide an accurate rate of yield cases for HBV DNA testing of Source Plasma donations. However, the Scientific Lead for the review of this BLA felt that the reviewers’ concerns could be addressed in a postmarket study since verification of at least 10 yield cases (based on serological evidence of infection) established the efficacy of the assay and since the rate of yield cases might change

anyway over time. In addition, review of the data submitted by NGI by management led to the conclusion that the detection of 93 yield cases in the clinical studies was likely correct, though not formally verifiable, supporting the efficacy of the assay. This issue is discussed in greater detail in Section 7 (Recommendations and Risk/Benefit Assessment) below.

b. BIMO Issues

The original BLA did not contain information on clinical investigators for the clinical studies and NGI did not indicate whether the pre-clinical studies were conducted according to GLP requirements. These issues were addressed satisfactorily in Amendment 4 submitted by NGI in response to the CBER CR letter of December 20, 2006. A bio-research monitoring inspection for the clinical data could not be done, as the clinical studies were conducted more than 10 years ago.

c. Statistical Issues

The clinical trial was performed by testing donations that were linked to donor identity at the Donor Center but such linkage was not available at the NGI testing site. NGI therefore did not know, and could not determine, the actual number of Source Plasma donors screened using the UltraQual™ HBV PCR Assay. The statistical reviewer concluded that it was necessary to know both the number of true positive donors and the number of total donors screened for the calculation of the clinical sensitivity and specificity. However, management determined that a report of clinical sensitivity and specificity based on donations, as in Table 6, remained meaningful. This issue is discussed in greater detail in Section 7 (Recommendations and Risk/Benefit Assessment) below.

6. Labeling

A package insert is not required for this test because the test is carried out only and entirely at NGI laboratories; a kit is not distributed. Informational and promotional material associated with this test includes a single sheet on specimen requirements and handling for pooled NAT screening (identical to that used with the currently licensed HIV-1 and HCV UltraQual™ assays). NGI intends to distribute a Validation Summary derived from this Summary Basis for Regulatory Action to customers in lieu of any other labeling for this test.

NGI's proposed proprietary name is "UltraQual™ HBV PCR Assay" for the biological device for screening pooled human Source Plasma for HBV DNA. The proposed name was reviewed by CBER's Advertising and Promotional Labeling Branch (APLB) and following internal discussions it was concluded that the name was acceptable. "UltraQual" is a trademark of NGI and is a prefix that has been used for other approved assays from NGI (e.g., UltraQual™ HIV-1 RT-PCR Assay and UltraQual™ HCV RT-

PCR Assay). The review committee recommended use of the proposed name “UltraQual™ HBV PCR Assay.”

7. Recommendations and Risk / Benefit Assessment

a. Recommended Regulatory Action

Two members of the review committee had significant concerns about the quality and consistency of data submitted from the clinical trials, such that they could not recommend licensure at this time. Rather, they recommended that NGI should conduct an additional clinical trial using a minimum of 500,000 donations to collect a complete dataset to support licensure of the UltraQual™ HBV PCR Assay. NGI subsequently made known its willingness to perform such a study as a voluntary post approval commitment. Management determined that acceptance of a voluntary commitment by NGI to perform a postmarket study as described would be appropriate for the following reasons:

- The review committee’s concerns were with the insufficiency of the clinical trials to determine sensitivity of the NAT assay. However, the purpose of the clinical trials was not to determine the sensitivity of the assay (unlike for a serological assay), but rather to demonstrate that there is clinical benefit of NAT as additive to serologic testing. In this case, clinical benefit was defined as the ability of the test to identify donations that would otherwise go undetected using currently required tests (in the case of Source Plasma donors, testing for HBsAg). Therefore, we were only asking that the clinical trials identify “yield” donations, i.e., from individuals who are NAT-reactive and HBsAg-negative, and who can be shown to be infected with HBV. This was demonstrated in the clinical trials, albeit with uncertainty about the rate of such yield cases.
- The combination of excellent analytical sensitivity (low LOD) and data from the clinical trial showing strong evidence that the assay detects donations that would otherwise be undetected without the use of HBV NAT and that would be used for further manufacture of plasma-derived products are sufficient grounds for licensure of this test.
- A voluntary postmarket study to determine the clinical sensitivity of the UltraQual™ HBV PCR Assay and the rate of “yield” cases of HBV detection in Source Plasma donations would provide accurate scientific information presently unavailable in the BLA that could be used in promotion of the assay.

b. Recommendation for Postmarket Activities

On August 19, 2011 NGI confirmed in writing their postmarket commitment as follows:

“NGI agrees to conduct a postmarket clinical study that will include at least 500,000 donations and the data will be submitted as a supplement within one year

of the approval date. The PMC study will be conducted using NGI's UltraQual™ HBV PCR Assay and currently available licensed HBsAg, anti-HBc tests, and other tests as needed. The data submission will include donor IDs, accurate enumeration of positive and negative tests, Master Pool IDs, Primary Pool IDs, bleed dates, identification of donor centers that supplied all specimens for testing, and follow-up testing data for all NAT-positive/HBsAg negative donors.”