Hepatitis B NAT virus–positive blood donors in the early and late stages of HBV infection: analyses of the window period and kinetics of HBV DNA

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Background and Objectives The Japanese Red Cross (JRC) carries out nucleic acid amplification testing (NAT) for hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus-1 (HIV–1) by using a multiplex (MPX) reagent. Screening is undertaken on serologically negative units. In this study we characterized HBV NAT-positive donations individually and analysed the window period and kinetics of HBV DNA, during acute infection, in follow-up studies.

Materials and Methods Two hundred and seventy-seven HBV DNA-positive donations have been identified in Japan since the introduction of NAT screening of 50-donation minipools. The viral loads and genotypes of these HBV DNA-positive donations were characterized. The doubling time and half-life of HBV was estimated from the data of 123 follow-up donors. The sensitivity of the NAT system (based on 50-donation minipools) was compared with the sensitivities of the enzyme immunoassay (EIA) and the chemiluminescence immunoassay (CLIA). Samples that were CLIA negative, but with > 10^4 copies/ml of HBV DNA, were analysed by sequencing the hepatitis B surface antigen (HBsAg) region.

Results Out of 277 HBV NAT-positive samples, 125 (45%) were found to have an increasing viral load and 45 (16%) a decreasing viral load. Forty per cent of HBV NAT-positive samples with an increasing viral load, and 33% of those with a decreasing viral load, were negative when tested by using the CLIA. No mutations related to escape mutants were found in the samples that were CLIA negative but with HBV DNA loads of > 10^4 copies/ml. The median HBV doubling time was 2·6 days (n = 93, 1·3–15·2 days) and the half-life was 1·6 days (n = 55, 0·9–6·3 days). Some kinetic difference was observed between genotypes A and B.

Conclusions HBV NAT screening detected HBV DNA in both early (the so-called serological window period) and late stages of acute HBV infection.

Key words: half-life of HBV, HBV DNA doubling time, HBV DNA kinetics, HBV genotypes, HBV mutations, window period.

Introduction

There are about 30 × 10^6 hepatitis B virus (HBV)-infected individuals in the world. Together with human immunodeficiency virus (HIV) and hepatitis C virus (HCV), HBV is an important agent of post-transfusion infection. Reports of the time course of HBV infection from hospitals relate to the late stage of HBV infection, as patients seek clinical care only when symptoms have developed [1,2]. Therefore, reports of HBV dynamics from the early stages of infection, or in asymptomatic cases, are few in number and usually relate to studies on a small number of experimental animals or seroconversion panels [3].
HBV DNA screening of blood donations is not routinely undertaken in most countries. Japan, however, is an exception, for two main reasons: first, the absence of effective technologies; and second, the licensing of highly sensitive serological hepatitis B surface antigen (HBsAg) screening assays, with subnanogram detectability, is considered by some to eliminate any benefits associated with HBV DNA testing [3–6]. We have previously reported the efficiency of HBV minipool nucleic acid amplification testing (NAT) in Japan [7–11]. Routine testing has identified chemiluminescence immunoassay (CLIA)-negative but minipool NAT-positive donations, with HBV DNA loads of > 10^4 copies/ml in the decreasing phase of HBV DNA. In this report we characterized these CLIA-negative, minipool NAT-positive samples by analysing the sequence of the HBsAg region of HBV DNA. In addition we calculated the doubling time and half-life of HBV DNA in both symptomatic and asymptomatic subjects, and we analysed the factors influencing the dynamics of viral loads from 123 follow-up or stored samples of 277 HBV DNA NAT-positive donations.

Materials and methods

Minipool NAT and serological tests

All blood collected by the Japanese Red Cross (JRC) is assurred for screening based on a questionnaire administered by the JRC blood centres in Japan. Samples from these donations are then screened for a number of infectious agents [7–11].

Serological screening for HBV is carried out by using reverse passive haemagglutination (RPHA) for HBsAg with reagents prepared by the JRC. The sensitivity of RPHA for HBsAg is ≈ 2 ng/ml. Testing for antibody to the hepatitis B core antigen (HBcAg) is undertaken by using an in-house haemagglutination inhibition (HI) technique [12]. Regarding the sensitivity of HI, samples that are diluted 1 : 200 and that are then positive by enzyme immunoassay (EIA) (AxSYM HBc; Abbott Laboratories, Abbott Park, IL) would have a titre of ≥ 2^5 by HI. The HI test detects both immunoglobulin G (IgG) and immunoglobulin M (IgM) against the HBV core antigen. IgM against HBV core antigen was detected by using an EIA (IMxHBc-MM; Abbott Laboratories).

The JRC criteria for serological positivity for HBV is HBsAg positivity by RPHA in initial screening, with this result subsequently confirmed by EIA (AxSYM HBsAg; Abbott Laboratories), and/or an HI titre of antibody to HBcAg of > 2^5 without antibody to HBsAg. When the titre of antibody to HBsAg by RPHA is > 2^4 (= ≥ 200 mIU/ml), donations are accepted for NAT screening, even when the HI titre of HBc antibody is > 2^5 [12].

All agglutination testing was carried out by using an automatic PK7200 (Olympus Co. Ltd, Tokyo, Japan). Reactive results were confirmed by specific inhibition tests. The end-point of the twofold dilution of test samples is expressed as an exponent of 2^n vs. the end titre of the specific inhibition test.

Serologically positive and alanine aminotransferase (ALT)-elevated (> 60 IU/l) donations are excluded from NAT screening [10,11].

The NAT screening system in Japan has been reported previously by Mine et al. [10]. In brief, NAT screening is undertaken by using a multiplex system capable of detecting HBV DNA, HIV RNA and HCV RNA simultaneously to reduce the cost and to ensure that testing is completed within 72 h. Samples are tested in minipools of 50.

Study samples

Two hundred and seventy-seven samples collected between 1 February 2000 and 30 November 2002 were found to be positive for HBV DNA in routine 50-donation minipool NAT. These samples were further tested for HBsAg by EIA (Auszyme II overnight methods; Abbott Laboratories) and by CLIA (PRISM™; Abbott) in order to compare the sensitivity of 50-minipool NAT with that of individual HBsAg testing.

An aliquot of all blood donations is stored at −30 °C or lower for 10 years in two facilities in Japan [10]. In the event that donations are found to be NAT positive in routine minipool testing, the stored samples from the donor are retrieved and retested individually.

We notify HBV NAT-positive donors and attempt to contact them to obtain informed consent for follow-up. The follow-up data on virus loads, ALT levels and levels of serological markers are sent to donors after each blood collection. The interval between blood collections is dependent on the donor.

Sequencing of the HBsAg region

The following primers, based on the HBsAg region, were used for the JRC standard method [11]: S1-1 (sense): 5′-TCTGTCT-ACAGGGGCTTT-3′; nucleotides 192–211, S2-2 (antisense): 5′-CGAACCTGAACAATGCC-3′; nucleotides 685–704, S2-1 (nested sense): 5′-CAAGGTTATGTGCCGTTTG-3′; nucleotides 455–474) and S2-2 (nested antisense: 5′-GGCACTAGAAAATGGGC-3′; nucleotides 668–687).

Sequencing was carried out directly by using the BigDye Terminator Cycle Sequencing Kit and the ABI PRISM 3100 Genetic Analyser (PE Applied Biosystems, Foster City, CA). To analyse the sequences, SEQUENCHER MAC Version 4·1 (Hitachi Software Engineering, Tokyo, Japan) or GENETYX MAC Version 9·0 (Software Development, Tokyo, Japan) were used.

The genotypes of HBV and precore or core promoter mutations were identified and characterized according to Okamoto et al. [13,14].

Fig. 1 The course of acute hepatitis B virus (HBV) infection in follow-up studies of nucleic acid amplification test (NAT)-positive donors. The vertical axis shows the HBV DNA load (copies/ml) and the horizontal axis shows the follow-up (in days) of blood collection. *a, NAT-positive day (day 0); *b, a slight increase is observed just before the peak HBV DNA load is reached; *c, peak HBV DNA load; *d, a slight decrease is observed after the peak HBV DNA load, which is followed by *e, a steep decrease in HBV DNA load; *f, a slight decrease in HBV DNA load is observed after the steep decrease observed in *e; *g, < 100 copies/ml, assumed to be = 60 copies/ml. Doubling times and half-lives, respectively: donor 01141, 2·2 and 1·7 days; donor 00050, 2·1 and 1·8 days; donor 01024, 3·2 and 1·6 days (Fig. 6); donor 01038, 3·3 and 1·9 days; and donor 01063, 15·2 and 1·8 days. The doubling time was calculated at the time of the NAT point *a.

Estimation of the doubling time and the half-life

The doubling time and the half-life were calculated from the slopes of the increasing and the decreasing phases of HBV DNA, respectively. Donors were excluded from the analysis when the interval between successive sampling exceeded 30 days.

The doubling time was measured from the time when the first NAT-positive sample was obtained (Fig. 1*a, day 0). The data for estimating the half-life was measured from the start of the sharp decrease of HBV DNA load (Fig. 1*e), which occurred after the slight decline of HBV DNA around the HBV DNA peak (Fig. 1*d). In the event that the HBV DNA load was < 100 copies/ml, an arbitrary figure of 60 copies/ml [95% CI (confidence interval)] [15] was used for calculating both the doubling time and the half-life. Where HBV DNA testing gave a negative result (below the detection limit) then an arbitrary figure of 1 copy/ml was used.

Qualitative and quantitative detection of HBV DNA

Out of the 277 HBV DNA-positive donations, we were able to perform follow-up or look-back on 192. The qualitative and quantitative detection of HBV DNA by individual NAT was reported previously by Minegishi et al. [11]. In brief, nucleic acid extracts were amplified and titrated by using a sequence detection system (TaqMan, ABI Prism 7700 Sequence Detector; PE Applied Biosystems). The primers and the fluorogenic probes were designed in-house. Primers used for the HBs region were as follows: primer TBF-1 (5′-AGACTCGGTGTTGCTCCTCCCAAG-3′; nucleotides 250–272), primer TBR-1 (5′-TGAGGAGAGAAGGACGGAGT-3′; nucleotides 409–428), and probe TP-02 (5′-TATCGCTGATGTCGCGGTGTT-3′; nucleotides 368–392). Primers used for the HBe region were as follows: primer HB106 (5′-CCCCTATCTATCTAAGTTG-3′; nucleotides 2310–2332) and primer HB105 (5′-TGGATTGCTCAAGG-3′; nucleotides 2463–2482). TP-05 (5′-AAGAAGAAGTTCTAGGCA-3′; nucleotides 2377–2399) was used as the fluorogenic probe. The quantity of HBV DNA was calculated from a working curve (10⁷, 10⁶, 10⁵, 10⁴, 10³ copies/ml) produced by domestic standard samples that were prepared based on an international standard sample (National Institute of Biological Standard and Control, Potters Bar, Hearts, UK). Calculation was carried out by using Sequence Detector version 1·7 (PE Applied Biosystems). The data were the average of quadruplicate tests.

Statistics

Comparisons of quantitative variables in two independent samples were made by using the Mann–Whitney U-test.

Results

Characterization of HBV NAT-positive donations

Eighty-five of the 277 NAT-positive donations were from donors who donated on one occasion only and therefore no follow-up or look-back was possible in these individuals. Of the remaining 192 donations, 125 were from the increasing phase of HBV DNA loads and 45 donations were from the decreasing phase. In 19 donors the stage of infection was not determined because either the period between donations
exceeded 50 days or little difference in HBV DNA loads was observed between samples. Three donors (a 53-year-old man, a 60-year-old man and a 60-year-old woman) were thought to be carriers, because the concentration of HBV was maintained at \( \approx 100 \) copies/ml for > 50 days, IgM antibody to HBeAg was not detected and ALT levels and the IgG antibody titre to HBeAg were normal or low during observation. Thus, 170 donations were suitable for evaluation (Table 1).

Sixty-eight of the 170 evaluable donations were used for calculating the doubling time, 30 were used for calculating the half-life and a further 25 were used for calculating both the doubling time and the half-life (five are shown in Fig. 1). The remaining 47 were not used to calculate the kinetics as the interval between blood collections was longer than 30 days.

The kinetics of acute HBV infection in NAT-positive donors

The calculated doubling times of HBV in the increasing phase and the half-life in the decreasing phase are shown in Figs 2 and 3, respectively. The median doubling time was 2·6 days \((n = 93, 1·3–15·2 \text{ days}, 25\%: 2·2 \text{ days and } 75\%: 3·2 \text{ days})\) and that of the half-life was 1·6 days \((n = 55, 0·9–6·3 \text{ days}, 25\%: 1·3 \text{ days and } 75\%: 2·3 \text{ days})\).

| Table 1 | Hepatitis B virus (HBV) infection phases when nucleic acid amplification testing (NAT) is positive and chemiluminescence immunoassay (CLIA) results are positive |
|---|---|---|---|
| **HBV NAT-positive phase** | **CLIA\(^a\) (HBsAg)** | **Total** |
| One donation | 49 | 36 | 85 |
| Carrier | 0 | 3 | 3 |
| Increase phase | 75 | 50 | 125 |
| Decrease phase | 30 | 15 | 45 |
| Undermined | 9 | 10 | 19 |
| Total | 163 | 114 | 277 |

\(^a\)PRISM\(^\text{TM}\) (Abbott laboratories). HBsAg, hepatitis B surface antigen.

Fig. 2 Distribution of the doubling time of the hepatitis B virus (HBV) in the increasing phase of infection. The doubling time was calculated from the slopes at the nucleic acid amplification test (NAT)-positive points (Fig. 1\(*\), day 0). A HBV DNA load of < 100 copies/ml was assumed to be 60 copies/ml, and a negative HBV DNA load (below the detection limit) was assumed to be 1 copy/ml. Data obtained from samples collected at intervals of > 30 days were excluded. The median was 2·6 days \((25–75\%: 2·2–3·2 \text{ days} \ (n = 93)). Six samples had a doubling time of longer than 6 days \((6·2, 6·4, 6·6, 7·7, 9·1 \text{ and } 15·2 \text{ days})\).

Fig. 3 Half-life of hepatitis B virus (HBV) in the decreasing phase. The half-life data were measured at the point when the HBV DNA load started to show a sharp decrease (Fig. 1\(*\), e). A HBV DNA load of < 100 copies/ml was assumed to be 60 copies/ml, and a negative HBV DNA load (below the detection limit) was assumed to be 1 copy/ml. Data obtained from samples collected at intervals of > 30 days were excluded. The median was 1·6 days \((25–75\%: 1·3–2·3 \text{ days} \ (n = 55)). Two samples had a half-life of longer than 4·0 days \((6·1 \text{ and } 6·3 \text{ days})\).
Ninety-three donations were given during the increasing phase and 55 were given in the decreasing phase. Donations were classified according to gender and age of the donors, genotype and whether they were wild type or mutant. The results are shown in Table 2. No significant differences were observed regarding gender, age and the presence or absence of a mutation. However, significant differences (\( P > 0.5 \); Mann–Whitney \( U \)-test) were identified in doubling time and half-life for those that were genotype A as opposed to genotype B. The doubling time of genotype A is shorter, and the half-life of genotype A is longer than that of genotype B. No significant differences were observed between wild-type strains and mutant strains. Significant differences (\( P > 0.5 \); Mann–Whitney \( U \)-test) were, however, observed between core promoter mutants and precore mutants. Core promoter mutations involved a change from A to T at nucleotide 1762 and/or from G to A at nucleotide 1764 (wild-type A, mutation T at nucleotide 1762; and wild-type G, mutation A at nucleotide 1764) [14]. The precore mutation involved a change from G to A at nucleotide 1896 (wild-type G, mutation A) [13]. The doubling times of promoter mutants were 2·8, 2·3, 2·2, and 2·2 days; those of precore mutants were 9·1, 7·7, 3·9, 3·6, 3·4, and 3·2 days.

Comparison of the sensitivity of a 50-minipool, 0·2-ml multiplex NAT for HBV with that of HBsAg testing by CLIA

About 40% of the minipool HBV NAT-positive samples were negative by CLIA, as described previously [11]. In the increasing phase, 40%, and in the decreasing phase, 33%, of HBV NAT-positive donations were negative by CLIA (Table 1 and Figs 4 and 5).

The typical course of acute HBV infection in the increasing phase (donor 01024) is shown in Figs 1 and 6 and the sequences of the hepatitis B surface antigen (HBsAg) region of donors 01024 and 00077 are shown in Fig. 8.

### Table 2 The effect of gender, mutations, genotypes and age on the doubling time and half-life of hepatitis B virus (HBV) DNA loads after acute HBV infection

<table>
<thead>
<tr>
<th>Gender</th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
<th>Wild-type</th>
<th>Mutant</th>
<th>Genotypes</th>
<th>Age</th>
<th>16–20</th>
<th>21–25</th>
<th>26–30</th>
<th>31–60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>93</td>
<td>59</td>
<td>34</td>
<td>81</td>
<td>12</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>28</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>Median (days)</td>
<td>2·6</td>
<td>2·5</td>
<td>2·8</td>
<td>2·6</td>
<td>3·0</td>
<td>2·1*</td>
<td>2·9*</td>
<td>2·6</td>
<td>2·8</td>
<td>2·7</td>
<td>2·4</td>
</tr>
<tr>
<td>Number</td>
<td>55</td>
<td>28</td>
<td>27</td>
<td>40</td>
<td>15</td>
<td>4</td>
<td>6</td>
<td>45</td>
<td>5</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>Median (days)</td>
<td>1·6</td>
<td>1·8</td>
<td>1·7</td>
<td>1·8</td>
<td>1·6</td>
<td>2·3*</td>
<td>1·3*</td>
<td>1·7</td>
<td>1·7</td>
<td>1·6</td>
<td>1·9</td>
</tr>
</tbody>
</table>

*Significant difference (\( P > 0.5 \), Mann-Whitney \( U \)-test) between genotype A and genotype B, in both doubling time and half-life.

Mutation from G to A at nucleotide 1896 of the precore region (wild-type G; mutation A) [13].

Mutation from A to T at nucleotide 1762 and/or from G to A at nucleotide 1764 of the core promoter region (wild-type A, mutation T at nucleotide 1762; and wild-type G, mutation A at nucleotide 1764) [14].
with a concentration of $> 10^4$ HBV DNA copies/ml were found to be negative by CLIA in the decreasing phase of HBV DNA loads (Fig. 5). The time course of one of these, donor 02084, is shown in Fig. 7. The level of HBsAg remained below the limit of detection. Antibody to HBcAg was not detected until after $\approx 10$ days.

**Sequencing of the HBsAg region of CLIA-negative, NAT-positive samples**

The sequences of the HBsAg region from nucleotide 475 to nucleotide 667 is shown in Fig. 8. The sequences of samples that were CLIA negative and NAT positive in the increasing phase (01024 and 00077) and in the decreasing phase (02084, 01078 and 00201) are shown in Fig. 8. Also shown is the sequence of a donor (00109) with borderline positivity in CLIA (s/co 1·27), and who was NAT positive with a high HBV DNA load (590 000 copies/ml) in the decreasing phase.

It has been reported that mutations in the ‘a’ epitope (codons 122–147) correlate with the absence of detectable anti-HBsAg, especially when analysed by CLIA or EIA by using monoclonal antibodies [16–26]. The hot-spot escape mutations have been previously reported, as follows: L110R [16], P120Q/S [17,18], T/I126N/V [17,19,20], P127S [18], Q129R [20], M133V [21], Y/F134S, F134Y [16,17], P135L [20], S136F/T [20], K141E [22] P142L [16], D144A/E [16,17,23], and G145R [19,20,23–26]. Codons 122 and 160 were the determinants of subtypes: d/y is K/R and r/w is R/K, respectively [27]. No escape mutations were observed in the six donors investigated during this study.
Discussion

In acute HBV infection, the ability to estimate the day of infection and the day of disappearance of HBV DNA from the circulation may assist in look-back studies and in providing advice to blood donors. In most cases it is difficult to identify the specific date of infection.

In our study, the date of polymerase chain reaction (PCR) negativity (an arbitrary figure of 1 copy/ml) in the phases of increased and in decreased HBV DNA loads was estimated as...
follows. Unless storage samples from previous donation were available then an estimate was made using the viral load on the NAT-positive donation and known doubling times. The time-period from being NAT positive to having < 100 copies/ml differed markedly, as shown in Fig. 1. The median time from the day of PCR negativity to reaching the HBV DNA peak in the increasing phase was estimated to be 72 days \((n = 54, 25–228\) days, 25%: 54 days, 75%: 83 days). Similarly, the median time from the HBV DNA peak to the day of PCR negativity in the decreasing phase was estimated to be 40 days \((n = 42, 18–206\) days, 25%: 27 days, 75%: 63 days). Then, the median time from PCR negativity to the increased phase of HBV DNA loads that in the decreasing phase is estimated to be = 112 days (precise data were not shown) (Fig. 9).

If a model donor (weight 65 kg and circulation blood volume 5 l) is infected by ≥ 1 copy of HBV, then the period from the day of infection to the day of PCR negativity \((\geq 1\) copy/ml) would be 31-9 days using the median doubling time of 2-6 days. Then, the period from HBV infection to the peak virus load is in the order of 104 days. The period from the day of PCR negativity to the day of disappearance of HBV from the circulation in the decreasing phase might be estimated to be 19-7 days using the median half-life of 1-6 days. Then, the period from the day of HBV DNA peak to the day of disappearance of HBV is = 60 days. Thus, the time from infection to cure would be = 164 days (Fig. 9).

The doubling time has been previously reported as 4 days by Busch et al. [28], as 3-7 days by Whalley et al. [1], as 2-8 days by Busch [29] and as 2-56 days by Biswas et al. [3]. The half-life was reported to be 1-6 days at the beginning and 4 days at the end by Chulanov et al. [2] and 3-7 days by Whalley et al. [1]. Our results generally concur with these published data despite differences in sample number, intervals between donations and HBV genotypes.

Busch et al. introduced the concept of a ‘preramp-up’ period before the period of exponential increase in HBV DNA – the ‘ramp-up period’ [28,29]. The doubling time might be expressed as the sum of replication and degradation of HBV. It might be reasonable to assume that there are different immune responses, i.e. the natural immune response during the early stage of infection and the specific immune response during the late stage of infection. If this is the case then the doubling time may be different in the ‘preramp-up’ period and ‘ramp-up’ phases. However, as the 95% CI of HBV DNA was 60 copies/ml [15], we were not able to investigate the ‘preramp-up’ stage. For example, in one case we had three stored samples in the 3, 6 and 8 weeks before the NAT-positive index donation. HBV DNA was negative on all three samples. In this report, we estimated the day of PCR negativity by applying the same doubling time at the exponentially increasing phase or NAT-positive point to the early stage of infection, the ‘preramp-up’ stage. The question as to whether or not we should consider different doubling times in the ‘preramp-up’ stage will need to be clarified by experiments in animals in which the day of infection and the infectious concentration of HBV are known.

We identified two donors with slow doubling times (7-7 and 15-2 days, these slow doubling times persisted for longer than 50 days from NAT-positive to peak viral loads), even in the exponentially increasing period (Fig. 1, see donor 01063). Similar results have been reported by Biswas et al. [3]. In four donors there was a phase of slow increase at = 100 copies/ml followed by an exponential rapid increase.

Whalley et al. [1] has described three stages of the declining phase. The first occurs around the peak HBV DNA load.

Window period and kinetics of HBV DNA

The efficacy of HBV DNA NAT screening using minipools has already been reported [1]. The relative merits of highly sensitive HBsAg assay methods (CLIA) and minipool NAT in shortening the window period have been debated [3–6]. This debate may be appropriate when considering the increasing phase of HBV DNA. However, our data show that HBV NAT screening can detect HBV DNA in both early (serological window period) and late stages of acute HBV infection. The window period risk associated with the increasing phase is greater than that seen in the decreasing phase as HBcAg antibody screening will reduce the risk in the latter. We exclude blood containing high-titre HBcAg antibody without HBsAg antibody, which may contain HBV DNA. By using this procedure, no post-transfusion infection of HBV was observed among 908 transfused individuals [36].

However, in some subjects, HBV loads of > 10^4 copies/ml were seen before HBcAg antibody became detectable. In these individuals, HBV DNA testing was superior to CLIA (Figs 5 and 7). In the decreasing phase, HBsAg might disappear before HBV DNA, or indeed HBsAg might not be detectable because of the presence of escape mutants. We therefore sequenced samples that were CLIA negative but which had > 10^5 copies/ml of HBV DNA. In the six available cases no escape mutations were identified.

A number of possible explanations might explain the discrepancies in the sensitivity and effectiveness of CLIA and minipool NAT. The first relates to differences in samples. We used donor samples in our studies in both the increasing and decreasing phases of HBV. In previous reports, seroconversion panels have been used to assess the increasing phase [3]. The second relates to differences in the epidemiology of HBV genotypes between Japan and the USA; genotype C predominates in Japan, whereas genotypes A and D are mainly observed in the USA and Europe. It has been reported that HBV genotype may influence the clinical relevance of viral dynamics [34,35]. The rate of HBsAg/HBV DNA production/disappearance may be different among genotypes and strains of wild-type/mutations during the early stages of infection.

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