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Evaluating the cost-effectiveness of low-level HBV DNA screening in occult hepatitis B infection donors: A study from Shandong Blood Center, China

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ABSTRACT

Objective: This study aimed to assess the efficacy of individual-donation nucleic acid testing (ID-NAT) in detecting occult hepatitis B virus infection (OBI) among *anti*-HBc positive blood donors, compared to minipool nucleic acid testing (MP-NAT).

Methods: The present study analyzed data from the Shandong Blood Center in China during the period from January 2018 to June 2022, where HBsAg-negative blood donors were screened using the 6-sample minipool nucleic acid testing (6-sample MP NAT) method. NAT-positive samples underwent subsequent *anti*-HBc and *anti*-HBs testing. Approximately 5000 samples that passed the nucleic acid mixing test were randomly selected for *anti*-HBc testing, and over 100 *anti*-HBc positive samples underwent individual donor nucleic acid testing (ID-NAT). Any HBV DNA positive samples detected by ID-NAT were subsequently confirmed using alternative nucleic acid testing methods.

Results: Among 220,445 HBsAg-negative blood donors, the positivity rate of HBV DNA detection using the 6-sample minipool nucleic acid testing (MP NAT) method was found to be 0.031% (69/220,445). Of the 67 HBV DNA positive samples, 55 (82.09%) and 25 (37.31%) were found to be positive for *anti*-HBc and *anti*-HBs, respectively, using the supplementary chemiluminescent microparticle immunoassay (CMIA). Among the 4797 HBsAg-negative/MP NAT-negative samples, 909 (18.95%) tested positive for *anti*-HBc. Further NAT testing was performed on 164 arbitrarily selected *anti*-HBc-positive/MP HBV DNA-negative samples, revealing a HBV DNA positivity rate of 1.22% (2/164).

Conclusion: Using individual donation nucleic acid testing can significantly increase the detection rate of occult hepatitis B virus infection in *anti*-HBc-positive blood donors, resulting in a detection rate of 0.22% (1.22×0.1895). This rate is 8.10 times higher than the detection rate achieved by mixed testing methods (0.031%) [calculated as $(0.22 + 0.031)/0.031$]. Therefore, it is recommended to perform single HBV DNA testing on *anti*-HBc-positive blood donors, discard plasma

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with weakly positive or negative *anti*-HBs but positive *anti*-HBc, or avoid transfusing *anti*-HBc-positive plasma to recipients with weakly positive or negative *anti*-HBs to prevent HBV infection.

1. Introduction

To minimize the residual risk of transfusion-transmitted infections, several blood centers in China initiated a pilot program of nucleic acid testing (NAT) in early 2010. This screening method is more sensitive than the conventional methods for detecting hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus 1/2 (HIV 1/2). By 2015, China had achieved full coverage of NAT screening for all unpaid blood donors. To control costs, a common practice in China is to perform NAT screening by pooling 6 or 8 blood samples together. Liu et al calculated the total number of NAT screenings performed in China using NAT reagents from 2010 to 2015, which utilized eight different reagents [1]. Among the top four reagents utilized, the Procleix Ultrio Assay had a utilization rate of 40.19% for single-sample testing, while Roche's 6-pooling had a utilization rate of 33.17% (Cobas Taqscreen MPX at 21.74%, Cobas Taqscreen MPX Test, Version 2.0 at 11.43%), Haoyuan's 8-pooling had a utilization rate of 12.21%, and Kehua's 8-pooling had a utilization rate of 12.06%. The second, third, and fourth reagents were all based on pooling methods, and their combined utilization rate exceeded 57%. Although the latter three NAT systems can also be used for single-sample testing, the cost is 6–8 times higher than that of the pooling method. The sensitivity of pooling analysis is much lower than that of single-sample testing, for example, Roche's NAT system has a limit of detection of 2.3 IU/mL for single-sample testing, which would translate to approximately 14 IU/mL for 6-pooling testing. Currently, the blood supply and demand in China are tight, with a high prevalence of previous HBV infection, making it difficult to eliminate *anti*-HBc positive blood. In addition, NAT pooling screening cannot detect occult HBV infection (OBI) with low viral load (2.3–14 IU/mL), which poses a risk of infection to susceptible individuals. We evaluated the proportion of these low viral load HBV infections in eligible blood donors and the screening cost, and discussed the optimization of screening strategies by adding *anti*-HBc testing. It is worth noting that this study is part of a larger body of research on HBV DNA testing of blood donors in China. Numerous similar studies have been conducted and published previously. However, this study focuses specifically on the cost of testing a sample with low levels of HBV DNA, an area that has been rarely reported on in previous studies.

2. Materials and methods

2.1. Blood samples

From January 1st, 2020 to June 30th, 2022, a total of 220,445 blood donor samples were collected from the Shandong Blood Center and tested using the Roche NAT system. Prior to sample collection, all donors provided informed consent for blood donation and underwent a health history questionnaire, physical examination, ALT rapid test, ABO blood group test, HBsAg rapid test non-responsiveness test, and hemoglobin test to ensure their eligibility. Blood samples were stored in a refrigerator at a temperature of 2~8 °C after collection. Nucleic acid samples were centrifuged within 4 h, while ELISA and blood group samples were centrifuged within 12 h. The ALT rate method, blood group forward and reverse typing, ELISA, and NAT tests were completed in the laboratory within 72 h.

2.2. ELISA screening

The blood samples were assayed for HBsAg, *anti*-TP, *anti*-HCV, and HIV/*anti*-HIV through the use of ELISA reagents obtained from two distinct manufacturers. Any samples that were negative with either ELISA reagent or positive with one reagent underwent nucleic acid testing (NAT).

2.3. Nucleic acid amplification testing (NAT) assays

In the initial detection mode of Roche NAT, 167 μL of plasma were extracted from 6 tubes using the STAR sampling instrument and placed into an S-tube, resulting in a total volume of 1.002 mL as a reaction pool. The plasma was then extracted and amplified using the Roche Cobas s 201 system (Cobas TaqScreen MPX test, version 2.0, USA). If the mixed sample tube showed a positive result, 1 mL of plasma was taken from each corresponding tube and tested individually, with the result of the single sample test serving as the final result of routine testing. Roche analyzed the WHO viral (NIBSC code 97/746) standard in single-test mode with a detection volume of 1.0 mL, and the results showed a detection sensitivity of 2.3 IU/mL (average 95% LOD). In addition, 1 IU/mL is equivalent to 5.6 copies/mL. In Roche nucleic acid testing, the critical criterion for determining a negative result is a cycle threshold (CT) value exceeding 60, indicating the absence of amplification within the specified number of cycles. To ensure quality control, we incorporated three weak positive control sera in each testing batch, including HBV DNA, HCV RNA, and HIV RNA control materials. The concentration of the HBV DNA control material was maintained at 50 IU/mL. Over the course of 19 testing batches conducted in 2023, the average CT value was determined to be 33.5 (Due to a six-fold dilution of the 50 IU/ML control material, the actual concentration is 8.33 IU/ML). Notably, for the negative control, the CT value surpassed 60, signifying the absence of amplification.

2.4. Anti-HBc screening

Some samples with qualified test results were randomly selected for *anti*-HBc ELISA (Wantai Diagnostics, Beijing, China) screening test , all *anti*-HBc positive samples were retested for *anti*-HBc by another domestic EIA kit (InTec PRODUCTS, INC) , only samples reactive to both tests were considered as *anti*-HBc positive samples.

2.5. NAT single-sample testing

Random selection of *anti*-HBc positive samples for Roche NAT single-sample testing.

2.6. NAT test result review

Roche single-sample test positive samples were further reviewed in both single-sample and mixed-sample modes using the Huayimei detection system (1.2 mL, LOD: 4.2 IU/mL for HBV DNA, 95% confidence interval: 2.0–2.8 IU/mL). A positive result in either Huayimei test pattern was considered positive, a negative result was considered indeterminate, and an indeterminate result was considered negative, and such samples were not included in this study.

2.7. NAT quantitative test

NAT positive samples were sent to Jinan KingMed Laboratory Center and subjected to quantitative detection of HBV DNA using the cobas 4800 system (Roche PCR-fluorescence method).

2.8. CMIA testing

We selected samples with HBV DNA mixed-sample mode positive and single-sample mode positive reactions, and performed HBsAg neutralization testing, HBsAg, *anti*-HBs, and *anti*-HBc CMIA testing using the Abbott Architect i2000 chemiluminescence detection system. Additionally, we repeated testing on some of the samples using HBsAg, *anti*-HBs, and *anti*-HBc CMIA reagents produced by Wantai (Beijing, China).

2.9. Statistical analysis

Statistical analyses were performed using SPSS 22.0 software. Differences between groups or years were assessed using the chi-square test, and statistical significance was defined as $p < 0.05$.

3. Results

3.1. Total number of Roche HBV NAT blood donation tests

Between January 2018 and June 2022, a total of 220,445 samples from seronegative donors at the Shandong Blood Center were screened using Roche NAT. Over these five years, the positive detection rate of Roche HBV DNA has increased annually, from 0.023% in 2018 to 0.062% in 2022, with an average annual detection rate of 0.031%. No significant differences were observed between groups ($\chi^2 = 6.784$, $p > 0.05$) (Table 1) .

3.2. Anti-HBc screening

4797 samples with qualifying test results were randomly selected and subjected to additional testing with the *anti*-HBc ELISA test, which revealed a positive rate of 18.95% (Table 2).

Table 1
Roche HBV DNA test results (n = 220,445) .

	Number of test samples	Number of positive pools of 6	Number of HBV DNA-positive samples upon retesting individuals	HBV DNA positive rate
2018	56,016	19	13	0.023
2019	69,663	39	17	0.024
2020	53,245	29	19	0.036
2021	35,039	33	16	0.046
2022	6482	7	4	0.062
total	220,445	127	69	0.031

Table 2
HBV DNA test results of Roche (n = 4797).

	Number of test samples	Number of *anti*-HBc positive cases	The positive rate of *anti*-HBc
College student	1045	47	4.50
General population (non-college students)	3752	862	22.97
total	4797	909	18.95

3.3. NAT single-sample result

A random selection of 164 *anti*-HBc positive samples were tested using the Roche NAT single-test system, resulting in the detection of 3 samples positive for HBV DNA (Table 3).

3.4. NAT recheck testing

Three Roche single-test reactive samples were retested using both the Huayimei system's 8-mixed tests and single-sample tests. One sample was positive in both single-sample mode and mixed-sample mode tests, another sample was positive only in single-sample mode test, and the third sample was non-reactive in both single and mixed tests and was deemed an indeterminate result, and therefore excluded from statistical analysis (Table 4). However, we still discussed the case of the third sample during our analysis. By conducting NAT single-sample testing on 164 HBV core-positive blood donors, two HBV DNA-positive donors were identified, resulting in a detection rate of 1.22% for HBV DNA low-level positive blood donors.

3.5. HBV DNA quantitative testing

The two Roche single-test reactive samples were sent to Jinan KingMed Medical Testing Center for analysis, and the results indicated that no HBV DNA was detected (Table 4).

3.6. Serological characteristics of seronegative and HBV DNA-positive blood samples

Out of the 164 samples that tested positive for *anti*-HBc, additional testing was conducted using ELISA and Abbott CMIA to assess *anti*-HBs levels. Results indicated that both *anti*-HBs and *anti*-HBc were present in 75.61% of the samples, while the rate of samples that tested positive for only *anti*-HBc was 24.39%.

3.7. CMIA testing for HBV DNA-positive blood donors of mix mode

During the period of 2018–2022, 67 samples were identified as HBV DNA positive but ELISA negative. These samples underwent hepatitis B five-item testing at the National Clinical Laboratory (NCCL) using Abbott CMIA, and 25 of them underwent confirmatory testing using Wantai CMIA, with both yielding consistent results. To better understand the serological pattern of these samples, we evaluated the results and presented them in Table 5. The results showed that in the 67 HBV DNA positive samples, both HBsAg and HBsAg neutralization tests were negative in CMIA testing. However, 55 samples (82.09%) were positive for *anti*-HBc (OBI), while 12 samples (17.91%) were negative for *anti*-HBc (window period infection, WP). Among the 67 samples, 17 (25.37%) carried both *anti*-HBc and *anti*-HBs, 38 (56.72%) only had *anti*-HBc, 8 (11.94%) only had *anti*-HBs, and 4 were completely negative. Table 6 shows the *anti*-HBs levels of the 67 HBV DNA positive samples. The results showed that 3 samples (4.48%) had *anti*-HBs levels greater than 200 IU/L, 7 samples (10.45%) had *anti*-HBs levels greater than 100 IU/L, and 19 samples (28.36%) had *anti*-HBs levels greater than 14 IU/L.

3.8. Cost calculation

The average cost to detect one HBV DNA positive sample using the NAT mixed test mode is 212,777 RMB.

The average cost of nucleic acid reagents for the detection of one HBV DNA positive sample in this study was 32,767 RMB (the number after the decimal point is ignored), plus the cost of *anti*-HBc ELISA reagents, totaling 35,017 RMB.

Cost of *anti*-HBc ELISA reagent: 75 RMB/box * 60 boxes = 4500 RMB.

Cost of detecting 1 HBV DNA positive sample (decimal numbers ignored): (4500 + 65,534)/2 = 35,017 (RMB).

Note: This article only calculates the basic costs of reagents and does not include additional costs such as positive and negative

Table 3
Single nucleic acid test results of 164 *anti*-HBc positive samples.

	Number of *anti*-HBc positive samples	Number of HBV DNA positive samples	Detection rate of HBV DNA positive samples (%)
anti-HBs negative	41	1	1.64
anti-HBs positive	123	2	1.94

Table 4
Supplementary test results of positive HBV DNA single sample (n = 3) .

Sample number	Roche 6-MP	Roche ID-NAT (CT value)	HYM 8-MP	HYM ID-NAT (CT value)	*anti*-HBc S/CO	*anti*-HBs S/CO	HBV DNA Quantitative detection (IU/ml)
202202241	negative	33.9	negative	37.51	0.01	0.15	–
202202242	negative	37.3	38.36	36.63	0.01	28.57	–
202203051	negative	37.4	negative	negative	0.01	28.57	Not submitted for inspection

Note: "–" indicates that no HBV DNA is detected.

Table 5
Serological characteristics of the HBV DNA positive and seronegative blood donations (n = 67).

HBsAg	*anti*-HBs	*anti*-HBc	quantity	Percent (%)
–	–	–	4	5.97
–	+	–	8	11.94
–	+	+	17	25.37
–	–	+	38	56.72

Table 6
Anti-HBs and *anti*-HBc characteristics of HBV DNA positive blood donors (n = 67).

anti-HBs(IU/L)	Number of *anti*-HBs (%)	Number of *anti*-HBc positives (%)	Number of *anti*-HBc negative samples (%)
200–500	3 (4.48)	1	2
100–200	4 (5.97)	2	2
20–100	12 (17.91)	8	4
10–20	6 (8.96)	6	0
< 10	42 (62.69)	38	4
total	67 (100)	55	12

Note: *Anti*-HBs concentration ≥10 mIU/mL is considered positive.

quality control, in-house quality control, labor costs, utilities, and equipment depreciation.

Cost comparison: Approximately 1 in 5 samples will test positive for *anti*-HBc. With a 20% positivity rate for *anti*-HBc, the cost of a single test using a mixed test approach is twice that of a pure mixed test [(4 + 6)/5 = 2](Table 7).

4. Discussion

HBV and HCV infections are major public health concerns in China, with China having the highest number of HBV and HCV infections in the world. In 2018, it was estimated that there were approximately 80 million cases of chronic HBV infection in China [2]. The economic burden of these infections is also significant, with the average annual cost of hospitalization for each HBV-infected patient estimated to be 4454.0 USD in direct costs, 924.3 USD in indirect costs, and 6611.10 USD in intangible costs [3].

In 2010, the Shandong Blood Center participated in a pilot project organized by the National Ministry of Health to introduce nucleic acid testing (NAT) as a screening modality for enzyme immunoassay negative samples. By 2015, the national blood collection and supply system had fully implemented NAT screening technology, enabling testing of all blood donors to mitigate the risk of transmitting HBV, HCV, and HIV through blood donations.

According to a study conducted by Chao Liu et al. from 2010 to 2015, a total of 20,084,187 seronegative blood donors underwent NAT screening in China. Among these, the number of HBV DNA-positive samples detected in seronegative blood donations was 1/1,482, resulting in a detection rate of 0.0675% (6.75 per 10,000) [1]. Our investigation unveiled that in Jinan, Shandong Province, there was a detection of 1 HBV DNA-positive blood donor for every 3195 (6-MP), equating to a detection rate of 0.031% (310 per

Table 7
NAT detection cost.

Nucleic acid detection model	unit price (RMB)	Test semples	total (RMB)	Number of HBV DNA positive cases	1 case of HBV DNA positive cost was detected (RMB)
6-MP	66.6	220,445	14,681,637	69	212,777
Single sample detection	399.6	164	65,534.4	2	32,767.2

Note: Roche charges based on the number of individuals for whom test reports are issued, at a rate of RMB 66.6 per sample. Roche's nucleic acid reagent can simultaneously detect HBV DNA, HIV RNA, and HCV RNA using a 3-in-1 specification, with a conventional detection mode of mixing 6 samples in one reaction pool for testing. In this study, the single detection reagent was also 3-in-1, therefore the cost was calculated as 66.6*7 RMB per unit.

million). This rate was slightly lower than the detection rate of 0.037% (103/280,818) from 2015 to 2017 [4], less than half of the national average, and higher than the rate of 0.025% in India (20/79,938) [5]. Comparing these findings to other regions, the detection rate of HBV DNA-positive blood donors from 2008 to 2010 was 20.9 per million in Japan [6], while in Europe, the detection rate was generally lower. For instance, the detection rates in Germany [7], France [8], and the Netherlands [9] were 0.64 per million, 0.88 per million, and 0.9 per million, respectively. The aforementioned findings further support the positive correlation between the positive detection rate of HBV DNA testing and the prevalence of *anti*-HBc in the population. For example, in our study, 18.95% of HBsAg-negative blood donors from Shandong, China were found to be positive for *anti*-HBc, while only 0.71% of blood donors from the Netherlands were identified as positive for *anti*-HBc during initial screening [9]. In Germany, there were 0.55 cases of *anti*-HBc positivity per one million blood donations [7].

Anti-HBc can be detected during asymptomatic infection and after recovery from HBV infection [10]. In contrast, *anti*-HBs may not always be produced as a result of HBV infection. Therefore, *anti*-HBc is the most critical serum marker for OBI, and screening for *anti*-HBc can exclude most OBI that cannot be detected by NAT [6,11–13]. Liu et al.'s research indicates that 77.36% of blood donors who tested negative for HBsAg but positive for HBV DNA were positive for *anti*-HBc [1]. This suggests that screening for *anti*-HBc can effectively prevent the clinical use of up to 77.36% of HBV DNA-positive blood products. In this study, the proportion of OBI among HBV DNA positive blood donors in Jinan, Shandong Province was 82.09% (55/67), which is higher than the national average and very close to the 83.57% (229/274) reported by the Blood Center of Zhejiang Province [14].

In accordance with the Blood Donation Law of the People's Republic of China, screening for *anti*-HBc is not a mandatory test for blood donors. This is mainly because China is an endemic country for HBV infection. Based on the results of a national epidemiological survey on viral hepatitis from 1992 to 1995, the prevalence of HBV infection in the Chinese population was 57.6%, with a HBsAg positive rate of 9.75% and an *anti*-HBc positive rate of 49.8%. Universal hepatitis B vaccination for newborns was implemented in China in 1992, resulting in a rapid decrease in the prevalence of hepatitis B. By 2006, the prevalence of HBV infection in the adult population had decreased to between 42.40% and 80.77%, with a HBsAg positive rate of 7.18%, representing a decrease of 26.36% [15]. Ling Ouyang et al.'s survey on HBV infection in blood donors showed that the prevalence of *anti*-HBc positivity was 63.4% among blood donors in Shenzhen between 1998 and 2000 [16]. In 2013, the prevalence rates of HBsAg and *anti*-HBc among children aged 1–14 years in Guangdong Province were 1.16% and 2.35%, respectively [17]. The fourth national hepatitis B serological survey in 2014 showed that the HBsAg positivity rate among children aged 1–4 years was 0.3%, a decrease of over 60% compared to 2006 [18]. In the present study, it was observed that the HBV core antibody positivity rate among non-student blood donors was 22.97% during the period from 2021 to 2022, which is 5.1 times greater than that observed among university students (4.50%). This reflects the benefits of universal hepatitis B vaccination in China since 1992. However, the overall positivity rate of core antibodies among the entire blood donor population was still 18.95%. Discarding blood that tests positive for HBV core antibody could exacerbate the existing shortage of blood products in Shandong Province.

To prevent transmission of OBI through blood transfusion, developed countries like those in Europe and the United States routinely screen blood donors for *anti*-HBc and discard blood from donors who test positive for *anti*-HBc. According to a WHO report, countries around the world employ three main strategies for screening blood donors for HBV: (a) 56% (98/176) of countries employ HBsAg testing; (b) 17.6% (31/176) of countries employ both HBsAg and *anti*-HBc testing (with 5 countries, including selective *anti*-HBc testing); (c) Another 8 countries implemented a 3-testing strategy that included selective *anti*-HBc testing [19]. In São Paulo, Brazil, 0.6% (6/976) of *anti*-HBc positive blood donors who were negative for HBsAg and 6 MP-NAT were positive for single-sample HBV DNA testing [20]. This rate is half as low as that found in Shandong Province, China, where 1.2% (2/164) of donors were positive for HBV DNA testing. The NAT single screening mode detects HBV DNA-positive samples in serologically negative cases with a detection rate of 0.22%, calculated as the product of the *anti*-HBV core antibody positive rate of 18.95% and the HBV DNA-positive rate of 1.22%. However, in mixed samples, the detection rate is markedly lower at 0.031%, suggesting that 85.90%【(0.22–0.031)/0.22】 of samples with a low viral load of occult HBV infection (OBI) were not detected in mixed tests. These findings were similar to those of a study by the American Red Cross. In that study, nucleic acid testing was performed on 142 OBI-positive blood donors, and 121 (85%) samples were negative for HBV DNA by multiplex nucleic acid testing using single-well, but positive by single-sample testing [21]. The Japanese Red Cross (JRC) analyzed blood from 4742 donors with low *anti*-HBc and *anti*-HBs antibody titers using individual-donation nucleic acid testing (ID-NAT), which increased the OBI detection rate from 3.9 to 15.2 per million, and confirmed the OBI transmission rate from 0.67 to 1.49 per million [6]. A study by Spreafico M et al. in Italy performed supplemental ID-NAT on previously retained blood from HBV DNA positive donors screened for 6-MP, and the results showed that 6-MP HBV DNA screening did not detect 14/28 (50%) viremic donations that were released for transfusion. HBV marker testing of the recipients of these blood products identified 2 cases of transfusion-transmitted HBV infection, recorded as donor-recipient genetic identity [22]. A study by Daniel Candotti et al. also demonstrated that HBV viral load as low as 2.3 IU/mL or even lower in serum could still cause 29% (9/31) of transfusion infections, including 7 infected individuals transfused with plasma and 2 infected individuals transfused with red blood cells containing 20 mL plasma [23]. In addition to false negatives for HBV MP-NAT, HIV and HCV also have infectious donations in which MP-NAT is not detected but ID-NAT is reactive [24]. The cost of one sample in Roche single-test mode is about 399.6 RMB. In 2021, Shandong Blood Center tested about 140,000 samples (100,000 whole blood samples and 40,000 apheresis platelet samples). With a grant of RMB 3 million per year from the financial department, if individual-donation supplemental ID-NAT was performed for all samples using Roche reagents, it would cost an additional RMB 52.94 million. However, Shandong Blood Center, which is a public charity relying solely on financial contributions, cannot afford the additional cost.

In this study, we first screened for *anti*-HBc and subsequently performed NAT single testing on *anti*-HBc positive samples. If we calculate the total cost of performing single testing on 20% of the samples (this study used two ELISA kits for detecting HBc antibodies, with a dual-kit positivity rate of 18.95%, but approximately 1% of single-kit positive samples were not included in the statistical

analysis, hence we used a positivity rate of 20% for the calculation) and mixed testing on 80% of the samples, the expected cost will double. (The cost of the *Anti*-HBc ELISA kit is very low, less than 0.1 yuan per person, and is not included in the total cost.) Based on the collection of 100,000 samples per year by the Shandong Blood Center, routine screening could detect only 31 HBV DNA positive samples (Table 1). However, theoretically, using the *anti*-HBc positive and NAT single testing mode, an additional 244 HBV DNA positive OBI samples could be detected (calculated as 100000*0.20*0.0122), resulting in a detection rate of 7.87 times higher than routine screening. Although this method incurs twice the cost, it is relatively cost-effective. Furthermore, according to the detailed data from this study, the prevalence of occult hepatitis B infection (OBI) among blood donors should be approximately 8.9 times higher than the detection rate of current screening methods. This was determined by the calculated detection rate of a single screening mode, which was found to be 8.9 times greater than that of mixed screening (calculated using formula $(0.22 + 0.031)/0.031$). It should be noted that the prevalence of occult hepatitis B infection (OBI) may vary depending on the specific population and screening methods used. For instance, in this study, confirmation was not performed on a sample that tested positive by Roche single assay but negative by other testing methods. Moreover, most of these low-level samples were below 10 IU/mL, which could potentially be missed even by the most sensitive single-sample NAT (Roche LOD 1.6 IU/mL). Additionally, the small sample size used in our single testing approach may have contributed to significant statistical errors. Therefore, further research is warranted to confirm these findings.

In situations where blood collection and supply institutions face financial constraints and cannot perform both *anti*-HBc screening and NAT single tests simultaneously, screening for *anti*-HBc alone can be sufficient. *Anti*-HBc negative blood can be transfused to recipients who are negative for *anti*-HBs and have low levels of *anti*-HBs antibodies. Furthermore, *anti*-HBc positive samples can be screened with supplemental *anti*-HBs testing, and blood products with high levels of *anti*-HBs can be allocated to clinical recipients. It is advisable to avoid giving blood that is only *anti*-HBc positive and/or has low levels of *anti*-HBs antibodies to recipients with weaker immune systems against HBV. For instance, Allain JP et al. demonstrated that in recipients who were unvaccinated and received *anti*-HBs-negative occult bloodborne infection (OBI) blood products, the prevalence of *anti*-HBc increased to 63.8% (28/44) in a background of 15% *anti*-HBc-positive recipients. In contrast, *anti*-HBc positivity was observed in only 15.4% (4/26) of *anti*-HBs-positive blood product recipients. The presence of *anti*-HBs (titer: 20–160 IU/L) in donors reduced the risk of HBV transmission via transfusion by approximately five-fold [25]. In Japan, blood centers discard blood products with low *anti*-HBc and *anti*-HBs titers, which represent 1.3% of total donations. Conversely, *anti*-HBc positive blood containing *anti*-HBs levels of 200 IU/L and above is considered to pose a low risk of transmitting HBV [6,26]. However, some countries like Germany and Austria consider blood with *anti*-HBs levels greater than 100 IU/L to pose a low risk of transmitting HBV [27]. Employing the criteria utilized by countries like Germany and Austria to ascertain low-risk blood, only 10% (Table 6 , 7/67) of occult blood-borne infections (OBI) cases in our study exhibited *anti*-HBs levels exceeding 100 IU/L, signifying their classification as low-risk samples. These findings imply that the presence of detectable *anti*-HBs in blood may not guarantee complete safety. Additionally, 17.91% (Table 6, 12/67) of HBV DNA positive patients were found to be in the window period, with 80% of those infected during this period being *anti*-HBc-negative individuals. Therefore, a single test for screening the low-load window period may not be cost-effective in *anti*-HBc-negative populations. A study by Ramachandran et al. in the USA also demonstrated that using the more sensitive Ultrio Plus assay (3.4 IU/mL) instead of Procleix Ultrio (10.4 IU/mL) for screening HBV DNA did not significantly reduce residual blood infectivity. However, individual nucleic acid detection is required for detecting occult bloodborne infection (OBI) in 92% (537/583) of OBI blood donors [28]. The choice of nucleic acid single or mixed detection has a significant impact on the detection rate of OBI, but has little impact on the detection of the window period. Unfortunately, due to limited funding, we did not examine the detection rate of the window period in *anti*-HBc-negative samples with HBV DNA levels ranging from 2.3 to 14 IU/mL in this study.

Currently, there is limited available data on the prevalence of HBV infections and corresponding statistics regarding blood donors in China with an HBV DNA load ranging from 2.3 to 14 IU/mL.Our study suggests that a conservative estimate of 509 blood donors with low-load HBV DNA levels (220,445*0.1895*0.0122) may have gone undetected over the past five years. It is important to note that this figure does not account for individuals with inconclusive test results or those in the window period of initial HBV infection. This may be due to inadequate awareness of the infection risks associated with low-load HBV DNA, as well as insufficient follow-up of blood recipients who received such blood units. However, transfusion-related infections caused by occult HBV infection (OBI) with very low viral loads are associated with the amount of transfused blood and blood components, and can be prevented by *anti*-HBs. Therefore, a rational transfusion strategy can be developed by conducting *anti*-HBc and *anti*-HBs tests in combination with NAT single tests for blood donors, as well as *anti*-HBc and *anti*-HBs tests for blood recipients, in order to minimize the risk of HBV transfusional transmission.

Author contribution statement

Jianfeng Chen: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Zili Ma: Performed the experiments; Wrote the paper.

Dandan Wu, Qi Zuo: Performed the experiments.

Fengtian Wang, Chen Xiao, Fuqiang Chen, Peng Li: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Data availability statement

Data included in article/supp. material/referenced in article.

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Declaration of competing Interest

I, Chen Jianfeng, hereby declare the following potential conflicts of interest associated with the submitted manuscript titled "Evaluating the Cost-effectiveness of Low-level HBV DNA Screening in Occult Hepatitis B Infection Donors: A Study from Shandong Blood Center, China" to the journal Heliyon:

1. The research conducted for this study does not involve any conflicts of interest that could bias or distort the research findings. This study received funding from the Shandong Health Science and Technology Development Plan and the Research Fund of Blood Screening Technology from the Chinese Medical Equipment Association Transfusion Medical Equipment Technology Professional Committee. The researchers involved in this study have no financial interests related to the research.

2. The data and analysis methods used in this study are accurate and reliable, and the conclusions are objective and unbiased. There was no manipulation of the research results during the study process.

3. All authors of this study have reviewed and approved the final version of the manuscript. The corresponding author, Chen Jianfeng, on behalf of all authors, has authorized the submission of the manuscript to Heliyon journal for review.

4. This manuscript has not been previously published in any other journal, nor is it under consideration for publication elsewhere. The content of this manuscript is original and does not involve any plagiarism or duplication of other research findings.

5. If the manuscript is accepted for publication in Heliyon journal, the copyright will be granted exclusively to the publisher for global publication. The authors agree that the abstract of the paper may be included in other journals and indexed in databases.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18609.

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