A mathematical model for estimating residual transmission risk of occult hepatitis B virus infection with different blood safety scenarios

Jos Weusten,1 Harry van Drimmelen,2 Marion Vermeulen,3 and Nico Lelie2,4

BACKGROUND: If anti-hepatitis B core antibody testing is not mandated blood donors with occult hepatitis B infection (OBI) may transmit hepatitis B virus (HBV) to a recipient in spite of the use of nucleic acid amplification technology (NAT) or pathogen inactivation (PI).

STUDY DESIGN AND METHODS: We developed a model to estimate OBI transmission risk based on three components: the probability distribution of the viral load (VL) in a randomly selected OBI donor, the probability that a given VL remains undetected, and the probability that this VL causes infection in the recipient. A subset of 217 South African OBI samples identified by individual donation (ID)-NAT screening were quantified by replicate testing using an ID-NAT assay (Ultrio Plus) against HBV DNA standard dilution series. The observed log VLs could be described by a Gumbel distribution. A correction was included to compensate for OBI samples missed by initial ID-NAT screening.

RESULTS: The model estimates that 3.3% of all OBI donations are undetected by ID-NAT (Ultrio Plus) and cause infection by a blood component containing 20 mL plasma, going up to 8.7% when using minipools of 6 (MP6)-NAT. For 200-mL plasma transfusion these risks were estimated at 14 and 28%, respectively, while PI with modest (2 log) reduction capacity would reach 4.8% without NAT and 1.3 or 0.4% when combined with MP6- or ID-NAT.

CONCLUSION: The model can be used to compare different screening and/or PI strategies in reducing viral transmission risk and could serve as a tool in evaluating efficacy of alternative blood safety scenarios.

Previously we developed a mathematical model for estimating residual risk of transfusion-transmitted virus infection by blood donations drawn in the early seronegative window period (WP) in spite of screening by individual donation (ID) or minipool (MP) nucleic acid amplification technology (NAT). This model was among others based on the concept of a doubling time in the early ramp-up phase of viremia as had been demonstrated for hepatitis B virus (HBV), hepatitis C virus, and human immunodeficiency virus (HIV), indicating random appearance of asymptomatic donors in the diagnostic WPs of NAT, hepatitis B surface antigen (HBsAg), and HIV p24 antigen assays. Other components of the model were the concentration-dependent detectability of the virions in the NAT assays, and the probability of infection in the recipient that depended on the absolute number of virions in the transfusion product.

The current article aims at adjusting the WP risk model for blood components from donors with occult HBV infection (OBI) in a setting where anti-hepatitis B -

ABBREVIATIONS: ID = individual donation; LOD(s) = lower limit(s) of detection; MID50 = 50% minimum infectious dose; MP = minipool; MP6 = minipool of 6 samples; OBI = occult hepatitis B virus infection; PDF = probability density function; PI = pathogen inactivation; VL(s) = viral load(s); WP = window period.

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core antibody (anti-HBc) testing is not performed. Many individual building blocks of the previously developed WP risk model can be applied once again, but an analysis is needed on the distribution of the viral load (VL) in OBI donations. These VLs can fluctuate and are generally low (mostly < 100 copies/mL) and not easily detectable using the standard HBV NAT procedures. Yet, as will be shown, such low HBV concentrations can be quantified and these data were used to mathematically characterize the probability distribution of VLs for this group of OBI donors. Based on this, a risk model was built.

**MATERIALS AND METHODS**

**Main building blocks of the risk model**

The four building blocks of the general risk model for a given subgroup of donors are reflected by a set of conditional probabilities:

a. The probability that a random donor is actually in the group of interest;
b. The probability that the blood donation has a certain VL given that Condition (a) is met;
c. The probability that the VL is not detected by NAT, given Condition (b);
d. The probability that the VL leads to an infection in the recipient, given Condition (b).

The probability of (a) follows from epidemiologic data, for as far as donors can be identified as such. Items (c) and (d) are conceptually no different from the situation in the WP donors we reported previously, albeit that the infectivity of a viral particle in an OBI donor is different from a WP donor, but that is only reflected by a different numerical value of the 50% minimum infectious dose (MID50). The main aim of the current article is the mathematical characterization of the VL distribution as indicated in Item (b).

**Assessing the overall risk**

Once the distribution of the OBI VLs is characterized, the overall risk can be assessed. Similarly as before, the overall risk of introducing a viral infection given the blood donor has a log VL equal to some value μ is the product of conditional probabilities:

Pr(Transmission|m) = Pr(NonDetection|m) * Pr(Infection|m).

(1)

The probability of nondetection includes the properties of the NAT assay employed, the use of MP or ID testing, and so forth, as described for the WP risk model. The probability of the donation being infectious depends on the infectivity of the virus (expressed as the MID50) and the blood product administered (most notably its plasma volume). The overall risk follows by taking the integral

Pr(Transmission|infectiousOBI) = \int_{-\infty}^{\infty} Pr(NonDetection|\mu) * Pr(Infection|\mu) * f(\mu)d\mu,

(2)

with f(\mu) being the probability density function (PDF) of the distribution of the OBI log VLs. The indication “infectiousOBI” is needed as a certain fraction of all OBI donations is not infectious at all, regardless the VL because of the presence of neutralizing anti-HBs. Characterizing this distribution function f(\mu) is one of the major topics of the current article. The integral of Eq. (2) is to be evaluated numerically, as before. The total risk for the donor population as a whole then follows from the current result in combination with the observed frequency of OBI donations as will be described under Results.

**VL estimation in OBI donations**

VL data on known OBI donors were collected by the South African National Blood Service. The first available data set of 55 OBI plasmas was used in a previous publication to estimate OBI transmission risk. These were anti-HBc- and HBV DNA–positive donations picked up by screening using the Ultrio test (Grifols). If VL was below the lower limit of quantification of the Roche TaqMan assay (<116 copies/mL), HBV concentration in copies/mL was estimated by probit analysis on replicate Ultrio Plus test data in comparison to those on dilutions of the Eurohep standard. However, it turned out that the previous Ultrio assay was deficient in detecting certain HBV strains leading to underdetection of samples, even in the range of 100 to 1000 copies/mL. We therefore also analyzed another group of 162 OBI donations identified during the first year of screening by the South African National Blood Service using the more sensitive Ultrio Plus assay (M. Vermeulen et al., manuscript in preparation). For samples below the lower limit of quantification of quantitative polymerase chain reaction we determined the proportion of reactive Ultrio Plus test results from replicate multiplex or discriminatory HBV tests routinely performed for confirmation testing. The proportion of reactive NAT results was compared with the probit curves established on the Eurohep standard with 50 and 95% lower limits of detection (LODs) of 4.5 and 43.1 copies/mL.

**RESULTS**

**Modeling the VL distribution in OBI donations, Step 1: the observed VLs**

To study the observed VL distribution in the OBI donations, empirical cumulative probability distribution plots
were constructed. Four groups of data were distinguished: OBI sample selection based on screening by Ultrio and by the more sensitive Ultrio Plus assay and for both groups a distinction was made between anti-HBs–negative and anti-HBs–positive samples. The result is shown in Fig. 1 and reveals that the four subsets show very similar distributions. Applying the Kolmogorov-Smirnov test to any combination of two distributions did not lead to any significant result (all \( p > 0.44 \)), indicating that potential distributional differences are small. For the current model building, we assumed that all data could be combined into a single group, the distribution of which is also indicated in Fig. 1. At the lower end, below the vertical reference line drawn at 0.35 log (2.23 copies/mL), quantification is very difficult and numerically not really trustworthy anymore, apart from being very low.

To come to a mathematical description of the complete distribution, albeit in approximation, the observed overall distribution is to be linearized by a proper transformation of the vertical axis. This is illustrated in Fig. 2 assuming a normal distribution (Fig. 2A) and a Gumbel distribution (Fig. 2B). For the normal distribution the probit transformation is used, and for a Gumbel distribution the transformed \( p \) value is given by \(-\ln(-\ln(p))\). The plots reveal that a normal distribution does not provide an adequate description of the data, whereas the Gumbel transformation gives an almost perfect linearization.

The Gumbel distribution is a two-parameter distribution, with cumulative probability distribution function \( F(x) \) of \( x \) according to

\[
F(x) = \exp \left( -\exp \left( \frac{x - \theta_1}{\theta_2} \right) \right),
\]

with \( x \) the log VL, \( \theta_1 \) the location parameter, and \( \theta_2 \) the scale parameter. The distribution is often referred to as the extreme value distribution, as it plays a central role in the field of extreme value statistics.

The Gumbel parameters can be assessed using the theory of maximum likelihood estimation, with the VLs at the lower end \((<0.35 \log)\) treated as being censored, meaning that the only reliable information is that the log VLs are below 0.35. The resulting parameters and some observed and predicted quantiles are shown in Table 1. A formal statistical test can be performed to study the null hypothesis that all four groups of data as shown in Fig. 1 follow the same Gumbel distribution versus the alternative that they do not using a likelihood ratio test. The result is nonsignificant (\( p = 0.34 \)).

**Modeling the VL distribution in OBI donations, Step 2: the underlying population of OBI VLs**

The OBI samples that ended up in the data set were obtained after routine ID-NAT screening of all donor samples for HBV. The median of the observed distribution is at 13 copies/mL, well below the 95% LOD of the NAT
screening assay, implying that a considerable fraction of all OBI donors can be missed by routine screening. This implies that the observed set is actually a selection of the total OBI population. For a proper risk assessment, it is required to reconstruct this total population distribution of which the current observed set is a concentration-dependent selection. The concept behind this is illustrated in Fig. 3. The black line presents the PDF of the observed log VL data, defined by the Gumbel distribution of Table 1. The dashed blue line indicates the detection probability of the VL (based on the Ultrio Plus assay with 50 and 95% LODs of 4.5 and 43.1 copies/mL). The red line reflects the hypothesized underlying distribution of the population of OBI donors. If this underlying distribution can be captured in an equation, then combining this PDF equation with the detectability relation would lead to the observed PDF (black line).

Let \( f_U(x) \) be the PDF of the underlying distribution of the OBI population VLs (red line in Fig. 3), with \( x \) the log VL. Let the expression for the detectability probit model be \( f_D(x) \) (blue line in Fig. 3), and let the PDF of the combined distribution for the observed data be \( f_O(x) \) (black line in Fig. 3). It follows

\[
    f_O(x) = \frac{f_U(x)f_D(x)}{\int_{-\infty}^{\infty} f_U(y)f_D(y)dy}.
\]

(3)

Given the usefulness of the Gumbel distribution for the observed data, it is an obvious choice to use the Gumbel distribution for the underlying distribution as well. Parameter estimation can be done using maximum likelihood estimation, with the values below the lower limit treated as being censored and with the integrals evaluated numerically during the optimization. The results are shown in Table 2. The distribution has shifted considerably compared to the distribution of the observed data; the median of the underlying distribution (estimated at 5.70 copies/mL) is numerically similar to the first quartile of the observed distribution (5.15 copies/mL). A graphical presentation is given in Fig. 4, indicating that the data and the fitted distributions correspond well to each other. The red line in Fig. 3 is actually the PDF of the optimized Gumbel distribution. Some additional analyses on the applicability of the Gumbel distribution for the underlying OBI distribution can be found in Appendix S1 (available as supporting information in the online version of this paper).

### Table 1. Gumbel distribution parameters of the observed log VLs in the OBI samples and some predicted quantiles

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>SE</th>
<th>Approx. 95% CI</th>
<th>Q1</th>
<th>Median</th>
<th>Q3</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \nu_1 )</td>
<td>0.8994</td>
<td>0.04149</td>
<td>0.8176-0.9812</td>
<td>0.71</td>
<td>1.11</td>
<td>1.61</td>
</tr>
<tr>
<td>( \nu_2 )</td>
<td>0.5743</td>
<td>0.03268</td>
<td>0.5099-0.6387</td>
<td>5.15</td>
<td>12.9</td>
<td>41.2</td>
</tr>
</tbody>
</table>

![Fig. 3. Concept—observed distribution in the experimental data set (black), underlying distribution of the OBI population (red), and HBV detectability curve (blue, right vertical axis).](wileyonlinelibrary.com)

### Modeling the occurrence of OBI donations in the donor population

The overall risk of a transfusion-mediated infection given the donor is actually an occult carrier can be assessed by combining Eq. (2) with some other considerations. In a given blood establishment over a given period of time, there are \( D_{total} \) donations. Of these donations, there are in total \( N_{OBI,total} \) OBI donations. Due to the low VLs only a fraction \( \delta_{detected} \) is detected, so the number of detected OBI donations is \( N_{OBI,detected} = \delta_{detected}N_{OBI,total} \). As noted, a fraction \( \delta_{aHBsNeg} \) is anti-HBs negative (<10 mIU/mL) and therefore potentially infectious (see Discussion). Combining these building blocks yields that the probability that a random donation leads to an OBI-related infection in a blood recipient can be calculated as

\[
    \Pr(OBI-related\ infection) = \frac{N_{OBI,detected}\delta_{aHBsNeg}}{D_{total}}\Pr(Transmission|infectiousOBI) = \frac{N_{OBI,detected}\delta_{aHBsNeg}}{D_{total}}\delta_{detected}\Pr(Transmission|infectiousOBI).
\]

(4)

Note that the fraction of all OBI donations that is detected is actually given by the denominator of Eq. (3), that
combines the probability distribution of log VL and the probability of detection, in the following equation

\[ \delta_{\text{detected}} = \int_{y=-\infty}^{\infty} f_U(y)f_D(y)dy. \]  

(5)

Given the current results as summarized in Table 2 and using a NAT assay with 50 and 95% LODs at 4.5 and 43.1 copies/mL, the value of \( \delta_{\text{detected}} \) can be calculated as being 0.57. When using a more sensitive NAT, with 50 and 95% LODs at 2.2 and 21.4 copies/mL, \( \delta_{\text{detected}} \) is estimated to be 0.70. Finally, the estimate for \( \delta_{\text{aHBsNeg}} \) was found to be approximately 0.50 in a large multiregional study.

**Examples of risk estimations**

A number of relevant scenarios based on current NAT sensitivities and virucidal capacity of some pathogen inactivation (PI) methods are compared to illustrate our OBI risk model for different blood components. Table 3 shows the residual risk as a percentage of the total number of OBI donations regardless the anti-HBs test result and detectability by the NAT assay (Table 3, second column). With the estimate for \( \delta_{\text{aHBsNeg}} \) of 0.50 this can be rephrased in terms of a percentage of the anti-HBs–negative donations (Table 3, first column). Similarly, the risk can be expressed in terms of the number of detected OBI donations by ID-NAT regardless the anti-HBs test result (Table 3, third column). For four of them, a graphical presentation is shown in Fig. 5 and the legend of this figure gives further explanation. Scenarios without NAT can be treated in the model by setting extremely high values for the 50 and 95% LOD levels.

**DISCUSSION**

In this article we modeled the risk of transfusion-transmitted infections caused by OBI donations, in a model that is very similar to the one we earlier developed for WP donations. An important issue concerns the probability distribution of the log VL in the population of OBI donors. For the WP donors we made use of the concept of the doubling time of the VL due to the viral dynamics observed in the ramp-up phase, leading to a uniform distribution of the log VL over a restricted range. For the OBI donations we found that the observed log VL distribution can satisfactorily be described by a Gumbel distribution. There is no theoretical foundation in virology that would predict a Gumbel distribution, but it apparently does work very well, as we noticed also for some other viral infections (data not shown).

The observed distribution is based on OBI samples that were detected in the routine ID-NAT screening. A correction for the undetected OBI samples requires an assumption concerning the underlying distribution of all potentially infectious occult carriers. Given that the observed data in ID-NAT–positive OBI donors could so very well be described by a Gumbel distribution, this seems an obvious choice for the underlying distribution as well. The calculations in Appendix S1 further support this. The estimated underlying Gumbel distribution covers very low VLs; Table 2 indicates that the first quartile is estimated to be 2.29 copies/mL and from the Gumbel distribution parameters in Table 2 it can be calculated that 1% of the population has VLs below 0.47 copies/mL. Whether or not the Gumbel distribution also applies to these very low concentrations is beyond measurement, but since these low VLs barely contribute to the OBI transmission risk it becomes irrelevant for the purpose of our model.

The risk as calculated with Eq. (2) reflects the risk given the donation is an OBI donation. As only about 50% of the OBI donations are anti-HBs nonreactive (<10 mIU/mL) and can be expected to be infectious, the value is to be multiplied by a factor to exclude noninfectious anti-HBs–positive OBI donations. This factor may differ regionally and is estimated at 0.50 (\( \delta_{\text{aHBsNeg}} \) in Eq. (4)) worldwide since 51% of OBI donations in a large

**TABLE 2. Gumbel distribution parameters of the underlying OBI distribution and some predicted quantiles**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>SE</th>
<th>Approx. 95% CI</th>
<th>Q1</th>
<th>Median</th>
<th>Q3</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \nu_1 )</td>
<td>0.5468</td>
<td>0.0796</td>
<td>0.3900-0.7037</td>
<td>0.361</td>
<td>0.756</td>
<td>1.26</td>
</tr>
<tr>
<td>( \nu_2 )</td>
<td>0.5704</td>
<td>0.0378</td>
<td>0.4957-0.6451</td>
<td>2.29</td>
<td>5.70</td>
<td>18.1</td>
</tr>
</tbody>
</table>

**Fig. 4. Fitted cumulative distributions (red = underlying distribution; black = observed distribution with experimental data) and detectability (blue line, right vertical axis).** [Color figure can be viewed at wileyonlinelibrary.com]
multicenter study\textsuperscript{7} were found to be anti-HBs reactive (\(\geq 10\) mIU/mL). Only few transmissions have been reported by OBI donations with low anti-HBs titers (<30 mIU/mL) and VLs too high to be neutralized.\textsuperscript{14-16} Hence, it is reasonable to assume that the proportion of anti-HBs–positive donations in a regional OBI population is representative for the fraction that is not infectious at all.

To get an overall estimate for the number of infections the frequency of OBI donations in the total blood bank population is required. The number of detected OBI donations only reflects a part of the total number due to the issue of nondetection; our analyses reveal that 43\% of the OBI population is not detected by Ultrio Plus testing in ID-NAT setting, implying that the result is to be divided by 0.57 to correct for this (\(\delta_{\text{detected}}\) in Eq. (5)). When correcting both for the undetected and for the 50\% anti-HBs–positive OBIs one has to multiply the OBI (Ultrio Plus) ID-NAT detection rate by a factor of 0.88 for estimating the rate of potentially infectious OBI donations.

The proportion of OBI donations that are predicted to be infectious with a certain scenario are given in Table 3 in this article and is expressed as a percentage of 1) the number of anti-HBs–negative (or potentially infectious) OBI donations, 2) all OBI donations including the fraction of anti-HBs–positive (or noninfectious) donations, and 3) only the number of OBI donations that were detected by ID-NAT screening (Ultrio Plus). When expressed to the number of all (detected and undetected) OBI donations these percentages vary between 3.3 and 28\% depending on the NAT protocol (ID- or MP6-NAT) and the plasma volume in the blood component. The proportions change depending on the analytical sensitivity of the NAT method applied. For example with a twofold more sensitive ID-NAT the residual risk reduces from 3.3\% to 1.9\% for red blood cell (RBC) transfusions.

In this article, we used VL data as observed in a selected set of OBI donor samples from South African origin, where HBV Genotype A1 is predominant. It is not known whether the VL distributions are the same for all OBI donors around the world. There may be genotype-dependent differences in HBV VL distributions in HBsAg-positive donations across the regions but for HBsAg-negative occult carriers it is expected that the variations are minor. For now we assume that the observed distribution in our study is applicable to VLs of HBsAg negative OBI donations worldwide.

In our example calculations, we used the assumption that the MID\(_{50}\) of HBV in anti-HBs–negative OBI donors is somewhere between 100 and 1000 virions, as could be deduced from a European lookback study among

### Table 3. Residual OBI transmission risk for different blood safety scenarios

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Screening method</th>
<th>50% LOD (copies/mL)</th>
<th>95% LOD (copies/mL)</th>
<th>PI reduction</th>
<th>Component plasma volume</th>
<th>Anti-HBs–negative OBI in population, (N_{\text{OBI, total, anti-HBs Neg}})</th>
<th>All OBI in population, (N_{\text{OBI, total}})</th>
<th>All OBI as detected, (N_{\text{OBI, detected}})</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>No NAT (7 log)</td>
<td>(8 log)</td>
<td>No PI</td>
<td>316</td>
<td>RBCs 20 mL</td>
<td>35</td>
<td>18</td>
<td>31</td>
</tr>
<tr>
<td>2</td>
<td>No NAT (7 log)</td>
<td>(8 log)</td>
<td>No PI</td>
<td>316</td>
<td>PC 50 mL</td>
<td>52</td>
<td>26</td>
<td>46</td>
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<tr>
<td>3</td>
<td>No NAT (7 log)</td>
<td>(8 log)</td>
<td>No PI</td>
<td>316</td>
<td>FFP 200 mL</td>
<td>80</td>
<td>40</td>
<td>70</td>
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<tr>
<td>4</td>
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<td>43.1</td>
<td>No PI</td>
<td>316</td>
<td>RBCs 20 mL</td>
<td>6.5</td>
<td>3.3</td>
<td>5.7</td>
</tr>
<tr>
<td>5</td>
<td>ID-NAT 4.5</td>
<td>43.1</td>
<td>No PI</td>
<td>316</td>
<td>PC 50 mL</td>
<td>13</td>
<td>6.5</td>
<td>11</td>
</tr>
<tr>
<td>6</td>
<td>ID-NAT 4.5</td>
<td>43.1</td>
<td>No PI</td>
<td>316</td>
<td>FFP 200 mL</td>
<td>28</td>
<td>14</td>
<td>25</td>
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<tr>
<td>7</td>
<td>MP6-NAT 4.5</td>
<td>43.1</td>
<td>No PI</td>
<td>316</td>
<td>RBCs 20 mL</td>
<td>17</td>
<td>8.7</td>
<td>15</td>
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<tr>
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<td>No PI</td>
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<td>PC 50 mL</td>
<td>31</td>
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<td>27</td>
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<tr>
<td>9</td>
<td>MP6-NAT 4.5</td>
<td>43.1</td>
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<td>FFP 200 mL</td>
<td>55</td>
<td>28</td>
<td>48</td>
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<tr>
<td>10</td>
<td>ID-NAT 4.5</td>
<td>43.1</td>
<td>2 log</td>
<td>31,600</td>
<td>RBCs 20 mL</td>
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<td>0.041</td>
<td>0.073</td>
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<td>11</td>
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<td>PC 50 mL</td>
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<td>0.10</td>
<td>0.18</td>
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<td>FFP 200 mL</td>
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<td>0.40</td>
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<td>1.9</td>
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<td>21</td>
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<td>21.4</td>
<td>No PI</td>
<td>316</td>
<td>FFP 200 mL</td>
<td>45</td>
<td>22</td>
<td>39</td>
</tr>
<tr>
<td>22</td>
<td>No NAT (7 log)</td>
<td>(8 log)</td>
<td>2 log</td>
<td>31,600</td>
<td>RBCs 20 mL</td>
<td>2.1</td>
<td>1.1</td>
<td>1.9</td>
</tr>
<tr>
<td>23</td>
<td>No NAT (7 log)</td>
<td>(8 log)</td>
<td>2 log</td>
<td>31,600</td>
<td>PC 50 mL</td>
<td>3.9</td>
<td>2.0</td>
<td>3.5</td>
</tr>
<tr>
<td>24</td>
<td>No NAT (7 log)</td>
<td>(8 log)</td>
<td>2 log</td>
<td>31,600</td>
<td>FFP 200 mL</td>
<td>9.7</td>
<td>4.8</td>
<td>8.5</td>
</tr>
<tr>
<td>25</td>
<td>No NAT (7 log)</td>
<td>(8 log)</td>
<td>4 log</td>
<td>3,160,000</td>
<td>RBCs 20 mL</td>
<td>0.074</td>
<td>0.037</td>
<td>0.065</td>
</tr>
<tr>
<td>26</td>
<td>No NAT (7 log)</td>
<td>(8 log)</td>
<td>4 log</td>
<td>3,160,000</td>
<td>PC 50 mL</td>
<td>0.15</td>
<td>0.074</td>
<td>0.13</td>
</tr>
<tr>
<td>27</td>
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<td>(8 log)</td>
<td>4 log</td>
<td>3,160,000</td>
<td>FFP 200 mL</td>
<td>0.41</td>
<td>0.21</td>
<td>0.36</td>
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</table>

\(^a\) Assuming NAT used for assessing historic frequency OBI donations has 50\% LOD = 4.5 copies/mL and 95\% LOD = 43.1 copies/mL.

PC = platelet concentrate.
OBI NAT-yield donors with relatively high detectable VL. However, the infectivity of HBV in a considerable fraction of occult carriers missed by ID-NAT screening may be much less because of escape mutations that render the virus less infectious. Only a few systematic lookback studies have been performed in OBI donors. The implicated OBI donors in these studies had detectable VL by ID or MP-NAT screening and the transmission rate by previous donations was found to be only 3% to 5% in the period before NAT was introduced (or less-sensitive NAT methods were used). Our model predicts an 18% OBI transmission risk by RBC transfusions if no NAT would be applied, which indicates that an average MID50 of 316 virions must be a worst-case assumption that does not hold for all anti-HBs–negative OBIs.

Fig. 5. Examples of calculations of OBI transmission risk (some scenarios of Table 3). Consider a HBV NAT with the 50 and 95% hit rate concentrations at 4.5 and 43.1 copies/mL, respectively. Consider an ID-NAT setting in which initial reactive donations are not used for transfusion, regardless the result of repeat tests. Let the infectivity of the virus be reflected by a MID50 of 316 virions or DNA copies. Finally, let the transfusion volume in a platelet concentrate be 50 mL. The corresponding graphical presentation is as presented in A. The blue line presents the probability that the given VL is not detected, and the red line presents the probability the blood product does lead to an infection. Both probabilities are plotted against the left vertical axis. The green line presents the PDF of the OBI donations. The area under this curve is, by definition, equal to 1. The black line with the colored area reflects the combination of the three, and it is the area under this curve that reflects the overall risk given the donation is from an OBI donor. This area is to be compared, visually, with the area under the green PDF curve. Both the PDF and the product are plotted against the right vertical axis. In this example the area equals 0.13, indicating that there is a transmission risk of 13%. The second example (B) concerns the same situation, but now minipools of 6 are used for NAT screening. As such, the curve reflecting the probability of non-detection shifts to higher values, and the overall risk increases to 31%. In the third example (C), the volume of the transfusion product is increased to 200 mL to represent an MP6-NAT screened FFP of an OBI donor, and as a result the curve reflecting the infectivity shifts to the left. The risk increases to 55%. The fourth example (D) shows the impact of adding PI reducing HBV infectivity by 2 log. The risk of the MP6-NAT screened FFP unit now drops to 2.6%. [Color figure can be viewed at wileyonlinelibrary.com]
Our model allows the calculation of residual risk estimates with different testing scenarios. We made no attempts to construct confidence intervals (CIs), mostly because the uncertainties on the individual terms in the model are unknown. In our opinion, predicting orders of magnitude of residual risk depending on screening choices is more important than giving exact values. In a model based on interpretation of lookback data Seed and colleagues used a formula similar to our Eq. (1) for estimating OBI transmission risk from ID-NAT (Ultrio and Ultrio Plus) screened blood donations in Australia. Despite the differences between our model and the one of the Australian investigators (and the limitations of both models) the residual risk estimates of OBI transmission by ID-NAT screened blood components are remarkably close (in the order of 2%-3%).

The mathematical model described in this article can also be used to study the impact of PI. In our examples, we assumed that a modest inactivation of 2 to 4 log (that may very well describe the HBV reduction capacity by some of the currently used PI systems) can be translated in a MID50 value that increases accordingly. The model can also be used to study the impact if NAT screening is not performed and one relies on PI only. We believe that models like the one we describe in the present and our previous articles are important in decision making concerning the efficacy of alternative NAT screening protocols or PI methods or combinations of these interventions.

We found that a Gumbel distribution of log VLs could also be applied to HBsAg-positive donors that were HBV-DNA nonreactive by initial ID-NAT screening using the less sensitive Ultrio assay (Appendix S2, available as supporting information in the online version of this paper). Our model predicts that 15% of HBsAg-positive donations that are initially nonreactive in the more sensitive Ultrio Plus assay will likely transmit HBV infection by RBC transfusion and 62% by fresh-frozen plasma (FFP). Hence, in an ID-NAT screening setting and in the absence of anti-HBc testing or PI, HBsAg testing still contributes to eliminating HBV transmission risk. Since the Gumbel distribution was found to be also applicable to other viral infection categories, such as for HIV elite controllers and donors infected by hepatitis E virus (data not shown), it is hoped that our mathematical model is instrumental for comparing the efficacy (and cost effectiveness) of different blood safety scenarios.

CONFLICT OF INTEREST

NL received financial support from Grifols Diagnostic Solutions to develop the manuscript. The other authors have disclosed no conflicts of interest.

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**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article at the publisher’s website:

**Appendix S1.** Some further analyses on the VL distribution in the OBI population.

**Appendix S2.** Viral load distribution in HBsAg+/DNA-donations.