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Citation	**Gut, 2010, v. 59 n. 10, p. 1389-1393**
Issued Date	**2010**
URL	**http://hdl.handle.net/10722/125086**
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Prevalence of occult hepatitis B infection in a highly endemic area for chronic hepatitis B: a study of a large blood donor population

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ABSTRACT

Background and aims The aim of the present study was to determine the population prevalence of occult hepatitis B (OHB) infection and its clinical profile in a highly endemic area of chronic hepatitis B virus disease.

Methods OHB was first identified by individual sample testing for hepatitis B surface antigen (HBsAg) followed by nucleic acid testing (NAT) and vice versa for 3044 (cohort 1, stored sera from donation within 1 year) and 9990 (cohort 2, prospective study) blood donors, respectively. OHB was confirmed meticulously by ≥2 out of 3 tests with detectable hepatitis B virus (HBV) DNA using a sensitive standardised assay. Detailed serology and viral load in the serum and liver were studied.

Results The prevalence of OHB was 0.13% (4/3044) and 0.11% (11/9967) for cohort 1 and 2, respectively. In cohort 2, 10 out of 11 OHB samples were positive for anti-HBc (hepatitis B core antigen) antibody (all were immunoglobulin G). Seven had detectable anti-HBs. The serum HBV DNA levels were extremely low (highest 14.1 IU/ml). Of the six donors who underwent liver biopsies, all had normal liver biochemistry, extremely low liver HBV DNA (highest 6.21 copies/cell) and nearly normal liver histology. For those with viral sequence generation, none had the common HBsAg mutant G145R.

Conclusions The prevalence of OHB in a highly endemic area of chronic HBV was very low, thus implying a low impact on transfusion services. To implement universal screening, the high cost of NAT should be taken into account. OHB blood donors had very low HBV replication, and normal liver biochemistry and histology, conferring a favourable prognosis.

INTRODUCTION

The prevalence of chronic hepatitis B (CHB) which affects 400 million people worldwide has been fully documented.[1] The highly endemic regions with carrier rates of >8% include Asia and Africa. However, there is a lack of systematic and population studies in Asia on the prevalence of occult hepatitis B (OHB) virus infection, defined as the presence of hepatitis B virus (HBV) DNA in the sera or livers in subjects who are negative for serum hepatitis B surface antigen (HBsAg). The paucity of population-based data on OHB may be due to two main reasons. First, the entity OHB has gained global attention only recently. Secondly, there is no

Significance of this study

What is already known about this subject?
- Occult hepatitis B, because of its low HBV DNA levels, is a notoriously difficult disease to study.
- Prevalence of occult hepatitis B varies greatly among different countries.
- The accuracy of detection of occult hepatitis B depends on the performance of tests.
- Occult hepatitis B blood donors may transmit hepatitis B virus through blood transfusion.

What are the new findings?
- Even in a highly endemic area of chronic hepatitis B, the prevalence of occult hepatitis B is low.
- Screening for occult hepatitis B by HBsAg testing followed by nucleic acid testing has the same yield compared with first screening by nucleic acid testing.
- Serum and liver HBV DNA levels in subjects with occult hepatitis B are extremely low and highly sensitive assays are required to diagnose the disease.
- Subjects with occult hepatitis B have normal liver biochemistries and liver histology.

How might it impact on clinical practice in the foreseeable future?
- The finding of low prevalence of occult hepatitis B in a highly endemic area of chronic hepatitis B provides useful information for planning and implementation of screening of this disease in transfusion services.
- The finding of the present study provides evidence that screening by HBsAg testing followed by nucleic acid testing may be the more cost-effective approach.
- Subjects with occult hepatitis B, because of the low viral levels and nearly normal liver histology, are expected to have a good long-term outcome.

generally accepted assay of HBV DNA detection for OHB in which serum HBV DNA levels are usually extremely low (<200 IU/ml).[2 3] However, studies on the prevalence of OHB have many implications for the blood transfusion services as the infectivity of blood products from donors with OHB remains

largely unknown. The prevalence documented in cohort studies is usually <1%.[4–7] Large population studies are required in order to define the prevalence with higher confidence of accuracy.

In addition, the serology, virology (in serum and in the liver) and histology of subjects with incidental identification of OHB have not been studied in detail.

We carried out the present large population study in Hong Kong (where 8% of the population has CHB) with the primary aim of determining the prevalence of OHB in our general population by using two different screening strategies. The secondary aims were to examine the viral and disease status of subjects with OHB.

PATIENTS AND METHODS

The present study was carried out in two stages with two cohorts. The first stage (cohort 1) involved testing of 3044 stored sera randomly selected by the Hong Kong Red Cross Transfusion Service. These sera were from blood donors who donated blood within 1 year of the start of the present study. The time of donation of these samples was between 1 June 2005 and 31 May 2006. The samples were retrieved from all the 18 governorates of Hong Kong. All donor sera were first tested negative for HBsAg (Abbott PRISM, Abbott Laboratories, Abbott Park, Illinois, USA), antibody to hepatitis C virus (HCV) and antibody to HIV. These samples were then tested individually (not by pooling) using the COBAS TaqScreen MPX (Roche Molecular Systems, Branchburg, New Jersey, USA) test on the s201 system (Roche Instrument Center, Rotkreuz, Switerland), a screening nucleic acid testing (NAT) for HBV, HCV and HIV. The lower limit of detection of this assay for HBV is 3.7 IU/ml with a 95% CI of 3.3 to 4.4 IU/ml. Initial positive samples were then quantified for the HBV DNA levels by a standardised commercial HBV DNA assay, Artus HBV RG test (QIAGEN, Hilden, Germany). When used with the QIAamp DSP Virus Kit (QIAGEN) for HBV DNA extraction, the Artus HBV RG test has a 95% lower limit of detection of 3.8 IU/ml, with a linear range of detection of between 1.1 and >4×10^9 IU/ml. Since the serum HBV DNA levels of occult HBV subjects were expected to be very low, in order to minimise the chance of false-positive or false-negative results, the Artus HBV test was performed three times on three separate occasions in all the samples which tested positive by NAT. Definite OHB was defined as two or more of the three runs of assays showing detectable HBV DNA levels. Further serological tests including antibody to HBsAg (anti-HBs), and total and immunoglobulin M (IgM) antibodies to hepatitis B core antigen (anti-HBc) using the Elecsys 2010 system (Roche Diagnostics, Mannheim, Germany) were performed.

In the second prospective stage (cohort 2) started in January 2006, subjects were recruited on site during blood donation. After obtaining written informed consent from the donors, 15 ml of blood were taken for serological tests (see below). The donors were interviewed with a questionnaire which included the following information: (1) personal history of previous donations, HBV vaccination, known hepatitis B, C and other liver diseases, jaundice, alcohol intake (>20 g/day), diabetes mellitus, hypertension, long-term medication and intake of traditional Chinese medications; and (2) family history of hepatitis B carriage, liver cancer and other chronic liver diseases.

During the recruitment period from 1 January 2006 to 3 June 2008, a total of 9990 blood donors were recruited. The sera were first tested by NAT using the s201 system mentioned above. NAT-positive samples were then tested for HBsAg to diagnose overt CHB. The remaining NAT-positive samples which were HBsAg negative were tested for OHB. Definite OHB was defined

by two out of three positive HBV DNA tests (Artus HBV RG test, QIAGEN) on samples negative for HBsAg with the same criteria as mentioned above. Tests for anti-HBs and anti-HBc (total and IgM) as described above were also performed.

Donors with OHB from cohort 2 were recalled. Liver biochemistry was performed after a second written informed consent. Liver biopsies were performed in subjects who consented. Liver tissues were assessed for histology which was graded according to the Ishak's criteria.[8] Total intrahepatic HBV DNA and covalently closed circular (ccc) DNA were assayed by real-time PCR, as described in previous studies.[9] [10] Briefly, primers and FRET (fluorescence resonance energy transfer) probes directed against the HBV surface region were used for real-time PCR quantification of the total intrahepatic HBV DNA in the Rotorgene 3000 Real-time Multiplex System (Corbett Research, Australia). For cccDNA detection (lower limit of detection 0.002 copies/cell), the extracted liver DNA was first treated with Plasmid-safe DNase (Epicentre, Madison, Wisconsin, USA), followed by real-time PCR using primers spanning the incomplete region in the HBV relaxed circular genome. Human genomic DNA content in the liver DNA extract was measured by real-time PCR using the β-globulin primers and probes in the LightCycler Control DNA Kit (Roche Applied Science, Mannheim, Germany). HBsAg mutations at amino acid 145 (G145R) were determined by PCR sequencing, using primers spanning the HBV surface gene at nucleotides 426−600 (HBsAg amino acid residues 134−148). The HBV DNA was amplified by semi-nested PCR, using the sense primer HBV406s (both first and second rounds; 5′-CTTCATCCTGCTGCTATGCCT-3′) and antisense primer HBV608a (first round; 5′-AAAGCCCAGGAT-GATGGGAT-3′) or HBV600a (second round; 5′-CCANGAT-GATGGGATGGGAATAC-3′). The 216 bp amplicons were then sequenced bi-directionally by the second round primers. HBV genotypes were determined by phylogenetic comparison of the same HBV DNA surface gene amplicons with HBV viral reference sequences in GenBank.

Both stages of the study were approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster.

Statistical analysis

The present study was descriptive in nature. We calculated the prevalence of OHB in the study populations. Continuous variables were expressed as the mean with SD and range. Comparisons of the demographic data between different groups of subjects were performed by Student t tests for continuous variables and χ^2 tests for categorical variables. Two-tailed p values of <0.05 were considered to be statistically significant.

RESULTS

In cohort 1 with 3044 HBsAg-negative donors (1525 males, 1519 females), the mean age was 33.1 years (SD 10.6, range 16.0−62.3). NAT by the s201 system identified 12 positive samples (0.4%). Four samples had detectable HBV DNA by the Artus HBV RG test (two had 2 out of 3 tests, two had 1 out of 2 tests (sample volume not adequate for the third run)). If the latter two cases were also regarded as having OHB, the prevalence of OHB was 0.13% with a 95% CI of 0.036% to 0.336%. The HBV DNA levels of these six runs were 1.10, 1.21, 1.70, 2.16, 2.47 and 19.40 IU/ml. Of the 12 NAT-positive samples, 10 with adequate volume for further anti-HBc testing, six were positive for anti-HBc (all were negative for IgM anti-HBc) of which two had OHB.

For the prospective cohort 2, the demographics and the report of the questionnaires of the 9990 donors recruited are listed in table 1.

Figure 1 shows the multistep approach by which the donors with OHB were identified. OHB was found in 11 donors with detectable HBV DNA (three in all 3 runs, eight in 2 out of 3 runs). The prevalence of occult HBV was 0.11% (95% CI 0.055% to 0.197%). There were another four donor samples with detectable HBV DNA only in 1 out of 3 runs. They were regarded as suspected OHB.

The demographics, serum viral markers and serum HBV DNA levels of the 11 confirmed occult HBV donors are listed in table 2. Of these, 10 donors had no family history of CHB; one donor was not sure. All had no family history of hepatocellular carcinoma or cirrhosis. Nine had given previous blood donations. Only two were known to have had HBV vaccinations (six had not received HBV vaccinations; three were not sure). When compared with blood donors without hepatitis B (n=9956) and donors with CHB (n=23), OHB donors were significantly older than donors without hepatitis B (mean age 39.9 years (SD 12.1; range 20.4—57) vs 31.5 years (SD 10; range 16—65.8), respectively, p=0.005) and donors with CHB (mean age 25.6 years (SD 7.7; range 18.3—49.0), p=0.003). There were no significant differences in the gender ratio between the three groups (all p>0.05).

Ten of the 11 samples of these OHB donors were positive for total anti-HBc but all were negative for anti-HBc IgM, indicating that these donors were not in the window phase of acute hepatitis B infection, when subjects are positive for HBV DNA and have yet to develop circulatory antibodies to HBsAg.

Nine donors agreed to undergo further liver biochemical tests and viral sequencing, and six consented to liver biopsies. All these OHB donors had normal liver biochemistry, extremely low serum and liver HBV DNA and nearly normal liver histology (table 2). The HBV surface regions of all nine donor samples were successfully sequenced. Three donors carried genotype B HBV and six carried genotype C HBV. All nine showed wild-type glycine at HBsAg amino acid 145, indicating the absence of G145R mutations (the vaccine escape mutants).

DISCUSSION

The present study documented a population prevalence of OHB of 0.13% and 0.11% from two large cohorts of blood donors in

Table 1 Demographics and report of questionnaires of the 9990 donors

No. of subjects	9990
M:F (%)	5550:4440 (55.6:44.4)
Mean age, years (SD, range)	31.5 (10.1, 16—65.8)
Report of questionnaires	
Past personal history	
Previous blood donation (yes: no: not sure)	7714: 2245: 31 (77.2: 22.5: 0.3%)
HBV vaccination (yes: no: not sure)	2657: 3502: 3831 (26.6: 35.1: 38.4%)
HBV infection (yes: no: not sure)	11: 9906: 73 (0.1: 99.2: 0.7%)
HCV infection (yes: no: not sure)	0: 9924: 66 (0: 99.3: 0.7%)
Jaundice (yes: no: not sure)	44: 9745: 201 (0.4: 97.5: 2%)
Significant alcohol intake (yes: no)	205: 9785 (2.1: 97.9%)
Diabetes mellitus (yes: no: not sure)	15: 9939: 36 (0.2: 99.5: 0.4%)
Hypertension (yes: no: not sure)	29: 9940: 21 (0.3: 99.5: 0.2%)
Long-term medication (yes: no)	117: 9873 (1.2: 98.8%)
Family history	
HBV infection (yes: no: not sure)	727: 9039: 224 (7.3: 90.5: 2.2%)
Liver cancer (yes: no: not sure)	179: 9639: 172 (1.8: 96.5: 1.7%)
Chronic liver disease (yes: no: not sure)	372: 9377: 241 (3.7: 93.9: 2.4%)

F, female; HBV, hepatitis B virus; HCV, hepatitis C virus; M, male.
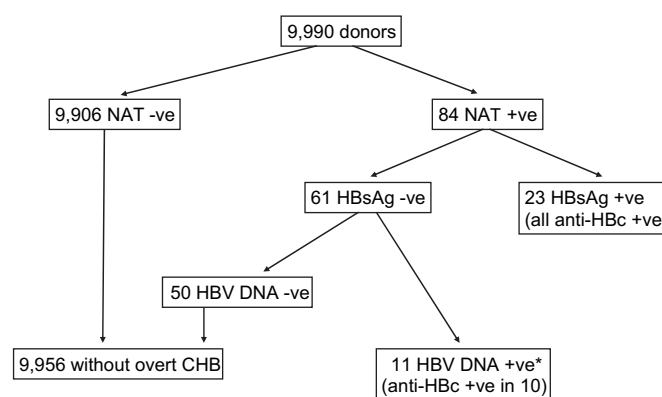
* Incidence of occult HBV: 0.11%

Figure 1 Diagram showing the steps of tests to identify occult hepatitis B donors (+ve, positive; −ve, negative).

Hong Kong, an endemic area for CHB with a prevalence rate of 8%. These figures should be highly accurate. First, in contrast to some previous studies which adopted pooled sample testing with a pool of 6—500 donor sera, our study tested single samples individually. It has been shown that the sensitivity of identifying OHB is 3—10 times lower in pool sample testing compared with individual single sample testing.[11][12] Secondly, in order to reduce the chance of false-positive results due to contamination or real-time PCR noise signals as well as to enhance the detection rate of the PCR test in the context of extremely low HBV DNA levels, at least two out of three runs by Artus HBV RG test had to be positive before the samples were regarded as positive for OHB. Although it is sometimes difficult to distinguish between occult chronic HBV infection and acute infection in the window period, the fact that nearly all OHB samples were positive for anti-HBc (all in IgG form) suggested that the subjects did not have acute HBV infection during the window period when the blood was donated. A Taiwan study has also shown that all the identified HBV DNA-positive and HBsAg-negative follow-up samples had OHB with low HBV DNA titres and none was in the window period.[13]

Previous studies using in-house HBV DNA testing with a smaller number of subjects reported a rate of 0.01—0.02% and 2—4% for OHB in non-endemic and endemic areas of CHB, respectively.[4][14–16] The present study indicates that the prevalence of OHB was in fact low even in an area of high endemicity for HBV. This is in accordance with a recent Taiwan study showing a prevalence of 0.11% by using commercial NAT.[13] NAT screening has been implemented in some countries in Europe, North America, Australia, Japan and southeast Asia.[17–21] The results of the present study may provide important information for individual countries to consider whether universal NAT screening should be adopted. The implementation of NAT screening should also take into account the risk of transmission of HBV from OHB donor blood products to recipients which was not assessed in the present study. The estimated transmission rate of HBV from blood transfusion is 1 in 300 000 in general.[22][23] This rate should be even lower in an OHB population not suffering from acute HBV infection, although the exact chance of HBV transmission from OHB donors remains to be determined. There are a few case reports documenting possible HBV transmission from OHB donor blood products.[24–26] A careful look-back study to determine the clinical outcome of recipients receiving blood products from occult HBV donors should be performed in the future.

Table 2 Demographics, liver biochemistry, serum viral markers, serum HBV DNA levels, liver histology and intrahepatic HBV DNA levels of 11 occult HBV donors

Donor	Age	Sex	Liver biochemistry			Serum hepatitis B marker		Serum HBV DNA levels (IU/ml)			Intrahepatic HBV DNA		Liver histology	
			Alb (g/l)	Bili (μmol/l)	ALT (U/l)	Anti-HBc (+/−)	Anti-HBs (IU/l)	First run	Secon run	Third run	Total (copies/cell)	cccDNA (copies/cell)	NI (0−18)	Fibrosis (0−6)
1	20	F	45	7	29	+	<2	12.3	4.0	11.1	0.22	UD	0	0
2	27	F	46	9	12	+	>1000	UD	0.9	2.4	UD	UD	1	0
3	31	M	49	7	15	−	<2	UD	0.8	1.0	NA	NA	NA	NA
4	34	M	50	3	16	+	<2	7.1	1.7	1.5	1.17	UD	1	0
5	34	M	46	9	20	+	9.5	3.2	3.4	14.1	6.21	UD	0	1
6	37	M	49	7	45	+	110.3	UD	0.6	0.6	0.23	UD	0	0
7	43	F	45	2	13	+	998.4	UD	0.3	2.5	UD	UD	1	1
8	50	F	NA	NA	NA	+	14.6	4.5	6.4	UD	NA	NA	NA	NA
9	53	M	NA	NA	NA	+	<2	UD	5.1	1.6	NA	NA	NA	NA
10	53	M	48	7	13	+	166.7	UD	1.4	1.8	NA	NA	NA	NA
11	57	M	42	9	40	+	82.0	3.2	UD	4.8	NA	NA	NA	NA

Alb, albumin (normal 38−48 g/l); ALT, alanine aminotransferase (normal <53 IU/l for males, <36 IU/l for females); Bili, bilirubin (normal < 19 μmol/l); ccc, covalently closed circular; F, female; HBc, hepatitis B core; HBs, hepatitis B surface; HBV, hepatitis B virus; M, male; NA, not available; NI, necroinflammatory score; UD, undetectable.

We carried out the present study in two separate cohorts by using two different strategies of screening for OHB. In the first cohort with 3044 donors, all the samples were first tested negative for HBsAg before being subjected to NAT and finally by sensitive HBV DNA assay. In the second prospective cohort with 9990 donors, the samples were first tested by NAT. NAT-positive samples were then tested for HBsAg. For NAT-positive and HBsAg-negative samples, HBV DNA assay was performed to identify OHB. Both strategies yield very similar prevalence rates. This suggests that initial screening by HBsAg followed by NAT has the same yield of detection as with initial screening by NAT. HBsAg testing followed by NAT is obviously cheaper. However, a practical advantage of adopting the second strategy of initial screening by NAT is that there will be a faster release of blood products for use once the NAT is negative since the sensitivity of NAT is superior to detection of HBsAg by enzyme immunoassay (EIA).[27] Another potential advantage of using initial NAT screening is its ability to detect HBV in the presence of antigenically modified HBsAg such as G145R mutants, which may give rise to negative results in HBsAg detection assays.[28] However, our study revealed that none of the occult HBV carried this G145R mutation, a finding consistent with other studies.[29 30]

Since the cost for universal NAT screening is high, anti-HBc screening has been suggested as an alternative. The positivity rate of isolated anti-HBc in the general population directly correlates with the endemicity of CHB. It is estimated to be up to 17% in areas of intermediate endemicity.[31] In areas which are highly endemic for HBV infection, such as Hong Kong, the prevalence rate of anti-HBs positivity was as high as 40% before the implementation of universal HBV vaccination.[32] A majority of these anti-HBs-positive subjects would also be positive for anti-HBc. In light of the low prevalence rate of OHB observed in the present study, a high proportion of blood donations may be discarded from usage unnecessarily if isolated anti-HBc positivity is used for screening HBV.

In the 11 OHB donors identified in cohort 2, all had normal liver biochemistry and nearly normal liver histology with no or insignificant necroinflammation and fibrosis. Performing liver biopsies on these subjects was to be more confirmatory of the diagnosis of OHB as we could not completely rule out false-positive HBV DNA results obtained by very sensitive assay. In addition, there are no data on the possible liver injury and intrahepatic virological status in these subjects. The serum HBV DNA levels were very low, with the highest value of 14.1 IU/ml

(19.4 IU/ml in cohort 1). This is consistent with the very low or undetectable total intrahepatic HBV DNA, indicating that HBV inside the liver is replicating at an extremely low rate. It has been shown that most patients with CHB with HBsAg seroclearance still have detectable total intrahepatic and cccDNA.[9 33] In the present study, the undetectable cccDNA in the liver tissues is likely to be related to the extremely low viral load. This suggests a favourable long-term prognosis in donors with OHB. However, there are still two main concerns for these OHB donors despite the low level of viral replication. There is a possibility of reactivation of the hepatitis B disease if these OHB donors should require immunosuppressive therapy in the future, especially in regimens containing rituximab.[34] Also the low viral load is still of a theoretical concern in transmitting HBV. It has been shown that as few as 1−10 HBV particles can infect chimpanzees.[35]

One limitation of the present study was that the prevalence of OHB elucidated by the present study may not be totally representative for the general population in Hong Kong since it recruited blood donors who were relatively young (mean age of 31.5 years, table 1) and healthy.

In conclusion, with the prevalence of only 0.1% of the population having OHB in an endemic area of high HBV prevalence, the impact on the transfusion services is expected to be low. Implementation of universal screening of OHB in blood transfusion services should be determined with the consideration of both the disease prevalence and the cost incurred by the programme.

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Editor's quiz: GI snapshot

An unusual inflammation of the colon

A 27-year Caucasian man received an unrelated donor bone marrow transplant for severe aplastic anaemia. Six weeks later he was re-admitted to hospital with a febrile illness. He developed profuse non-bloody diarrhoea and cervical lymphadenopathy was detected. Blood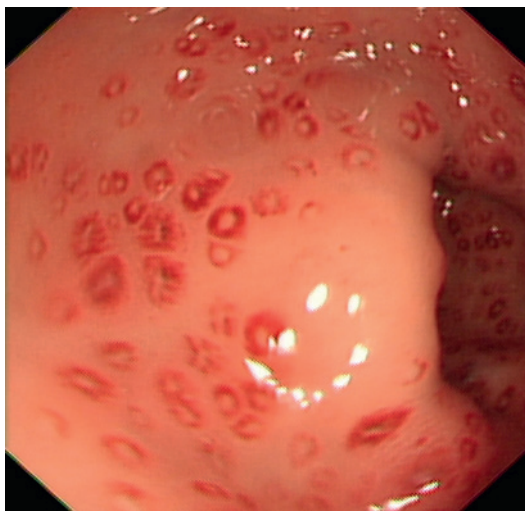 tests showed pancytopenia and peak concentrations of C-reactive protein 100 mg/l, alanine aminotranferase 357 U/l and alkaline phosphatase 197 U/l. Standard stool microscopy and culture did not reveal any abnormalities and tests for Clostridium difficile toxins a and b were negative. Flexible sigmoidoscopy revealed the following appearances from the rectum to the extent of the examination (figure 1).

QUESTION

What is the cause for this atypical colitis and how would you treat it?

See page 1427 for the answer

Figure 1 Endoscopic view of the rectal mucosa.

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Prevalence of occult hepatitis B infection in a highly endemic area for chronic hepatitis B: a study of a large blood donor population

Man-Fung Yuen, Cheuk-Kwong Lee, Danny Ka-Ho Wong, et al.

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Notes