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A mathematical approach to estimate the efficacy of individual-donation and minipool nucleic acid amplification test options in preventing transmission risk by window period and occult hepatitis B virus infections

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Abstract

BACKGROUND—Sensitivity data from a head-to-head comparison study in South Africa were used to compare the efficacy of the Ultrio Plus assay in individual-donation (ID) and minipool (MP)4 and MP8 formats with that of TaqScreen MP6 in preventing hepatitis B virus (HBV) transmission risk.

STUDY DESIGN AND METHODS—The replicate nucleic acid test (NAT) results on 106 HBV NAT (Ultrio)-yield samples and 29 HBV DNA (Ultrio)-negative, hepatitis B surface antigen (HBsAg)-positive samples were used to determine the viral load in copies/mL against the Eurohep HBV standard by probit analysis. Random viral load distributions were established in 32 pre-HBsAg window period (WP), 15 post-HBsAg WP, and 56 occult HBV infection (OBI) donations. Regression analysis of log viral load and Poisson distribution statistics of infectious HBV particles in blood components was used to predict infectivity and efficacy of NAT options in removing HBV transmission risk.

RESULTS—For red blood cell transfusions (20 mL of plasma), the modeling predicted an Ultrio Plus ID-NAT efficacy of 68 and 83% in removing WP and (antibody to hepatitis B surface antigen-negative) OBI transmission risk, respectively, compared to 52 and 49% by TaqScreen MP6. For 200 mL of fresh-frozen plasma the estimated efficacy levels by these ID- and MP6-NAT options reduced to 57 and 44% for WP and to 67 and 34% for OBI donations, respectively.

CONCLUSION—The efficacy of the currently available commercial NAT systems in reducing HBV transmission risk is mainly driven by the pool size and the transfusion plasma volume. The modeled OBI transmission risk and NAT efficacy levels were in line with those recently reported

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CONFLICT OF INTEREST

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in three lookback studies and give more insight in the incremental safety provided by HBsAg and antibody to hepatitis B core antigen testing of ID-NAT screened blood.

Recently the South African National Blood Service compared the sensitivity of the Ultrio, the newer Ultrio Plus assay version (Novartis Diagnostics, Emeryville, CA), and the TaqScreen 1.0 assay (Roche Molecular Systems, Pleasanton, CA) for detection of hepatitis B virus (HBV) DNA.¹ For this purpose 106 HBV individual-donation nucleic acid test (ID-NAT) yield samples originally detected by the Ultrio assay were tested in six replicate assays by the NAT methods on undiluted, 1:4, 1:6, and 1:8 diluted samples. These replicate NAT assays mimicked ID and minipool (MP) testing of 4 and 8 donations for Ultrio and Ultrio Plus and MP6 testing for TaqScreen. In addition 29 undiluted samples of low viral load hepatitis B surface antigen (HBsAg) carrier donors missed by initial screening with the Ultrio assay were tested in replicate assays for comparison of the sensitivity of the three NAT methods.

Since significant differences in sensitivity existing between the old Ultrio and TaqScreen assays disappeared with the new Ultrio Plus version, the authors concluded that the clinical sensitivity of the currently available commercial NAT systems on South African HBV-yield samples was mainly driven by the MP size.¹ However, what is the efficacy of the NAT systems in preventing HBV transmission risk? And what is the impact of the MP size on the transmission risk for window period (WP) and occult HBV infections (OBI), the latter known to have a reduced infectivity?² To answer these questions we performed a mathematical analysis that allowed for translation of the clinical sensitivity of the different NAT options¹ into an estimated level of efficacy in risk reduction of posttransfusion hepatitis B infection. In this study efficacy is defined as the proportion of transmission risk by HBsAg-negative donations from different stages of acute or chronic HBV infection that can be avoided by screening with the ID- and MP-NAT methods. For this analysis we needed to examine the viral load distributions in the samples from WP and OBI donations. Since 83% of the HBV NAT-yield samples detected by the Ultrio assay had a viral load below the quantification limit of the quantitative TaqMan polymerase chain reaction (PCR) assay,¹ we used the available replicate Ultrio Plus test results to determine the low viral load. This was possible by comparing the proportions of reactive results with those on dilutions of the Eurohep HBV Genotype A2 standard³ calibrated in copies/mL. Extrapolation of the distribution of the viral load in WP and OBI samples and Poisson distribution statistics on infectious virus particles in plasma⁴ enabled us to predict which proportion of donations would be infectious, but not detected by the ID- and MP-NAT options. We then compared the theoretical infectivity of OBI donations with those observed in recent European and Australian lookback studies.^{2,5} Finally we compared the modeled ID-NAT efficacy levels on the South African WP and OBI donations with those observed in a recently reported Japanese lookback study.⁶

MATERIALS AND METHODS

HBV NAT- and HBsAg-yield samples

In the study of Vermeulen and colleagues,¹ the sensitivity of the NAT systems was compared on 1) 106 HBV NAT-yield samples detected by screening with the Ultrio assay and 2) 29

HBsAg confirmed-positive samples initially missed by screening with the Ultrio assay. Confirmatory testing in the index and follow-up samples of the donors allowed for further classification of the 106 HBVNAT–yield samples in the following categories:⁷ 1) 22 pre-HBsAg WP samples; 2) 10 probable vaccine breakthrough samples; 3) 15 immunoglobulin (Ig)M antibody to hepatitis B core antigen (anti-HBc)-positive late acute-phase WP samples; 4) 29 anti-HBc positive, antibody to hepatitis B surface antigen (anti-HBs)-negative OBI samples; 5) 27 anti-HBc– and anti-HBs–positive OBI samples; and 6) three unclassified HBV NAT–yield samples. For analytical sensitivity and viral load determination a dilution panel of the Eurohep Genotype A2 standard calibrated in copies/mL³ was prepared by BioQControl, DDL Diagnostic Laboratories (Rijswijk, The Netherlands).

Viral load determination by probit analysis

Since 83% of the confirmed HBV NAT–yield donations were quantitative PCR (qPCR) negative, we used for those samples the replicate test results in the Ultrio Plus assay on the undiluted and 1:4 and 1:8 diluted samples to estimate the viral load. For this purpose the proportions of positive results in the undiluted and diluted samples were compared with those on dilutions of the Eurohep HBV DNA reference standard. Since TaqScreen was only tested on 1:6 diluted HBV NAT–yield samples, we could not use these results for viral load determination. The method used to estimate the viral load in copies/mL was parallel line probit analysis using a statistical package (SPSS, Version 17.0, SPSS, Inc., Chicago, IL). Once the criteria for parallel slopes of the probit curves have been accepted the relative potency (or concentration) of the test samples against the Eurohep reference standard was deduced from the shift between parallel lines. When Ultrio Plus was none of six reactive in the undiluted sample the concentration could not be determined. In these cases we assumed an Ultrio Plus reactive rate of one in 12 and a value of 0.62 copies/mL was imputed. The low viral loads in the HBsAg-positive, Ultrio-nonreactive donations were also determined by probit analysis. In these samples the proportion of positive results in both the Ultrio Plus and the TaqScreen assays could be compared against the respective probit curves on the Eurohep standard dilutions. The geometric mean concentration was calculated from the values obtained in the analyses on the Ultrio Plus and TaqScreen data. In cases where both Ultrio Plus and TaqScreen were none of 12 reactive we assumed a reactive rate of one in 24 and a value of 0.3 copies/mL was imputed.

Random viral load distribution

A random distribution of log viral load is a prerequisite for estimation of transmission risk in the pre-NATWP according to mathematical methods described by Weusten and coworkers⁴ and Busch and coworkers.⁸ The fact that donors appear randomly over time in the WP ramp-up phase has been established for human immunodeficiency virus NAT–yield donations in South Africa.⁹ A random appearance of early infected blood donors has also been examined and confirmed for HBV in this study. For OBI donations a similar mathematical approach has been followed to determine the random distribution of log viral loads in potentially infectious donations. This was done by plotting the increasing log viral load values against the case number and applying linear regression analysis to determine the correlation.

Transmission risk analysis

For each of the different categories of HBV-yield samples the log viral load was plotted on the Y-axis against the case number on the X-axis. Assuming an equal distribution of the log viral load in the range of detected HBV NAT-yield cases, the viral loads of the nondetected cases could be extrapolated by linear regression analysis until the lowest possible concentration of one virion per 2500 mL of body plasma volume was reached. The probability of a red blood cell (RBC) unit (20 mL of plasma) to be infectious was estimated from the actual determined viral load of the detected cases and the projected lower viral loads of the nondetected cases using the infectivity formulas described by Weusten and coworkers.⁴ The infectivity of a donation to be infectious depends on the viral load and Poisson distribution of infectious virus particles in plasma and is calculated by the formula

$$\text{Pr (infectious)} = 1 - \exp(-N \cdot p_v),$$

where N is the expected number of viral particles in the blood product and p_v is the individual probability of a viral particle to cause infection. From this equation a relation can be derived between the viral load corresponding to a 50% infectivity rate (N_{50}) and p_v .

$$N_{50} \cdot p_v = \ln(2) \approx 0.69.$$

For our risk analysis we assumed a 50% minimum infectious dose (N_{50} or ID_{50}) of 3.2 virions in anti-HBc-negative WP samples^{10,11} and a 100-fold lower infectivity in later anti-HBc-positive stages of infection.^{2,11,12} To determine the probability that the viral load in a donation would transmit HBV to a recipient the probability of infection was multiplied with the probability of nondetection by the NAT option evaluated. For this analysis we did not use the observed proportion of nonreactives in the NAT option, but the normalized probability of detection deduced from the viral load according to probit analysis. The probit curves of the NAT options used for this analysis were those calculated on dilutions of the clinical NAT-yield samples with 50% limits of detection (LODs) of 10.6 copies/mL for Ultrio, 4.3 copies/mL for Ultrio Plus, and 4.6 copies/mL for TaqScreen.¹ The probability of HBV transmission was calculated for RBC units (20 mL of plasma), platelet concentrates (PCs; 50 mL of plasma) and fresh-frozen plasma (FFP) units (200 mL of plasma) and for ID_{50} values of 3.2 and 316 virions in anti-HBc-negative and anti-HBc-positive NAT-yield samples, respectively.^{2,4,10-12} For the different categories of HBV-yield samples we then calculated the sum of the probabilities of infectivity for detected and projected undetected viral loads multiplied by the transfusion plasma volume (a = sum cases infectious). The overall transmission risk that remains for a particular NAT option is the sum of the probabilities of cases to be infectious and nondetected (b = sum cases infectious and not detected). This residual risk was then represented by the formula b/a and expressed as a percentage.

Efficacy analysis

The efficacy is the proportion of the residual risk that can be removed by a NAT option. When b/a is the residual risk the efficacy or risk reduction is represented by the formula $(a - b)/a$ and was also expressed as a percentage.

The efficacy of screening options can also be calculated in general for all infection stages prevalent in the donor population and this also includes the large proportion of concordant HBsAg and HBV DNA–positive donations. In this study the efficacy calculations are restricted to certain acute or chronic HBsAg-negative stage of HBV infection, such as pre-HBsAg WP or post-HBsAg WP or OBI. We also assessed the efficacy of NAT options on the subgroup of low-viral-load HBsAg carriers that were nonreactive on initial screening by the Ultrio assay. Finally we compared the estimated theoretical efficacy levels provided by ID-NAT (Ultrio Plus) in this study for RBC and FFP transfusions with those observed and recently reported by Taira and colleagues⁶ for TaqScreen and AmpliNAT (Roche Molecular Systems) when tested in ID-NAT format on the stored donation samples in a Japanese lookback study.

RESULTS

Distribution of viral load in HBV-yield samples

Of the 106 HBV NAT–yield samples only 18 (17%) had detectable viral loads varying between 400 and 10,000 copies/mL in the Roche TaqMan real-time PCR assay. The viral load in the rest of the samples was determined by replicate Ultrio Plus testing of undiluted and 1:4 and 1:8 diluted samples and comparison against the Eurohep standard dilutions using parallel line probit analysis. The distribution of the log viral load in the different categories of HBV NAT–yield samples is shown in Fig. 1. The measurable HBV DNA concentration in WP and possible vaccine breakthrough samples varied between 7 and 10,000 copies/mL. Figure 2 shows again the distribution of the log viral loads of the WP samples with the linear regression line. The high correlation coefficient (0.99) confirms that donors are likely to present randomly over time in the WP ramp-up phase. The vast majority of HBV DNA concentrations in IgM anti-HBc–positive donors in the early recovery phase and in OBI donations was below the qPCR quantification limit and only quantifiable by comparison of replicate Ultrio Plus results to the Eurohep HBV DNA standard probit curve. In Fig. 3 the viral load distribution in all 56 OBI donations with and without detectable anti-HBs is presented. The correlation coefficient (0.99) of the regression line confirms an equal distribution of log HBV DNA concentrations in the range between five and 100 copies/mL.

As expected, the viral loads in the HBsAg-yield samples (Fig. 1) are even lower than in OBI samples and are also likely to be equally distributed in the range between 0.6 and 100 copies/mL. In one sample the HBV DNA concentration could not be determined because none of 12 Ultrio Plus and none of 12 TaqScreen results were reactive. Here a concentration of 0.3 copies/mL was imputed, corresponding to an estimated reactivity of one in 24 replicate assays.

Residual HBV transmission risk with NAT options

As described under Materials and Methods we determined the HBV transmission risk that remains with the different NAT options compared to the total number of projected infectious donations in the absence of NAT screening as the denominator. This analysis was based on the observation of random distribution of log viral loads in the preacute phase as well as in the later anti-HBc–positive phases of HBV infection. The theoretical risk analysis was based

on an ID₅₀ of 3.2 virions in the preacute phase and 316 virions in the anti-HBc-positive phases of infection. Table 1 gives the projected total number of HBV transmissions and the combined probability of nondetection and infectivity for each of the ID- and MP-NAT options and for each of the HBV infection categories. The percentage residual HBV transmission risk is shown in Table 1A for RBC transfusions (20 mL of plasma), Table 1B for PC transfusions (50 mL plasma), and Table 1C for FFP transfusions (200 mL of plasma).

For example, as is illustrated in Fig. 1, we extrapolated from the viral loads in 22 WP NAT-yield samples interdicted by Ultrio that another 27 WP donations had viral loads of one or more virions in their body volume of plasma (2500 mL). From the probabilities of infectivity of the viral load in the 22 interdicted and 27 projected undetected cases it was calculated for RBC transfusions that a total of 31.7 WP donations would be infectious. Of these the Ultrio Plus assay would not prevent HBV transmission in 10.0 WP cases (32%). Likewise we were able to determine and plot the probability of HBV transmission for OBI donations with an estimated 100-fold lower infectivity against log HBV DNA concentration (Figs. 4 and 5). The maximum probability of infectivity and nondetection of OBI donations by the Ultrio Plus ID-NAT assay reaches approximately 10% at a viral load of 10 copies/mL and is still 7% at a concentration of 2 copies/mL, the lowest concentration detected by Ultrio. From the viral load distribution in 29 interdicted OBI cases it can be projected that another 40 would have viral loads higher than one virion/2500 mL (Fig. 5). Taking the sum of the probabilities of infectivity into account of the 29 interdicted and the 40 projected but not interdicted donations of lower viral load, a total of 14.7 donations are calculated to be infectious. Note that for RBC transfusions this number is less than the number of interdicted donations and represents 14.7 of 29 (51%) of the Ultrio ID-NAT-yield cases, higher than the 24% estimated by Allain and colleagues² in a recent European lookback study. For FFP transfusions with a 10-fold higher plasma volume than RBCs 30.3 of 29 (104%) of Ultrio ID-NAT yields is expected to be infectious, comparable to the 100% observed on only a few FFP transfusions in the European lookback study² (Table 1C). Of the estimated 14.7 OBI transmission cases by RBC transfusion that would occur without NAT it was calculated that 17% would not be detected by Ultrio Plus in ID-NAT configuration. A higher proportion of 51% of OBI transmissions would go unrecognized by TaqScreen MP6-NAT (Fig. 5, Table 1A). Likewise in a theoretical scenario where no HBsAg screening would be performed 16% of the infectious HBsAg-positive, Ultrio-negative donations would not be interdicted by Ultrio Plus in ID-NAT and 45% would be missed by TaqScreen MP6 NAT (Table 1A).

The residual HBV transmission risk for the NAT options becomes higher when the amount of plasma in the blood components increases. This can be compared from the estimated proportions of infectious donations not detected by the screening options in Tables 1A, 1B, and 1C for transfusions of RBCs (20 mL of plasma), PCs (50 mL of plasma), and FFP units (200 mL of plasma), respectively. In WP donations the residual risk of Ultrio Plus ID-NAT screened donations increases from 32% (RBCs) to 43% (FFP) with 10-fold higher plasma transfusion volume, whereas the risk remaining with TaqScreen MP6 increases from 48% to 56%. In OBI donations the transmission risk with Ultrio Plus ID-NAT increases from 17% by RBCs to 33% by FFP transfusions, whereas for TaqScreen MP6 the risk increases from 51% to 66%.

The risk remaining with TaqScreen MP6 lies between that of Ultrio Plus MP4 and MP8 for all HBV-yield categories (Tables 1A, 1B, and 1C).

Efficacy of NAT options

Instead of the proportion of the transmission risk that remains for each infection stage (Tables 1A–1C) one can also calculate the efficacy or proportion of risk reduction, that is, 100% minus percentage of residual risk presented in Table 1. Tables 2A and 2B compare the Ultrio Plus ID-NAT efficacy in WP and OBI donations as determined by the mathematical modeling in this study with the observed ID-NAT (AmpliNAT/TaqScreen) efficacy in a recent lookback study conducted by the Japanese Red Cross (JRC).⁶ The ID-NAT efficacy calculated from the JRC study data in Table 2 may be an overestimation of the true efficacy because only 98 established HBV transmission events in MP20 to MP50 NAT-negative donors were taken into account, while an additional 691 unconfirmed possible transmission events in hospital reports from donations with undetectable viral load were ignored. In the Japanese RBC and PC/FFP recipients (>160 mL of plasma), infected by MP20 to MP50 NAT-nonreactive WP donations, 25 of 27 (93%) and 18 of 28 (64%) were detected by ID-NAT in the lookback procedure, respectively. In our modeling of HBsAg-negative WP donations this was 68 and 57% for RBC and FFP recipients, respectively. Likewise, the modeled ID-NAT efficacy levels in South African OBI donations (based on 100-fold reduced infectivity compared to WP²) were somewhat lower than those estimated from the established Japanese OBI transmission cases. ID-NAT (TaqScreen) detected 17 of 17 (100%) OBI transmissions in the Japanese RBC recipients and 19 of 26 (73%) in PC/FFP recipients. In the modeling on the viral load distribution in South African OBI donations the ID-NAT (Ultrio Plus) efficacy was estimated at 83 and 67% in RBC and FFP recipients, respectively.

DISCUSSION

The replicate testing of serial dilutions of 106 South African HBV NAT– and 29 HBsAg-yield samples by the Ultrio Plus and TaqScreen assays allowed us not only to compare the sensitivity of these NAT methods,¹ but also to estimate the HBV DNA concentrations below the quantification limit of the Roche TaqMan viral load assay. Comparison of the proportions of reactive results with those on the Eurohep HBV standard by probit analysis enabled quantification of HBV DNA in copies/mL, equivalent to (defective or infectious) virions/mL. The viral loads in 22 WP and 10 probable vaccine breakthrough samples showed an equal distribution between fewer than 10 and 10,000 copies/mL. However, the vast majority of HBV DNA concentrations in 15 IgM anti-HBc–positive WP samples and 56 OBI donations was lower than 100 copies/mL. Only six of 62 anti-HBc–positive HBV NAT (Ultrio)-yield samples (10%) had a viral load above 100 copies/mL, the rest showing an equal distribution of HBV DNA concentrations between five and 100 copies/mL (Fig. 3). The range of viral loads in 29 Ultrio-nonreactive HBsAg carrier donors was even lower and varied between 0.6 and 240 copies/mL with a similar random distribution of the HBV DNA levels below 100 copies/mL (Fig. 1). One confirmed HBsAg- and anti-HBc–positive donor had nondetectable viral load because none of 12 Ultrio Plus and TaqScreen replicates were reactive.

Linear regression analysis on the log viral load distribution indicated a random distribution of HBV DNA concentrations in OBI-yield samples detected by the Ultrio assay. One would have expected that the frequency of OBI donations picked up by Ultrio would become less when the viral load approaches the 50% LOD (10 copies/mL),¹ but this was not obvious (Fig. 3). A recent analysis of HBV DNA concentrations in yield plasma samples detected by Ultrio and by Ultrio Plus¹³ indicated that the former assay is likely to underdetect HBV NAT-yield donations over a wider viral load range (also above 100 copies/mL) and that as a consequence the OBI- and WP NAT-yield rate was 1.7-fold higher with the newer assay version.¹³ It is therefore possible that for the more consistently sensitive Ultrio Plus assay^{14,15} the frequency of donations with viral loads below the 95% LOD (<50 copies/mL) would become less and that a correction factor needs to be applied for projecting the frequency of lower viral loads. It is not known whether the log viral loads of prevalent OBI donations that are undetectable by ID-NAT are also evenly distributed. Probably the OBI donations with HBV concentrations of fewer than 0.3 copies/mL are abundantly prevalent and at some minimal concentration the viral load distribution reaches an asymptote, but these extreme low levels do not contribute to the OBI transmission risk (see Figs. 4 and 5). Hence, for the projection of the frequency of WP and OBI donations not detected by Ultrio the distribution of detectable viral loads in the lower viral load range was not corrected for the probability of detection by Ultrio. This may have been a limitation of our viral load distribution and risk analysis. It is unfortunate that the study has not been done starting from yield samples detected by the more sensitive Ultrio Plus assay so that correction for the probability of detection for concentrations below the 95% detection limit could be computed and included in the projections of undetected WP and OBI cases.

With this limitation of the variable probability of detecting HBV NAT yields by the previous Ultrio assay version in mind, we performed a theoretical transmission risk analysis based on Poisson distribution statistics on the concentrations of infectious HBV particles in the low-viral-load donations. For low-viral-load HBsAg carrier donations we assumed a 100-fold reduced infectivity of HBV particles as was found in infectivity studies of late-acute HBsAg- and anti-HBc-positive chimpanzee samples in human liver chimera mice.¹⁰ According to this analysis the Ultrio Plus assay would not detect 16% of the HBV transmissions caused by RBC transfusions of the infectious HBsAg carrier donations missed by the Ultrio assay. Note that the probability of transmission would reduce to 7% if all 29 HBsAg positive Ultrio nonreactive yields instead of the estimated 13.4 infectious HBsAg carrier donations (Table 1a) would be used as the denominator. However with introduction of the Ultrio Plus assay the proportion of HBsAg reactive, HBV-DNA nonreactive donors has reduced 2.4 fold from 4.6% to 1.9% of all HBsAg positive donors.¹³ With this adjustment of the denominator (from 29 to 11.9 serology yields) 18% of HBsAg carrier donations missed by the Ultrio Plus assay are predicted to be infectious. In other words, in the hypothetical scenario that HBsAg testing in South Africa would be discontinued, the efficacy of the Ultrio Plus assay in identifying the infectious donations in the subgroup of low viral load HBsAg carrier donors that were missed by the less sensitive Ultrio assay is estimated at 93% for RBC transfusions and 18% of the HBsAg positive donations missed by Ultrio Plus assay are predicted to be infectious.

More interestingly we performed the same risk and efficacy analysis on the HBsAg-nonreactive OBI donations. Like for the HBsAg- and anti-HBc-positive samples we assumed a 100-fold higher 50% minimum infectious dose than in the WP, that is, an ID₅₀ of 316 virions as was recently confirmed by Allain and colleagues² in a European multicenter lookback study. For each of the observed viral loads in the OBI-yield samples and an additional number of undetected OBI yield samples with lower viral loads (projected by linear regression analysis), we estimated the probability of infectivity of RBC, PC, and FFP transfusions with 20, 50, and 200 mL of plasma, respectively. This mathematical analysis showed that approximately half of the number of OBI NAT-yield donations interdicted by Ultrio is expected to be infectious in RBC recipients, but with 10-fold higher plasma volume in FFP transfusions more than 100% of anti-HBs-negative OBI donations are predicted to be infectious. These modeled transmission risk levels are somewhat higher than those observed by Allain and coworkers² in the above-mentioned European lookback study. They deduced from the anti-HBc prevalence in susceptible recipients of anti-HBs-negative OBI blood that approximately 25% of RBC and 85% to 100% of FFP transfusions were infectious. The twofold lower HBV transmission risk in this lookback study² than estimated by us for RBC transfusions may indicate that the mean ID₅₀ in OBI blood is lower than the 316 virions used for the modeling. Our viral load and transmission risk analysis would predict that 17% of the ID-NAT-screened RBC transfusions of anti-HBs-negative OBI donors would be infectious. When the number of anti-HBs negative OBINAT yields that are detected by the Ultrio assay would be used as the denominator 2.5/29 (8.6%) would be infectious. However if Ultrio Plus would have been used for screening the number of OBI yields would have been 1.7 fold higher¹³ so that 2.5/49.3 (5.1%) would be infectious. In a recent Australian lookback study⁵ of Ultrio-nonreactive donations from OBI donors that have been identified by Ultrio on a later donation the transmission rate has been estimated from the anti-HBc prevalence at 3.8% (1.7%–6.0%). The Australian investigators have included OBI donors with detectable anti-HBs below 30 IU/L in their lookback analysis. If these donors with a lower likelihood to be infectious would not be included the probability of OBI transmission would probably be slightly higher and closer to the estimated 5% of detected Ultrio Plus OBI yields (and 17% of infectious OBI transfusions) according to our modelling. Knowing that the variables in our modeling were in line with the observed OBI transmission rates in the European and Australian lookback studies, we were confident to translate the relative sensitivity of the different ID and MP-NAT methods on the viral load distributions into efficacy in removing OBI transmission risk. For Ultrio Plus ID-NAT the efficacy was estimated at 83%, whereas TaqScreen MP6 was predicted to prevent 49% of OBI infections. The efficacy in preventing HBV transmission of OBI donations is lower with higher plasma volume and was estimated at 67 and 34% for Ultrio Plus ID and TaqScreen MP6 NAT, respectively, in case of FFP transfusions. When TaqScreen was used in ID-NAT configuration for testing of established OBI transmission events missed by MP20 to MP50 NAT in Japan, 100% of infections caused by RBC and 73% caused by PC/FFP transfusions (>160 mL plasma) would have been interdicted⁶ (see Table 2B). These observed efficacy levels by Taira and colleagues⁶ are somewhat higher than the estimated proportions of infectious OBI transfusions detectable by Ultrio Plus in our modeling exercise, but in the Japanese study only MP20 to MP50 NAT-nonreactive and anti-HBc low-titer donations were taken into account. Probably the true efficacy in the Japanese lookback study is lower since

691 possible HBV transmission events in hospital reports could not be confirmed by sequence analysis because of a too low viral load in the donations. On the other hand in our modeled efficacy levels we ignored the known immune neutralization in anti-HBs–positive OBI donations.^{16,17} When analyzing OBI donations with detectable anti-HBs in our study we estimated 89 and 64% efficacy by Ultrio Plus ID and TaqScreen MP6 NAT, respectively, for RBC transfusions, but probably the infectivity of HBV in this subcategory of OBI donations is completely neutralized by anti-HBs and anti-pre-S.^{16,17} In addition we did not apply a correction factor for recipient susceptibility and HBV neutralization by anti-HBs in cotransfused units in our modeling. In South Africa a relatively high proportion of donors and transfusion recipients may be anti-HBs positive, either from endemic HBV circulation or from childhood vaccination.

The efficacy of ID-NAT in reducing transmission risk by WP donations based on extrapolation of the viral load distribution was estimated at 68% by Ultrio Plus ID-NAT and 52% by TaqScreen MP6 NAT. The lower theoretical efficacy of ID-NAT in preventing WP than OBI transmissions is caused by the 100-fold higher infectivity of HBV in the WP than in OBI^{2,10,11} (Fig. 5).

In conclusion, since the Ultrio Plus and TaqScreen assays were found to be equally sensitive on the South African yield samples, the efficacy in preventing HBV transmission is mainly a function of the pool size. When taking OBI transfusions with and without detectable anti-HBs together more than 90% of OBI transmissions can be prevented by ID-NAT screening, but the efficacy diminishes significantly to less than 50% in MP-NAT settings and with larger plasma transfusion volumes. The results of three lookback studies are in line with the theoretical estimates for the probability of OBI transmission by NAT screened blood in our modelling. Therefore this theoretical analysis of transmission risk may be useful in providing the input variables for comparison of the (cost) efficacy of different screening scenarios based on ID- or MP-NAT with or without HBsAg and/or anti-HBc testing.

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ABBREVIATIONS

ID	individual donation
ID₅₀	50% minimum infectious dose
JRC	Japanese Red Cross
LOD(s)	limit(s) of detection
MP	minipool
OBI	occult hepatitis B virus infection
PC(s)	platelet concentrate(s)
qPCR	quantitative polymerase chain reaction

WP window period

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Fig. 1.

Distribution of measured and estimated log HBV DNA concentrations in different types of HBV NAT–yield and HBsAg–yield samples. Samples with HBV DNA concentrations above the upper dotted line had measurable viral load in qPCR and viral loads between the lower and upper dotted lines were determined by probit analysis (see Materials and Methods). HBV DNA concentration could not be estimated in samples with values below the lower dotted line since none of six or none of 12 replicate Ultrio Plus tests were reactive. HBV DNA concentrations in anti-HBc–negative acute-phase yield samples are depicted in order of increasing viral load, whereas anti-HBc–positive yields are presented in order of decreasing viral load. (♦) WP; (■) vaccine breakthrough; (▲) early recovery; (×) OBI anti-HBs positive; (*) OBI and anti-HBs negative; (●) HBsAg yield.

Fig. 2.

Distribution of log viral load in 22 HBVWP donations and regression line for extrapolation of WP donations of lower viral load down to one virion per 2500 mL of body volume of plasma of which a summation of 10.0 are estimated to be infectious with an ID₅₀ of 3.2 virions. (♦) Measured; (—) extrapolation.

Fig. 3.
Random distribution of log HBV DNA concentrations in 56 OBI donations confirmed by linear regression analysis.

Fig. 4. Probability of infectivity (red line) and nondetection by Ultrio Plus ID-NAT (blue line) of OBI donations as a function of the log viral load and the product of the two probabilities (green line) representing OBI transmission risk.

Fig. 5. Modeling the probability of residual HBV transmission by RBC transfusions derived from 22 HBVWP donations and 29 OBI donations, when screened by Ultrio Plus ID-NAT or TaqScreen MP6 NAT. The dotted line represents the extrapolation of WP transmission risk of undetected WP and OBI donations of lower viral load.

Estimation of the HBV transmission risk with different NAT screening options expressed as a percentage of the total number of projected infectious donations *

TABLE 1

Clinical status	Ultrio ID-NAT interdicted	Projected infectious cases (% of interdicted cases)	Projected number of undetected HBV transmission cases (% of projected infectious cases)				
			Ultrio Plus ID-NAT	Ultrio Plus MP4	Ultrio Plus MP8	TaqScreen MP6	
A. RBC transfusions (20 mL of plasma)							
WP	22	31.7 (144)	10.0 (32)	13.9 (44)	15.8 (50)	15.1 (48)	
Vaccine break	10	11.7 (117)	3.6 (31)	5.0 (43)	5.7 (49)	5.4 (47)	
Early recovery	14	6.2 (44) [‡]	1.0 (16)	2.2 (36)	3.0 (49)	2.7 (44)	
OBI anti-HBs	27	14.2 (53) [‡]	1.5 (11)	4.0 (28)	5.8 (41)	5.1 (36)	
OBI	29	14.7 (51) [‡]	2.5 (17) [§]	6.1 (41)	8.3 (56)	7.5 (51)	
HBsAg yield	29	13.4 (46)	2.1 (16)	4.8 (36)	6.7 (50)	6.0 (45)	
B. PC transfusions (50 mL of plasma)							
WP	22	34.2 (155)	12.5 (37)	16.4 (48)	18.4 (54)	17.6 (52)	
Vaccine break	10	12.6 (126)	5.0 (40)	6.0 (48)	7.4 (59)	6.4 (51)	
Early recovery	14	8.5 (61) [‡]	1.9 (22)	3.7 (44)	4.8 (56)	4.4 (52)	
OBI anti-HBs	27	18.3 (68) [‡]	2.7 (15)	6.5 (36)	8.9 (49)	8.0 (44)	
OBI	29	21.4 (74) [¶]	4.7 (22)	10.3 (48)	13.4 (63)	12.2 (57)	
HBsAg yield	29	19.1 (66)	4.1 (21)	8.5 (45)	11.1 (58)	10.1 (53)	
C. FFP transfusions (200 mL of plasma)							
WP	22	38.1 (173)	16.4 (43)	20.3 (53)	22.2 (58)	21.5 (56)	
Vaccine break	10	14.0 (140)	6.0 (43)	7.4 (53)	8.1 (58)	7.8 (56)	
Early recovery	14	10.9 (78) [‡]	3.4 (31)	5.8 (53)	7.1 (65)	6.6 (60)	
OBI anti-HBs	27	23.2 (86) [‡]	5.3 (23)	10.4 (45)	13.2 (57)	12.1 (52)	
OBI	29	30.3 (104) [¶]	9.9 (33)	17.7 (58)	21.6 (71)	20.1 (66)	
HBsAg yield	29	27.9 (96)	9.0 (32)	15.6 (56)	19.0 (68)	17.7 (63)	

* Data are reported as number (%). The data are based on extrapolation of the viral load distribution in observed HBV NAT-yield samples (Fig. 1) and the estimated probabilities of transmission of detected and projected undetected donations (Figs. 4 and 5). Tables 1A, 1B, and 1C compare the HBV transmission risk for transfusion of RBC, PC, and FFP units containing 20, 50, or 200 mL of plasma, respectively.

⁷ Ignoring probable complete neutralization by anti-HBs.
⁸ Estimated at 24% in European lookback study.²
⁹ Estimated at 3.8% in Australian lookback study.⁵
¹⁰ Estimated at 51% in European lookback study.²
¹¹ Estimated at 85% to 100% in European lookback study.²

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TABLE 2

Comparison of observed ID-NAT (AmpliNAT/TaqScreen) efficacy in 98 established HBV transmission events missed by MP20 to MP50 NAT screening (as adapted from a recent report of JRC lookback studies⁶) with the estimated ID-NAT (Ultrio Plus) efficacy levels in preventing transmission risk in WP and occult stages of HBV infection as determined by mathematical modeling in this study^{*}

JRC screening period	ID-NAT efficacy	
	RBCs	FFP/PC [†]
<i>A. WP transmission cases</i>		
WP donations		
MP50 AmpliNAT	15/15 (100)	13/18 (72)
MP20 AmpliNAT	8/10 (80)	4/8 (50)
MP20 TaqScreen	2/2 (100)	1/2 (50)
Whole screening period	25/27 (93)	18/28 (64)
Modeled on viral load distribution in current study	21.7/31.7 (68)	21.7/38.1 (57)
<i>B. OBI transmission cases</i>		
MP50 AmpliNAT	5/5 (100)	8/12 (67)
MP20 AmpliNAT	7/7 (100)	5/6 (83)
MP20 TaqScreen	5/5 (100)	6/8 (75)
Whole screening period	17/17 (100)	19/26 (73)
Modeled on viral load distribution in current study	12.1/14.6 (83)	20.5/30.4 (67)

^{*} Data are reported as number (%). Note that the 55 WP and 43 OBI transmission events in the JRC lookback study were established by HBV DNA sequence analysis from a total of 789 hospital reports of potential HBV transmission events and therefore the true ID-NAT efficacy levels may be lower.

[†] More than 160 mL of plasma.