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Two HBV DNA+/HBsAg− blood donors identified by HBV NAT in Shenzhen, China

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ABSTRACT

Background: In order to further improve blood safety, mini-pool (MP) nucleic acid testing (NAT) was implemented to screen samples negative for hepatitis B surface antigen (HBsAg), anti-hepatitis C virus (anti-HCV), anti-human immunodeficiency virus (anti-HIV), syphilis (anti-Treponemal antibody) and with normal ALT.

Study design and methods: From August 2006 to February 2008, 41,301 donations were screened using commercial HIV/HCV RNA and HBV DNA Real-Time PCR NAT assays in pools of 8. Reactive pools were re-tested as individual samples using the appropriate screening test and confirmed using an alternate commercial NAT assay. Donors reactive on both NAT assays were considered 'confirmed' positive for the virus concerned and recalled for additional follow-up testing and counseling.

Results: Of the 41,301 samples screened, no HIV or HCV RNA-positive/seronegative donations were detected but two HBV DNA positive/HBsAg negative blood donors (Donors 1 and 2) were identified. Their respective hepatitis immunological markers were: Donor 1 – anti-HBc positive/anti-HBe positive/HBeAg negative/ALT normal and HBV DNA viral load of 112 IU/ml; Donor 2 – anti-HBc positive/anti-HBe negative/HBeAg negative/ALT normal and HBV DNA viral load 2750 IU/ml.

Conclusions: MP NAT identified two HBsAg negative donors with presumed occult infection but no HIV or HCV seronegative/NAT positive (yield) donors. The HBV yield rate of 1 in 20,650 (95%CI – 1 in 5663 to 1 in 75,303) is comparatively high, exceeds the predicted rate based on previous modeling for the population and demonstrates the incremental blood safety value of NAT in countries where HBV is highly epidemic. The low viral load of the two yield samples underscores the importance of optimizing the sensitivity of the HBV NAT assay selected for screening.

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1. Introduction

Nucleic acid testing (NAT) for HIV and HCV RNA has been widely implemented for donor screening based on its improved ability to detect early infection [1]. A recent multi-Chinese blood center evaluation of HIV-1/HCV NAT confirmed its potential to reduce the residual risk of HCV in China [2]. Although HBV NAT has now been implemented in some countries there are no published

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studies assessing its performance in a Chinese donor population.

The potential for HBV DNA screening to improve the detection of hepatitis B virus (HBV) infection is important as HBV remains a major global health challenge with an estimated 350 million people worldwide chronically infected with the virus. Although prevalence of hepatitis B surface antigenemia has declined as a result of the effectiveness of its vaccination program, HBV infection is still endemic in China. The prevalence of hepatitis B surface antigen (HBsAg) was estimated as 7–9% in the 1980s and 50–60% of adults showed evidence of present or past HBV infection [3]. The latter makes the use of anti-HBc testing impractical as a donor screening test in China because the predicted deferral rate of approximately 40% for anti-HBc positive donors could potentially compromise the sufficiency of the blood supply [4].

Occult HBV infection is defined by serologically undetectable HBsAg despite the presence of HBV DNA in the bloodstream or liver [5]. Although the mechanism and clinical implications remain unclear, occult HBV infection has its own risks of disease transmission and may contribute to acute exacerbation and development of HBV-associated diseases such as hepatic inflammation, cirrhosis, and hepatocellular carcinoma (HCC) [6–8]. Occult HBV infection is common in subjects with risk factors for HBV infection. For instance, HBV DNA was detected in approximately 36% (12 of 33) of haemodialysis patients with chronic hepatitis C virus (HCV) by polymerase chain reaction (PCR) and in 45% (81 of 180) of HCV RNA-positive injection drug users by nested PCR [9,10]. Since viral DNA levels in occult hepatitis B are generally very low, the sensitivity of the HBV DNA assay applied is critically important. Several reports have demonstrated HBV DNA levels in serum or liver are usually less than 10^4 copies/ml in occult hepatitis B by nested or Real-Time PCR [10–16].

Many studies have shown that the prevalence of occult HBV infection in HBV endemic areas such as China, Japan, and Taiwan is higher than in Western countries, and the differences in its prevalence might be closely related to the endemicity of HBV infection [11,17]. Therefore, the majority of the population in countries with a high HBsAg prevalence rate has already been exposed to HBV and may have occult HBV infection [18].

The aim of this study was to assess the frequency of HBV, HIV and HCV serologically negative but MP NAT positive (yield) donations and to follow up these donors to determine their infection status.

2. Materials and methods

2.1. Blood donors

The study was conducted in two non contiguous periods; from August 2006 to December 2006 and July 2007 to February 2008, during which all blood donations collected in Shenzhen, China were tested in parallel for anti-HCV (Ortho Clinical Diagnostics, Raritan, NJ and Lizhu Diagnostics, Zhuhai, China), HBsAg (Murex Biotech. Ltd., England and Shanghai Kehua Diagnostics, Shanghai, China), and anti-HIV1/2 (Vironostika HIV Uni-Form II Plus O and Lizhu Diagnostics, Zhuhai, China), Syphilis (Lizhu Diagnostics, Zhuhai, China and Rongshen Trust, China) and alanine aminotransferase (ALT) with kinetic methods (Roche Refletron and AusBio Biotech., China). Only donors negative for serological viral markers, syphilis and ALT levels within the normal range for blood donors were analyzed in this study. All donors were voluntary and non-remunerated. Each was medically assessed and denied any known risk factors for viral infection listed in the pre-donation questionnaire.

2.2. NAT methods

2.2.1. Screening

All donations were initially tested using commercial Real-Time HIV RNA, HCV RNA and HBV DNA NAT assays (Shanghai Kehua, Shanghai, PRC).

2.2.1.1. RNA/DNA extraction. Master pools of 8 donations were created by combining 150 μL of plasma from each donation. RNA/DNA was extracted using a magnetic bead adsorption method included in the test kit. Briefly, viral particles were pelleted by high speed centrifugation (15,000*g* for 60 min) of the 1200 μL master pool. The pellet was resuspended in lysis reagent and magnetic beads were added to absorb target DNA/RNA. Subsequent washing steps removed unbound material and impurities before elution resulted in a final volume of 80 μL of concentrated DNA/RNA.

2.2.1.2. Amplification/detection. The 15 μL of extracted RNA/DNA was amplified in three separate (i.e. one for each virus) reaction wells containing the corresponding HIV, HCV or HBV specific primers and their matching fluorescence labeled probe. Real-Time PCR amplification for 50 cycles was performed on each reaction mixture using the ABI 7300 Real-Time PCR system (Applied Biosystems, Foster City, USA).

2.2.1.3. Reactive pools. RNA/DNA from each of the eight samples within a reactive pool was extracted and re-tested using the specific primer/probe combination that reacted initially with the pool (i.e. HIV, HCV or HBV). Reactive individual samples were referred for confirmatory testing.

2.2.1.4. Analytical sensitivity. The manufacturer claims an analytical sensitivity (95% limit of detection – LOD) of 18.8 IU/ml for HBV DNA, 75 IU/ml for HCV RNA and 75 IU/ml HIV RNA for individual donation testing. Assuming a linear relationship between dilution and concentration, this translates to a LOD for individual samples within a pool of eight of approximately 150 (i.e. 18.8×8), 600 and 600 IU/ml for HBV, HCV and HIV, respectively.

3. Confirmatory testing

Screening test reactive samples were retested (undiluted) using the appropriate Cobas Taqman PCR assay (Roche Molecular Diagnostics, Raritan, USA). Reactive samples were considered as 'confirmed' positive for either HBV, HIV or HCV infection dependent on the reactive confirmatory assay.

3.1. Follow-up testing

Donors of confirmed positive donations were recalled and invited to attend for weekly follow up samples. Ultimately only HBV follow-up testing was required as there were no HIV or HCV confirmed positive samples detected.

For HBV, index and follow up samples were re-tested using the Cobas Taqman HBV PCR with a claimed individual donation (ID) LOD at of 6 IU/ml [19]. Furthermore, in order to quantitate the viral load, samples were initially concentrated by ultracentrifuge and then subjected to individual donation HBV PCR (HBV PCR fluorescence quantitation kit, PG Biotech., PRC). Where HBV DNA viral load was sufficient the HBV genotype was resolved using an HBV genotyping EIA (Institute of Immunology Co. Ltd., Japan).

3.2. HBV serological markers

The presence or absence of serological HBV markers was determined in confirmed HBV DNA-positive index and follow up samples. For HBsAg, the Abbott Murex HBsAg EIA (Murex Biotech Ltd., Dartford, UK) and Shanghai Kehua HBsAg EIA (Shanghai Kehua Biotechnology Diagnostics, PRC) were used in parallel. For anit-HBe, HBeAg, anti-HBs and anti-HBc Shanghai Kehua Biotechnology Diagnostics assays were used in accordance with the manufacturers' instructions.

3.3. Counseling of yield donors

Donors with detectable HBV DNA were re-questioned for risk factors by a physician. In addition they were asked whether or not they had been vaccinated for HBV, and if so when.

3.4. Statistical methods

Ninety five percent confidence intervals (95%CI) for the observed HBV yield rate were derived using the score method as described by Agresti and Coull [20].

4. Results

4.1. Seronegative/MP NAT positive (yield) rate

A total of 41,301 samples from HBsAg, anti-HIV, anti-HCV seronegative blood donations were tested for HBV DNA, HIV/HCV RNA in pools of 8. At the end of July 2007 and again at the end of August 2007, two HBV DNA+/HBsAg− samples, Donor 1 (first time donor aged 34) and Donor 2 (first time donor aged 25) respectively were detected (refer Table 1 for details). This gives an observed HBV yield rate of 1 in 20,650 (95%CI – 1 in 5663 to 1 in 75,303). No HCV RNA-positive/seronegative or HIV RNA-positive/seronegative donations were detected.

4.2. Yield donors

Donor 1's index HBV DNA+/HBsAg− sample had very low viral load (112 IU/ml). DNA was detected in the first two follow-up (FU) samples both with similarly low viral loads which precluded sequencing. DNA was subsequently undetectable in the last two FU samples. He was vaccinated after his last FU sample (EngerixB™, GlaxoSmith Klein Biologicals, Belgium), however one month after the vaccination his anti-HBs level remained undetectable, while his anti-HBc was still positive.

During his initial interview Donor 2 volunteered that he had a previous history of hepatitis B infection (HBsAg, HBeAg and anti-HBc positive) and had been prescribed lamivudine for a 2 year period. At the conclusion of treatment his HBsAg and HBeAg were undetectable and despite being vaccinated at the time his anti-HBs remained undetectable. One year subsequently he donated at Shenzhen blood center for the first-time and was identified as HBV DNA+/HBsAg−. He attended twice for follow-up testing and HBV DNA was detectable in both samples at similar viral loads (2750 and 2860 IU/ml). Although he was HBsAg negative on his index sample, HBsAg was detected in his second FU sample. This allowed for genotyping which subsequently determined him to be genotype B.

Table 1
Index and follow-up (FU) molecular and serological HBV markers for Donors 1 and 2.

	HBV DNA screening[a]	HBV DNA confirmatory[b]	Viral load (IU/ml)	Genotype	HBV markers[d]				
					HBsAg	Anti-HBc	Anti-HBs	HBeAg	Anti-HBe
Donor 1									
Index	REA	POS	112	U	NEG	REA	NEG	NEG	REA
FU 1	REA	POS	114	U	NEG	REA	NEG	NEG	REA
FU 2	REA	POS	175	U	NEG	REA	NEG	NEG	REA
FU 3	NR	NEG	NA	NA	NEG	REA	NEG	NEG	NR
FU 4	NR	NEG	NA	NA	NEG	REA	NEG	NEG	NR
Vaccinated[c]	NR	NEG	NA	NA	NEG	REA	NEG	NEG	NR
Donor 2									
Index	REA	POS	2380	B	NEG	REA	NEG	NEG	NR
FU 1	REA	POS	2750	NA	NEG	REA	NEG	NEG	NR
FU 2	REA	POS	2860	NA	POS	REA	NEG	NEG	NR

REA – reactive; POS – positive; NR – non reactive; NEG – negative; NA – not applicable; U – unquantifiable.
[a] Shanghai Kehua HBV DNA PCR reactive in a pool of 8 and subsequently reactive at individual donation.
[b] Roche Taqman HBV DNA.
[c] Donor was vaccinated for HBV subsequent to FU 4.
[d] Refer 'Section 2' for assay details.

5. Discussion

In this study of more than 40,000 blood donations from Shenzhen, commercial HIV-1, HCV and HBV NAT assays detected two HBsAg negative/HBV DNA positive ('Yield') donations but no HIV or HCV seronegative/RNA positive donations. The interdiction of two potentially infectious donations confirms the value of HBV DNA screening in donor populations like Shenzhen, where the HBV donor prevalence is high (approximately 1% based on pre-donation HBsAg testing of prospective donors – data not shown). This is particularly important given that anti-HBc screening, the alternative approach to HBV blood safety used in countries such as France, Germany, the United States, and Canada, could not be considered in Shenzhen because the predicted deferral of over 40% of anti-HBc-reactive donations may potentially compromise the sufficiency of the blood supply [4].

In a previous study of Shenzhen blood donors we predicted yield rates for MP NAT based on 2003/2004 serological screening data of 0.65 (0.09–1.39), 14.7 (8.0–21.3) and 9.5 (8.3–10) per million donations for HIV, HCV and HBV, respectively [4]. The observed HBV yield rate of 48.4 (95%CI – 13.3–176.6) per million donations in fact exceeds our predicted yield for both MP and even ID NAT (28.3 [27.2–29.2]) per million donations, although the confidence intervals do overlap for ID NAT. The lower boundary of our yield predictions for HIV and HCV translate to rates of 1 in 11.1 million and 1 in 125,000 donations. Therefore our finding of zero yields for both HIV and HCV is not inconsistent with our prediction given the sample size (41,301) does not exceed either rate for one yield case. There are limited published studies documenting yield rates for MP HBV NAT in countries with intermediate/high HBV prevalence. However, our HBV yield rate of 1 in 20,650 (95%CI – 1 in 5663 to 1 in 75,303) is comparable to the rates observed in such countries including Italy and Spain (combined yield rate of 1 in 38,000) [21], Hong Kong (1 in 5000 – pools of 6) [22] and Japan (1 in 52,788 – pools of 50) [23].

Follow-up testing of the two yield donors indicated that both represented occult infections. Both had comparatively low viral loads (less than 10^4 copies/mL) which is consistent with the findings of others in respect of occult carriers and underscores the need to implement highly sensitive HBV DNA assays in order to effectively interdict them [22,24–27]. While the two yield donors in this study were detected using pools of 8, the impact of pooling on yield rate in other studies suggests that smaller pool sizes may have lead to a higher yield rate. For example in a study of 10,397 Hong Kong blood donors, Margaritis et al. [22] reported that ID NAT screening using the Cobas Taqscreen MPX test detected three additional HBV yield cases in the same donor samples which were not identified by initial screening using pools of 6.

Lookback studies of Japanese donors indicate that the HBV transmission risk associated with components from donors with occult infections is approximately 10 fold lower than those in the pre-HBsAg and/or MP NAT window phase. Importantly, in this lookback study Satake et al. [26] identified one of 33 (3%) HBsAg Negative/HBc positive/MP NAT positive components that resulted in HBV infection in the recipient. Furthermore, Inaba et al. [28] reported clinical HBV in recipients of some components from an HBsAg negative/anti-HBc positive donor with fluctuating DNA levels. These findings demonstrate that despite the lower infectivity risk in comparison with acutely infected donors, components from donors with occult infection are capable of transmitting HBV infection and therefore from a transfusion safety perspective it is important to identify and exclude them.

Interestingly one of the yield donors in this study had previously been diagnosed and treated for HBV infection and despite this did not disclose this information when he donated. During the follow up interview he indicated that this related to his assumption that a negative HBsAg test reported to him during his treatment indicated a full recovery. Had he not been tested for HBV DNA his blood would have been acceptable for transfusion. Notably he was HBsAg positive at his second follow up sample demonstrating the difficulty in consistently detecting such occult infections when reliant on HBsAg screening alone. Furthermore this highlights the need to ensure that donors are aware of the importance of disclosing a history of HBV infection prior to donation. This case suggests a need to improve understanding of this message among potential donors in Shenzhen although further investigation is required to determine if this limitation extends to other Chinese donor populations.

In summary, HBV MP NAT identified two HBsAg negative donors with presumed occult infection but no HIV or HCV seronegative/NAT positive (yield) donors. Consistent with other studies of occult HBV infection in blood donors, both had low viral loads. Our study indicates that a sensitive HBV MP NAT assay can interdict potentially infectious donations and consequently reduce the residual risk of transfusion transmitted HBV infection in Shenzhen, China. Given the lack of yield donors for HIV and HCV we were unable to make conclusions in respect of the value of NAT for either of these viruses in our donor population.

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