TABLE 2. Analysis of 26 HBV NAT–positive donations from donors who could be followed-up from early stage of infection to end of viremia

	Length of period (days)*				Peak or maximum value and the time (days from pool NAT-positive [>1200 copies/mL])†					
	HBV DNA (copies/mL)			ALT (IU/L)	HBV DNA		ALT		Anti-HBc	
No Donor	>1200	>60	HBsAg-positive	>100	Copies/mL	Days	IU/L	Days	HI: 2ⁿ	Days
1	*49*	*90*	*0‡*	0	2.0×10^4	*40*	48	—	>5§	*69*
2	*23*	*43*	*0*	0	2.9×10^4	*14*	14	—	10	*45*
3	*29*	*52*	*0*	0	6.8×10^4	*23*	16	—	6	*41*
4	*25*	*59*	*11*	0	8.2×10^3	*19*	10	—	>8	*>61*
5	**26**	*45*	**23**	0	8.0×10^4	*17*	36	—	>8	*>61*
6	*36*	*59*	*24*	0	1.2×10^5	*29*	19	—	>6.5	*>74*
7	*36*	*58*	*28*	0	1.4×10^5	*25*	10	—	>7.5	*>46*
8	33	53	28	26	5.5×10^4	*23*	323	*44*	10	*44*
9	29	46	29	0	1.4×10^5	*17*	22	—	7	*45*
10	**64**	80	**31**	0	1.7×10^6	*54*	47	—	9	*68*
11	*50*	*69*	*34*	0	5.6×10^6	*29*	23	—	11	*58*
12	56	75	34	0	9.8×10^5	*36*	>71	—	>8.5	*>64*
13	*39*	*56*	*40*	>4	7.5×10^5	*22*	139	>45	>8	*>45*
14	49	*62*	*44*	>7	4.6×10^6	*34*	>257	>54	>8	*54*
15	**49**	*73*	**45**	6	9.8×10^4	*30*	142	*44*	>7	*>58*
16	*42*	*66*	*46*	23	1.5×10^6	*22*	1705	*38*	>10	*38*
17	52	*83*	51	0	1.4×10^6	*37*	50	—	>9	*>68*
18‖	*75*	*112*	*61*	48	2.5×10^8	*49*	265	*49*	11	*78*
19	**60**	*87*	**68**	17	9.6×10^6	35	893	**49**	11	**49**
20‖	73	*116*	69	33	1.2×10^7	*28*	1395	*58*	11	*58*
21‖	*77*	*110*	**82**	31	2.4×10^7	**60**	2973	**71**	10.5	**79**
22‖	93	*121*	105	50	4.8×10^8	**60**	1467	**72**	11	**72**
23	108	*170*	98	>8	8.4×10^6	*85*	>309	*>107*	>10	*>107*
24‖	**99**	155	**129**	64	2.8×10^7	*77*	1707	**100**	11.5	**93**
25	*98*	*155*	*104*	43	6.8×10^8	*55*	408	*87*	11.5	*87*
26‖	*113*	*213*	*175*	38	1.1×10^9	**58**	2792	**71**	8	**76**

* Data in bold when slopes of curves in increasing and decreasing phase are based on actual viral load or s/co measurements with an observed HBV DNA or HBsAg peak (the intervals of sequential samples of around peak were within 15 days) and estimates are obtained by intrapolation and not by extrapolation to lower assumed ID-NAT concentration. Data in lightface when slopes of curves in increasing and decreasing phase are based on assumption of timing of viremia peak and estimates are obtained by intrapolation with assumption of the viral load such as approximately 60 copies per mL or approximately 1 copy per mL in quantitative PCR-negative donations with and without AMPLINAT reactivity. Data in italics when slopes are based on assumption of timing of viremia peak and estimates are obtained by extrapolation with assumption of the viral load such as approximately 60 copies per mL or approximately 1 copy per mL.

† Data in bold show that intervals of sequential samples around the HBV DNA or HBsAg peak are within 15 days (most of samples were within an interval of 7 days). Data in italics show that MP-positive date was estimated by intrapolation or extrapolation.

‡ Zero means occult HBV infection (HBsAg EIA is negative during observation).

§ > = the data are not the peak but maximum values during observation. Because of the incomplete follow-up, no peaks could be obtained.

‖ — = ALT was less than 100 IU per L during observation. Some samples are obtained from hospitals with informed consent.

position known to be associated with occult HBV infection.[22] In fact we are not certain that this donor has been infected with mutant virus and developed acute HBV infection, because the only indication is a borderline detectable titer in the JRC HI screening method and a drop in HBV DNA titer. Further follow-up testing is required to confirm delayed anti-HBc and anti-HBs conversion in this particular case. The laboratory results may also represent chronic occult HBV infection without detectable anti-HBc and fluctuating viral load. Whatever is the natural course, detection of HBV DNA was the only tool to identify occult infection in this donor. Our study shows that NAT is not only valuable for detection of low HBV DNA levels in the pre- and post-HBsAg window periods, but also may be capable of identifying higher levels of viremia in anti-HBc–negative donors with occult HBV infection in either an acute or chronic stage. The reduction in pool size from 50 to 20 donations in our MP NAT screening system from August 2004, may be instrumental in interdicting such donations with moderate levels of HBV DNA and so further reduce the risk of post-transfusion HBV infection in Japan.

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TABLE 3. Change of HBV-related markers of serial samples from pool NAT-positive donors who could be looked-back and followed-up

No Donor	Time (days) from MP-NAT–positive day*	ALT Transaminase HR-II (IU/L)	HBV DNA ID-NAT TaqMan (copies/mL)	HBsAg EIA Auszyme II (s/co)†	HBsAg EIA AxSYM (s/co)†	HBsAg CLIA Architect (IU/mL)‡	HBsAg CLIA PRISM (s/co)†	HBcAb EIA HI (2^n)	HBcAb EIA AxSYM (% Inh)§	HBcAb EIA IMx HBc-M indexII	HBcAb EIA PHA (2^n)¶	HBcAb EIA AxSYM (mIU/mL)
1	0	48	2.3×10^2	0.06	NT**	0.01	0.50	0	9.30	0.04	0	NT
	21	45	3.1×10^2	0.10	NT	NT	NT	0	NT	0.04	0	NT
	43	33	1.9×10^3	0.04	NT	NT	NT	0	NT	0.04	0	NT
	74	41	2.0×10^4	0.08	NT	0.01	NT	0	24.32	0.04	0	NT
	105	36	—	0.04	NT	NT	NT	5	NT	0.05	0	NT
2	–30	10	—	0.18	NT	NT	NT	0	2.40	0.06	0	NT
	0	10	2.9×10^4	0.46	NT	NT	0.99	0	NT	0.07	0	NT
	31	14	—	0.1	NT	NT	NT	10	87.70	2.55	0.5	0.1
	62	8	—	0.14	NT	NT	NT	8.5	93.00	2.02	4.5	199.2
3 (Fig. 1)	0	13	6.8×10^2	NT	0.56	0.02	0.37	0	NT	0.07	NT	0
	26	10	6.8×10^4	NT	0.79	0.04	NT	0	22.05	0.07	0	0
	44	14	—	NT	0.61	0.03	NT	6	65.55	1.49	0	0
	75	16	—	NT	0.57	0.00	NT	5	75.51	1.11	4	97.9
10 (Fig. 2)	–20	10	$<1.0 \times 10^2$	0.02	0.75	NT	NT	0	NT	0.04	0	0
	0	10	8.1×10^3	0.12	NT	NT	0.40	0	NT	0.05	0	NT
	25	8	4.3×10^4	1.00	1.23	NT	NT	0	NT	0.05	0	0
	47	12	1.7×10^6	>40	65.69	NT	NT	0	NT	0.05	0	0
	61	47	$<1.0 \times 10^2$	0.1	0.66	NT	NT	9	NT	2.06	0	0.9
	92	20	—	0.06	0.58	NT	NT	7.5	NT	1.31	1	36.5
21 (Fig. 3)	–24	9	1.0×102	0.32	0.38	NT	NT	0	NT	0.06	0	0
	0	11	3.0×10^3	5.24	NT	NT	6.92	0	NT	0.07	0	NT
	14	9	8.1×10^3	28.62	6.68	NT	NT	0	NT	0.07	0	0
	28	10	9.1×10^4	>40	26.83	NT	NT	0	NT	0.06	0	0
	44	10	7.9×10^6	>40	53.00	NT	NT	0	NT	0.06	0	0
	52	54	2.4×10^7	>40	80.37	NT	NT	0	NT	0.06	0	0
	60	2567	2.7×10^6	>40	73.29	NT	NT	8	NT	1.66	0	0
	63	2973	2.2×10^4	>40	12.1	NT	NT	8.5	NT	2.2	0	0.8
	66	2199	1.7×10^3	1.18	0.61	NT	NT	9.5	NT	2.36	0	1.4
	71	635	1.0×10^3	0.18	0.33	NT	NT	10.5	NT	2.44	0	1.7
	78	179	2.0×10^2	0.04	0.40	NT	NT	10	NT	2.50	0	1.3
	81	131	$<1.0 \times 10^2$	0.04	0.39	NT	NT	9.5	NT	2.44	0	1.0
	121††	20	—	0.02	0.45	NT	NT	8.5	NT	1.89	0	10.4

* Day (0) = 50-pool NAT-positive day; – = looked-back by stored samples.
† s/co: ≥1 = positive; <1 = negative.
‡ IU/mL: ≥0.05 = positive; <= 0.05: negative.
§ % inhibition: ≥50% = positive; <50% = negative.
‖ Index: ≥1.2 = positive; 0.8-1.2 = indeterminate; <0.8 = negative.
¶ 2^4 corresponds to 200 mIU/ml.
†† The interval was more than 32 days.
NT = not tested.

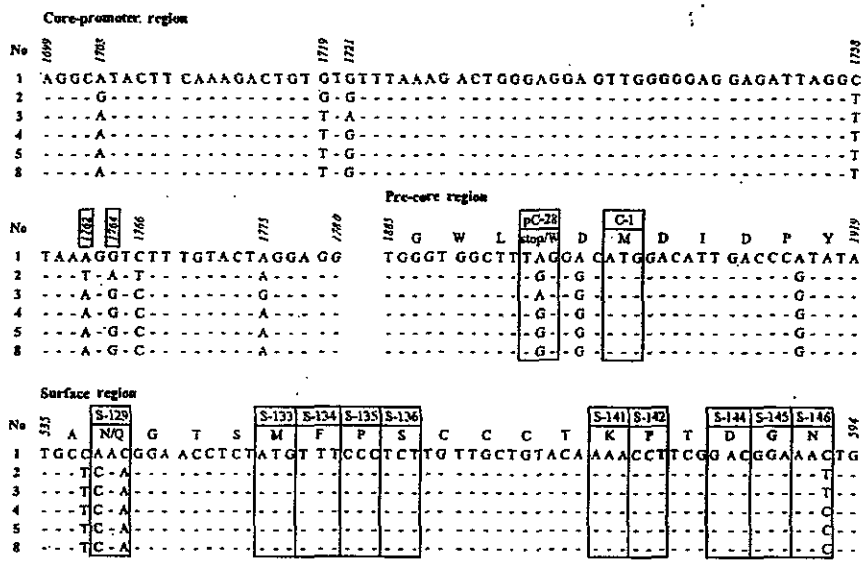

Fig. 4. The sequence of the HBV core-promoter (nucleotides 1699-1780), precore (nucleotides 1885-1919), and surface (nucleotides 535-594) region of Donors 1 to 5 and 8. The characters of HBV of all donors were genotype C and subtype adr. Donor 2 has core-promoter mutations (nucleotides 1762:A/T and 1764:G/A; nucleotides number are boxed). Donors 1 and 3 have precore mutations (nucleotide 1896; precore codon 28). The amino acids of precore and core are shown at the top of the sequences and precore codon 28 and first core codon: methionine are boxed. Donor 1 has an escape mutation (nucleotides 539:C/A and 541:A/C, codon 129:Q/N). The amino acids reported as escape mutations[5] are boxed and the number is shown at the top of the box. Nucleotide was numbered according to Okamoto and coworkers.[13]

REFERENCES

1. Biswas R, Tabor E, Hsia CC, et al. Comparative sensitivity of HBV NATs and HBsAg assays for detection of acute HBV infection. Transfusion 2003;43:788-98.
2. Stramer SL, Brodsky JP, Preston SB, et al. Comparative sensitivity of HBsAg and HBV NAT. Transfusion 2001;41: 8S-9S.
3. Biswas R, Busch M, Wright D, et al. Early detection of hepatitis B virus infection: comparative sensitivity of HBV DNA detection by PCR and HBsAg detection by a new generation of assays. Hepatology 2001;34:610A.
4. Kleinman SH, Busch MP. HBV amplified and back in the blood safety spotlight. Transfusion 2001;41:1081-5.
5. Yoshikawa A, Gotanda Y, Itabashi M, et al. Hepatitis B virus NAT positive blood donors in the early and late stage of HBV infection: analysis of the window period and kinetics of HBV DNA. Vox Sang 2005;88:77-86.
6. Minegishi K, Yoshikawa A, Kishimoto S, et al. Superiority of minipool nucleic acid amplification technology for hepatitis B virus over chemiluminescence immunoassay for hepatitis B surface antigen screening. Vox Sang 2003;84: 287-91.
7. Mine H, Emura H, Miyamoto M, et al. High throughput screening of hepatitis B virus, hepatitis C virus and human immunodeficiency virus type-1 by nucleic acid amplification testing with specific and sensitive multiplex reagent in Japan. J Virol Methods 2003;112:145-51.
8. Yugi H, Mizui M, Tanaka J, et al. Hepatitis B virus (HBV) screening strategy to ensure the safety of blood for transfusion through a combination of immunological testing and nucleic acid amplification testing—Japanese experience. J Clin Virol 2006;36 (Suppl. 1): S56-S64.
9. Japanese Red Cross NAT Screening Research Group. The first large scale nucleic acid amplification testing (NAT) of donated blood using multiplex reagent for simultaneous detection of HBV, HCV, HIV-1 and significance of NAT for HBV. Microbiol Immunol 2001;45:667-72.
10. Iizuka H, Ohmura K, Ishijima A, et al. Correlation between anti-HBc titers and HBV DNA in blood units without detectable HBsAg. Vox Sang 1992;63: 107-11.
11. Meng O, Wong C, Rangachari A, et al. Automated multiplex assay system for simultaneous detection of hepatitis B virus DNA, hepatitis C virus RNA and human immunodeficiency virus type 1 RNA. J Clin Microbiol 2001;39:2937-45.
12. Okamoto H, Yotsumoto S, Akahane Y, et al. Hepatitis B viruses with precore region defects prevail in persistently infected hosts along with seroconversion to the antibody against e antigen. J Virol 1990;64:1298-303.
13. Okamoto H, Tsuda F, Akahane Y, et al. Hepatitis B virus mutations in the core promoter for an e antigen-negative phenotype in carriers with antibody to e antigens. J Virol 1994;68:8102-10.
14. Fukuda R, Ishimura N, Kushiyama N, et al. Hepatitis B virus with X gene mutation is associated with the majority of serologically "silent" non-B, non-C chronic hepatitis. Microbiol Immunol 1996;40:481-8.
15. Allain JP. Occult hepatitis B virus infection: implications in transfusion. Vox Sang 2004;86:83-91.
16. Yuki N, Nagaoka T, Yamashiro M, et al. Long-term histologic and virologic outcomes of acute self-limited hepatitis B. Hepatology 2003;37:1172-9.
17. Günther S. Genetic variation in HBV infection: genotypes and mutants. J Clin Virol 2006;36 (Suppl 1):S3-S11.
18. Robinson WS. Hepatitis B virus and hepatitis D virus. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. 4th ed. New York: Churchill Livingstone; 1995. p. 1406-39.

19. Ogata N, Miller RH, Ishak KG, et al. The complete nucleotide sequence of a pre-core mutant of hepatitis B virus implicated in fulminant hepatitis and its biological characterization in chimpanzees. Virology 1993;194: 263-76.
20. Sato S, Suzuki K, Akahane Y, et al. Hepatitis B virus strains with mutations in the core promoter in patients with fulminant hepatitis. Ann Intern Med 1995; 122:241-8.
21. Kojima M, Shimizu M, Tsuchimochi T, et al. Posttransfusion fulminant hepatitis B associated with precore-defective HBV mutants. Vox Sang 1991;60:34-9.
22. Weinberger KM, Bauer T, Böhm S, et al. High genetic variability of the group-specific α-determinant of hepatitis B virus surface antigen (HBsAg) and the corresponding fragment of the viral polymerase in chronic virus carriers lacking detectable HBsAg in serum. J Gen Virol 2000;81: 1165-74.

医薬品 研究報告 調査報告書

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研究報告の概要

○遡及調査で特定されたB型肝炎ウイルスDNA値の低い血液製剤の感染性

背景:日本赤十字社(JRC)は、HBc抗体スクリーニングを1989年に、50検体ミニプール(MP)核酸増幅検査を2000年に導入した。B型肝炎表面抗原(HBsAg)発現前やMP-NATウインドウ期に採血された献血血液並びにオカルトHBV感染献血者由来の献血血液のB型肝炎ウイルス(HBV)伝播リスクを調べるために、系統的遡及調査が実施されている。

試験デザイン及び方法:JRCは、1996年以降、各献血血液の一部を保管している。この完全な保管検体システムに基づき、1997年～2004年の期間に得られた複数回献血者の全献血血液の遡及調査を行った。複数回献血者がHBVウイルスマーカー陽性となったときには、当該献血者の過去の献血血液の保管検体を個別(ID)-NATにより検査した。ID-NATのみ陽性である献血血液の頻度と、そのような血液由来の血液製剤の輸血によるHBV伝播リスクを検討した。

結果:15,721本の保管検体にHBV ID-NATを実施したところ、158検体 (1.01%) がHBV DNA陽性となった。この158検体のうち、95 (60%) はHBc抗体価の低い献血者由来であった。ID-NATのみ陽性の献血血液由来製剤を輸血された63名のうち、HBV感染が確認されたのは12名(19%)であった。HBc抗体価の低い血液製剤33製剤のうち感染性が特定されたのは1製剤のみであったが、HBc抗体陰性製剤では、22製剤中11製剤に感染性が認められた。HBs抗体陽性であると特定された16製剤のうち、血清学検査により感染の証拠を示した製剤はなかった。

結論:JRCのスクリーニングシステムをすり抜けたHBc 抗体価の低いオカルトHBV感染者由来の血液製剤を原因とする臨床的に観察されたHBV感染リスクは、HBsAg発現前やMP-NATウインドウ期に献血された血液の伝播リスクよりも10倍以上低い。

報告企業の意見	今後の対応
日本赤十字社のスクリーニングシステムをすり抜けたHBc抗体価の低いオカルトHBV感染者由来の血液製剤を原因とするHBV感染リスクは、HBsAg発現前やMP-NATウインドウ期に献血された血液の伝播リスクよりも10倍以上低いとの報告である。	日本赤十字社では、HBs抗原検査及びHBc抗体検査を実施することに加えて、HBVについて20プールでスクリーニングNATを行い、陽性血液を排除している。HBV感染に関する新たな知見等について今後も情報の収集に努める。また、これまでの凝集法と比べて、より感度の高い化学発光酵素免疫測定法(CLEIA)の導入を予定している。NATの精度向上についても評価・検討している。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

Infectivity of blood components with low hepatitis B virus DNA levels identified in a lookback program

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BACKGROUND: Japanese Red Cross (JRC) blood centers implemented anti-hepatitis B core antigen (HBc) screening in 1989 and 50-minipool (MP)-nucleic acid testing (NAT) in 2000. A systematic lookback study has been conducted to determine the hepatitis B virus (HBV) transmission risk of donations drawn in the pre-hepatitis B surface antigen (HBsAg) and/or MP-NAT window phase and by donors with occult HBV infection.

STUDY DESIGN AND METHODS: JRC blood centers have been storing aliquots of every blood donation since 1996. On the basis of the complete repository tube archives, all donations from repeat donors received from 1997 to 2004 were subjected to a lookback study. When repeat donors turned positive for HBV viral marker(s), repository tubes from their previous donations were tested for HBV with individual-donation (ID)-NAT. The frequency of ID-NAT-only–positive donations and the HBV transmission risk by the transfusion of those components were investigated.

RESULTS: HBV ID-NAT was performed on 15,721 repository tubes, and 158 tubes (1.01%) were found positive for the presence of HBV DNA. Of these 158 ID-NAT-only–positive donations, 95 (60%) were derived from carriers with low anti-HBc titers. Of 63 patients transfused with ID-NAT-only–positive components, 12 (19%) proved to be infected with HBV. Only 1 of 33 components with low anti-HBc titers could be identified as infectious, whereas 11 of 22 anti-HBc–negative components proved to be infectious. None of the 16 identified hepatitis B surface antibody–positive components showed serologic evidence of infection.

CONCLUSION: The clinically observed HBV infection risk caused by blood components from occult HBV carriers with low anti-HBc titers who slip through the JRC screening system is more than 10-fold lower than the transmission risk by donations in the pre-HBsAg and/or MP-NAT window phase.

Nucleic acid testing (NAT) for hepatitis C virus (HCV) and human immunodeficiency virus (HIV) has been implemented in developed countries for screening blood donated during the window period and plays a critical role in excluding infectious blood components.[1,2] For hepatitis B virus (HBV), Japanese Red Cross (JRC) blood centers have been screening blood with hemagglutination for hepatitis B surface antigen (HBsAg) detection combined with anti-hepatitis B core antigen (anti-HBc) and hepatitis B surface antibody (anti-HBs) testing since 1989 and implemented 50-member-pool NAT (50-NAT) for HBV, HCV, and HIV in February 2000.[3,4] There is still a residual risk of transfusion-transmitted viral infection (TTI), however, because of the limited sensitivity and use of pooled samples in current NAT systems.[5] A precise evaluation of residual risk after NAT implementation is essential in determining whether further strategies for preventing TTI are warranted (e.g., individual donation [ID]-NAT or pathogen reduction).[6,7]

To investigate the cause of reported TTI and to identify and retrieve virus-containing components, JRC has been storing aliquots of every blood donation since 1996 with a plan to preserve repository aliquots for 11 years. A

ABBREVIATIONS: 50-NAT = 50-member-pool nucleic acid testing; HBs = hepatitis B surface antibody; HI = hemagglutination inhibition; ID = individual donation; JRC = Japanese Red Cross; MP = minipool; TTI = transfusion-transmitted viral infection.

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systematic lookback study has been conducted in which repository tubes from previous donations were investigated for the presence of HBV, HCV, or HIV sequences by ID-NAT when repeat donors turned positive for viral antigens, anti-viral antibodies, or screening 50-NAT.[8] In the present lookback study, the proportion of ID-NAT–reactive donations that are not detected by the current JRC blood screening algorithm has been established and the clinically observed HBV transmission risk of the components derived from these donations has been investigated.

MATERIALS AND METHODS

In JRC blood centers, all donated blood samples are first screened for HBsAg by reversed passive hemagglutination,[9] the sensitivity of which is 3 ng per mL. HBsAg-negative samples are further screened for anti-HBc and anti-HBs by hemagglutination inhibition (HI) and particle agglutination, respectively. Only blood having a high anti-HBs titer ($\geq 2^4$ dilution equivalent to 200 mIU/mL) or no or a low anti-HBc titer (HI titer $\leq 2^4$ dilution) is defined as seronegative. The combination of the above serologic screening tests excludes HBsAg-positive donors and most occult carrier donors. Because the cutoff point for HI is set at a higher level than that for enzyme immunoassay (EIA) anti-HBc, there are donations that are accepted while having low anti-HBc titer detectable by EIA but not by HI. Fifty seronegative samples are then pooled for NAT. JRC NAT screening employs a polymerase chain reaction (PCR) system (AmpliNAT, Roche, Indianapolis, IN) that utilizes multiplex reagents for detecting HBV, HCV, and HIV genomes. The detection range limit (95% confidence interval) for HBV is 22 to 60 copies per mL.[10] The sensitivity of current 50-NAT is expected to be 50-fold lower than that described above, that is, 1100 to 3000 copies per mL.

All donations from repeat donors received from 1997 to 2004 were subjected to a lookback study when a subsequent donation turned positive for a 50-NAT, HBsAg, or anti-HBc. Repository samples from donors immediately preceding donations that became positive for the presence of the markers described above were routinely analyzed by ID-NAT. Donors exhibiting a subsequent donation with anti-HBc reactivity above 2^4 dilution in the absence of anti-HBs, or donors with a decrease in their anti-HBs reactivity (to below 2^4 dilution) in the presence of a positive anti-HBc result, were also defined as anti-HBc converters and included in the study. The process of ID-NAT was as follows. Test samples were first screened with the same reagent and method as AmpliNAT except that the system contained a capture probe only for HBV. Positive samples were then subjected to dual-repeat ID-NAT. Doubly positive (+/+) and singly positive (+/–) samples were defined as ID-NAT–positive and further subjected to quantitative NAT. The HBV genome was quantified by a JRC in-house method that utilizes TBF-1 (nucleotides 250-272; 5′-AGACTCGTGGTGGACTTCTCT CA-3′), TBR-1 (nucleotides 428-409; 5′-TGAGGCATAGCA GCAGGATG-3′), and TP-02 (nucleotides 368-392; 5′-TATC GCTGGATGTGTCTGCGGCGTT-3′) as F-primer, R-primer, and probe, respectively. The sensitivity of the quantitative NAT was 100 copies per mL. Samples verified to be ID-NAT–positive were reevaluated for anti-HBc by EIA (AxSYM, Abbott Japan, Tokyo, Japan) as well as semiquantitative HI and for anti-HBs by EIA (AxSYM). Only samples exhibiting concordant results for anti-HBc with $2^1 \leq$ HI $\leq 2^4$ and EIA positivity (>50% inhibition) were defined as samples with low anti-HBc titers. To substantiate the infectious status of the donors in the ID-NAT-only–positive donation, repository tubes obtained in the subsequent donations were also evaluated for HI, EIA-anti-HBc, EIA-HBsAg, and EIA-anti-HBs.

The risk analysis for HBV contamination in blood components was limited to donations obtained from February 1, 2000, to January 31, 2004, all of which had been qualified by the current screening algorithm including 50-NAT. For the analysis of the infectivity of HBV-containing units, components that had not been tested by 50-NAT were also used in the study.

ID-NAT results for the preceding donation were sent with an evaluation form for viral contamination risk to the medical facility that used the donation. On the form, the degrees of viral contamination risk were described with the following classification: the donation was 1) ID-NAT–positive; 2) ID-NAT–negative but with probable HBV contamination because the blood was donated during the potential window period (the window period was defined as 46 days according to the Japanese guideline for lookback investigation issued by the Ministry of Health, Labour and Welfare); 3) ID-NAT–negative but with possible HBV contamination even though the time interval between the preceding donation and the marker conversion was long (>46 days); or 4) ID-NAT–negative and unlikely to have viral contamination because the test result for the subsequent donation was verified to be positive because of the alteration of the cutoff point for anti-HBc during the period between the two donations. The clinical outcome of patients transfused with ID-NAT–positive components was collected by local blood centers. The testing of blood samples from transfused patients, the initial diagnosis of HBV-TTI, and the report of suspected cases to blood centers were basically conducted by physicians who treated the patients. The diagnostic bases for TTI was therefore not uniform regarding the HBV markers tested, the observational period after index transfusion, and the time interval between transfusion and blood testing. In most medical institutions, the presence of anti-HBc or anti-HBs in pretransfusion samples is usually not evaluated. In all documented HBV transmission cases, the diagnosis of HBV infection was established on the basis of

TABLE 1. Numbers of ID-NAT–positive donations and positivities for anti-HBc in repository tubes

Converted markers	Projected number of converted donations for 4-year period	Number of repository tubes positive for ID-NAT within 4 years	Anti-HBc status of ID-NAT–positive repository sample		
			Low titer	Negative	Not tested
50-NAT	329	28 (8.5%)*	13	13	2
HBsAg†	730	16 (2.2%)*	3	13	0
Anti-HBc	14,662	114 (0.78%)*	79	34	1
Total	15,721 (observed number)	158 (1.01%; 39.5/year)	95 (60%)	60 (38%)	3 (2%)

* Percentage of observed ID-NAT–positive samples among projected number of samples for which ID-NAT was performed.
† HBsAg conversion includes those accompanied by anti-HBc seroconversion.

the HBsAg conversion, anti-HBs seroconversion, or HBV DNA conversion.

TABLE 2. Relationship between EIA-anti-HBs and HBV genomic copy number

EIA value for anti-HBs (mIU/mL)	Copies/mL (number of samples)
EIA = 0	<100 (14), 160, 200, 220, 230 (2), 300 (2), 380
0 < EIA < 5.0	<100 (11), 100, 120
EIA ≥ 5.0 (range, 5.7-179.2; median, 24.9)	<100 (18)

RESULTS

Lookback study for HBV

Of the repository tubes that had been aliquoted from the donations obtained from February 1, 2000, to January 31, 2004, a total of 15,721 were subjected to ID-NAT (Table 1). All the donations had been qualified with the current screening algorithm including 50-NAT. Although the total number of tubes investigated by ID-NAT is evident because ID-NAT was performed in one laboratory, the numbers for each of the HBsAg–, 50-NAT–, or anti-HBc–converted repeat donors are not available because of a data management problem where the detail of lookback process for each conversion case was not recorded on a centralized computer system. The exact number for each of the three categories was summed up only for the period between June 13, 2002, and July 21, 2003. From the extrapolation of these 404 days of data, the number of repeat donors who became positive for the presence of the three markers during the 4-year period was estimated (Table 1).

Of the estimated 329 prior donations that became positive for the presence of HBV DNA by 50-NAT in the subsequent donation, 28 (8.5%) were verified to be positive for the presence of HBV DNA by ID-NAT (Table 1). Viral concentrations were available for 18 samples and were fewer than 380 copies per mL. A low anti-HBc titer below the screening cutoff value ($\leq 2^4$ by HI test) was detected in 13 of 26 cases evaluated but not in the remaining 13 (Table 1). This result suggests that a significant number of occult carriers have a fluctuating low-level viremia. Thirteen of the 16 ID-NAT–positive donations in which subsequent donations became HBsAg-positive were thought to have been obtained during the window period because they were anti-HBc–negative (Table 1). The remaining three contained a low anti-HBc titer and were considered to be derived from chronic carriers. Seventy-nine of the 114 ID-NAT–positive donations exhibiting a subsequent donation with anti-HBc seroconversion were considered to have been derived from occult carriers having low anti-HBc titers (Table 1); 82 percent (65/79) of these carrier donors were more than 50 years of age at the time of the repository sample. Thirty-four donations with negative anti-HBc reactivity were thought to have been obtained during the window period of acute infection. These units were donated mostly by young people, with 68 percent (23/34) less than 50 years of age.

In summary, during the 4-year study, we identified 158 HBV ID-NAT-only–positive blood donations that corresponded to 1.01 percent of samples for which ID-NAT was performed. Ninety-five (60%) of them were donated by occult carriers with low-titer anti-HBc and 60 (38%) by anti-HBc–negative or window-period donors (Table 1). Thus, of the repeat donors in Japan, there are an estimated 39.5 donations every year that are ID-NAT-only–positive. Viral concentrations were generally very low in occult carrier donors with 75 (95%) of the 78 samples studied having fewer than 100 copies per mL and only three having at least 100 copies per mL (data not shown). Donations obtained during the window period contained higher viral DNA levels with 12 of the 46 samples studied containing at least 100 copies per mL (range, 100-860 copies/mL).

Anti-HBs titer was measured with EIA in 53 repository tubes for which clinical outcome as a result of transfusion were available (see below). When anti-HBs titer was divided into three categories (=0, between 0 and 5, and ≥5 mIU/mL), there was no sample that contained at least 100 copies per mL of HBV DNA in the category of anti-HBs of at least 5 mIU per mL (Table 2).

Infectivity of ID-NAT–positive component

To establish the infectivity of the ID-NAT-only–positive components, we included donations obtained from 1997

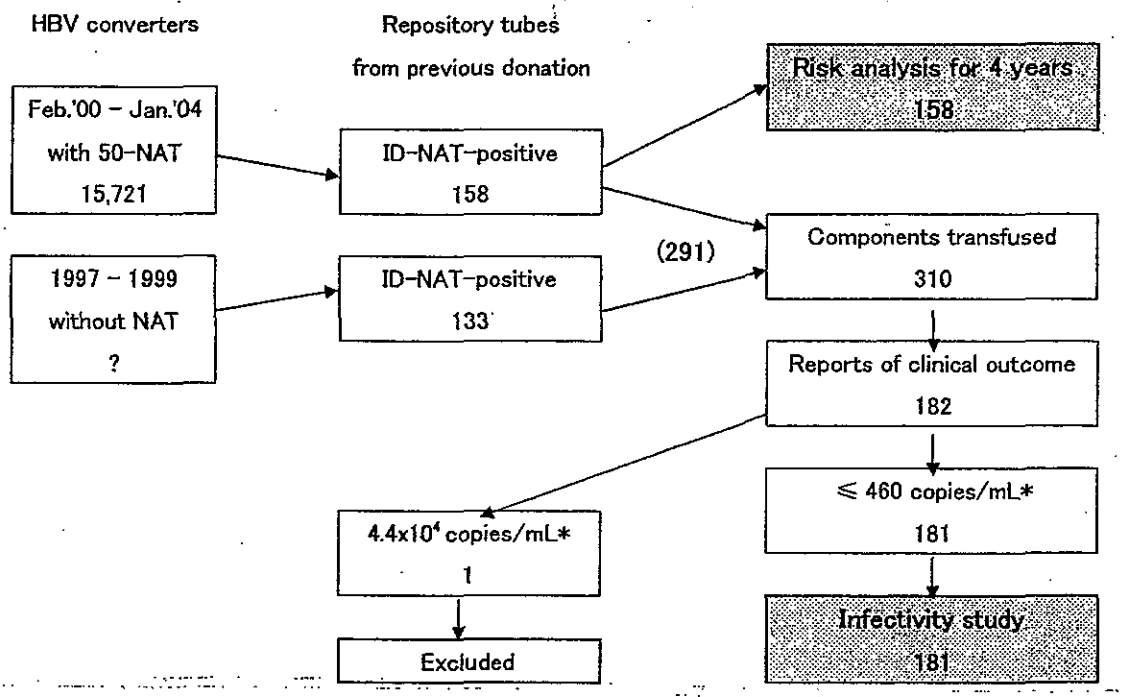

Fig. 1. Donations with HBV conversion obtained from 1997 to 2004. *HBV DNA copy number in repository tubes from prisero-conversion donation.

to 2004 in the analysis (Fig. 1). A total of 291 donations were determined to be ID-NAT–positive and an estimated 310 components were prepared from these donations and distributed to medical institutions. Through the hemovigilance system, we received 182 reports as of August 2005 that showed the clinical outcomes of the transfusions of the components. Although the donations given before February 2000 were not screened by 50-NAT, it is highly unlikely that the ID-NAT–positive donations before February 2000 would have been disqualified by 50-NAT screening considering the very low viral load in these samples (≤460 copies/mL, except for one case). Therefore, they are also referred to in this article as ID-NAT-only–positive donations. One sample donated before NAT screening that contained 4.4×10^4 copies per mL HBV DNA was excluded from this study.

TABLE 3. Clinical outcome of 181 patients transfused with ID-NAT-only–positive components

Clinical outcome	Number of patients (n = 181)
Infected through transfusion	12
Positive for the presence of HBsAg	7
Positive for the presence of HBV DNA	2
Positive for the presence of anti-HBs	3
Positive after transfusion without pretransfusion testing	7
Infected before transfusion	7
No evidence of infection	51
Expired	104

Twelve of the 181 reports were confirmed to be HBV-TTI cases (Table 3); of these, 7 became positive for the presence of HBsAg, 2 became positive for the presence of HBV DNA, and 3 showed anti-HBs seroconversion. All these patients had negative results for either HBsAg or HBV DNA before transfusion. HBV DNA sequences were identical between the blood donors and the transfused patients in all the 8 pairs studied. Seven patients who were positive after transfusion for the presence of anti-HBc (2 patients), anti-HBs (3 patients), or both markers (2 patients) were not classified as TTI cases because of the lack of pretransfusion HBV testing data. The other 7 patients who were also positive for the presence of HBV markers after index transfusion were actually found to have anamnestic HBV infection before transfusion. Fifty-one patients showed no evidence of HBV infection after index transfusion. Because no anti-HBc testing was performed after transfusion in any of the 51 patients, we may have overlooked cases that showed positivity only for anti-HBc as a result of infection. A total of 104 patients had died without any test results recorded regarding HBV infection. Thus, 12 of 63 (of 12 infected and 51 noninfected) patients acquired HBV infection as a result of the transfusion of ID-NAT-only–positive components, suggesting the infectivity of the components to be 19 percent. If all of the 7 positive patients without pretransfusion data were