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Blood donor screening: how to decrease the risk of transfusion-transmitted hepatitis B virus?

Christoph Niederhauser[a], *Behrouz Mansouri Taleghani*[a,b], *Mauro Graziani*[a], *Martin Stolz*[a], *Caroline Tinguely*[a], *Philippe Schneider*[b]

[a] Regional Blood Transfusion Service Berne, Berne, Switzerland
[b] Department of Haematology and Central Haematology Laboratory, Division of Transfusion Medicine, Inselspital, University Hospital and University of Berne, Berne, Switzerland
[c] Regional Blood Transfusion Service Vaud, Lausanne, Switzerland

Summary

Questions under study: The risk of transfusion-transmitted HBV remains significant in Switzerland, where routine screening for hepatitis B virus (HBV) in blood donations relies solely on serological hepatitis B surface antigen (HBsAg) testing. This study was designed to determine the prevalence of anti-hepatitis B core (anti-HBc) and HBV nucleic acid testing (NAT) positive donations in two different Swiss donor populations, to help in deciding whether supplemental testing may bring additional safety to blood products.

Methods: In a first population of donors, 18143 consecutive donations were screened initially for HBsAg, anti-HBc (with one EIA assay) and with HBV NAT in minipools of 24 donations. The screening repeatedly reactive anti-HBc donations were then "confirmed" with two supplemental anti-HBc assays, an anti-hepatitis B surface assay (anti-HBs) and with single donation HBV NAT.

In a second population of donors, 4186 consecutive donations were screened initially with two different anti-HBc assays in addition to the mandatory HBsAg screening test. The screening repeatedly reactive donations with at least one anti-HBc assay were tested for anti-HBs.

Results: In the first subset of 18143 donations, 17593 (97.0%) were negative for HBsAg, anti-HBc and HBV NAT in minipools. 549 (3.0%) were HBsAg and HBV NAT negative, but repeatedly reactive for anti-HBc. Of these 549 donations, 287 could not be "confirmed" with two additional anti-HBc assays and were negative with an anti-HBs assay, as well as with single donation HBV NAT. Only 211 (1.2% of the total screened donations) were "confirmed" positive with at least one of two supplemental anti-HBc assays. One repeatedly reactive HBsAg donation, from a first-time donor, was confirmed positive for HBsAg and anti-HBc, as well as with single donation HBV NAT.

In the second subset of 4186 donations, 4014 (95.9%) were screened negative for HBsAg and for anti-HBc, tested with two independent anti-HBc assays. 172 donations (4.1%) were HBsAg negative but repeatedly reactive with at least one of the two anti-HBc assays. Of these 172 samples, 86 were reactive with the first anti-HBc assay only, 13 were reactive with the second anti-HBc assay only and 73 (1.7% of the total screened donations) were "confirmed" positive with both anti-HBc assays.

Conclusion: The prevalence of anti-HBc "confirmed" positive donations in the two Swiss blood donor populations studied was low (<2%) and we found only one HBV NAT positive (HBsAg positive) donation among more than 18000. Concerning blood product safety, an increase in the deferral rate of less than 2% of anti-HBc positive, potentially infectious donors, would in our opinion make routine anti-HBc testing of blood donations cost-effective. There is however still a need for more specific assays to avoid an unacceptably high deferral rate of "false" positive donors. In contrast, the introduction of HBV NAT in minipools gives minimal benefit due to the inadequate sensitivity of the assay.

It remains to evaluate more extensively the value of individual donation NAT, alone or in addition to anti-HBc, as supplemental testing in the context of several Swiss blood donor populations.

Key words: blood transfusion; HBV screening; HBsAg; anti-HBc; anti-HBs; HBV NAT

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Introduction

Expanded blood donor selection procedure and improved laboratory detection of viral markers have reduced the risk of transfusion transmitted viral infections. Among the most relevant viruses, ie hepatitis C virus (HCV), human immunodeficiency viruses 1 and 2 (HIV-1, HIV-2) and hepatitis B virus (HBV), the current calculated theoretically residual risk of a transfusion transmitted infection is highest for HBV, namely approximately 7 per million donations in Switzerland [1]. The main reason for this relatively high risk is that screening for HBV relies solely on HBsAg testing. In contrast, the present residual risk for HCV and HIV-1 is less than 1 per 2 million donations, because of the implementation of corresponding nucleic acid testing (NAT) in the late 1990s. In the light of these successes, attention has returned recently to HBV, prompting significant efforts to understand and estimate residual risk for this virus and to develop improved HBV screening strategies.

For many years it has been known that there are several reasons which may account for the residual risk of transmission of HBV through transfusion. HBsAg assays are not sensitive enough in the very early phase (window phase of 45–56 days), in the early convalescence phase (core window) of acute HBV infections and in chronic HBV infections, where HBsAg is often present at very low levels [2–15]. Further, mutants with genetic differences in the "a" determinant region of the gene of the virus may allow HBsAg to escape detection by the currently available HBsAg screening assays [16–23].

Potential HBV infectious blood donations, which are negative for HBsAg, may be identified by either anti-HBc assay or HBV NAT [24]. Anti-HBc testing was introduced in several countries (eg USA, Japan and France) in the 1980s as a surrogate test for so-called non-A, non-B hepatitis. However, in other western countries, where the prevalence of HBV infections is low, a large proportion of anti-HBc reactive blood donations may be false positive due to lack of specificity of the available assays [25–27]. On the other hand, HBV NAT was introduced in some countries to overcome the window phase of HBsAg assays [28–34].

The aim of the present study was to determine the prevalence of anti-HBc/HBV DNA "confirmed" positive donations in a population of more than 22 000 Swiss blood donors from two different regions. These data are essential for strategic decisions on revision of the HBV screening algorithm for blood donations in Switzerland.

Material and methods

Donations and donors

All blood donations were given by volunteer donors of the Swiss Red Cross Blood Transfusion Service (SRC BTS). Autologous donations were excluded from the study. Repeat donors were defined as persons who were already tested in a BTS and first time donors as persons who were not yet tested. All donors gave informed consent to inclusion in the study at the time of donation and follow-up and contributed only one donation. The project was approved by the Government Ethics Committee of the State of Berne.

In the first part of the study a total of 18 143 consecutive donations collected from 18 January to 23 March 2005 from repeat and first-time donors of the Blood Transfusion Service Berne (BTS BE) were screened for anti-HBc (one anti-HBc EIA assay) and HBV DNA in minipools of 24 blood donations, in addition to the mandatory HBsAg screening test. Of these donations, 17 361 (95.7%) were from repeat donors and 782 (4.3%) were from first-time donors. Since 1999, all first-time donors of BTS BE have been tested for anti-HBc. From 1999 to 2002 all anti-HBc positive donors, with anti-HBs concentrations below 100 IU/ml were deferred. Since summer 2002, all anti-HBc positive donors, regardless of anti-HBs concentration, have been deferred.

In the second part of the study a total of 4186 consecutive donations collected between 13 June and 11 August 2005 from repeat (90%) and first-time donors (10%) of the Blood Transfusion Service Vaud (BTS VD), not previously tested for anti-HBc, were screened with two different anti-HBc assays, in addition to the mandatory HBsAg screening test.

Testing

All laboratory tests were performed at the BTS BE.

HBsAg screening

All donations from the BTS BE and the BTS VD were screened with the Enzygost HBsAg Integral 5.0 assay (Dade Behring, Marburg, Germany). HBsAg repeatedly reactive donations were confirmed by a neutralisation assay (Axsym HBsAg Confirmatory, Abbott, Delkenheim, Germany).

Anti-HBc screening

Donations from the BTS BE were screened with the Enzygnost anti-HBc monoclonal assay (Dade Behring, Marburg, Germany). Donations from the BTS VD were screened in parallel with the Enzygnost anti-HBc monoclonal assay and the Monolisa anti-HBc Plus (Biorad, Marnes la Coquette, France).

NAT screening

Donations from the BTS BE were screened in minipools of 24 with the Cobas Ampliscreen HBV PCR test (Roche Diagnostics, Rotkreuz, Switzerland).

Minipooling

Minipools consisting of a maximum of 24 samples were generated overnight with two Tecan Genesis

RSP 200/8 pipetting machines (Tecan Schweiz AG, Männedorf, Switzerland) which transferred 150 µl from each sample into a barcoded 13 ml sample tube. Two back-up plates were filled with 850 and 700 µl respectively of the corresponding plasma samples, to resolve any HBV NAT positive minipools found. Positive samples were confirmed by single donation NAT.

Nucleic acid extraction and PCR

Minipool tubes were vortexed at full speed for several seconds. Subsequently, minipool tubes were centrifuged for five minutes at 2300 g in order to prevent the tips in the pipetting machine from clotting. One millilitre (1000 µl) was used for the nucleic acid extraction procedure. Extraction was performed with the QIAamp 96 Virus BioRobot testkit (Qiagen, Hilden, Germany) adapted for use on a Tecan pipetting machine.

Fifty µl of DNA extract was mixed with 50 µl of prepared mastermix and subjected to polymerase chain reaction (PCR), followed by DNA detection on the COBAS Amplicor test system (Roche Diagnostics, Rotkreuz, Switzerland). The sensitivity limit of this system is 240 copies (45 IU)/ml for an individual donation in the minipool. Sample identification and results management were performed with the NADIS pooling management software (Grawunder Software & Kommunikationstechnik GmbH, Kassel, Germany).

Anti-HBc "confirmation"

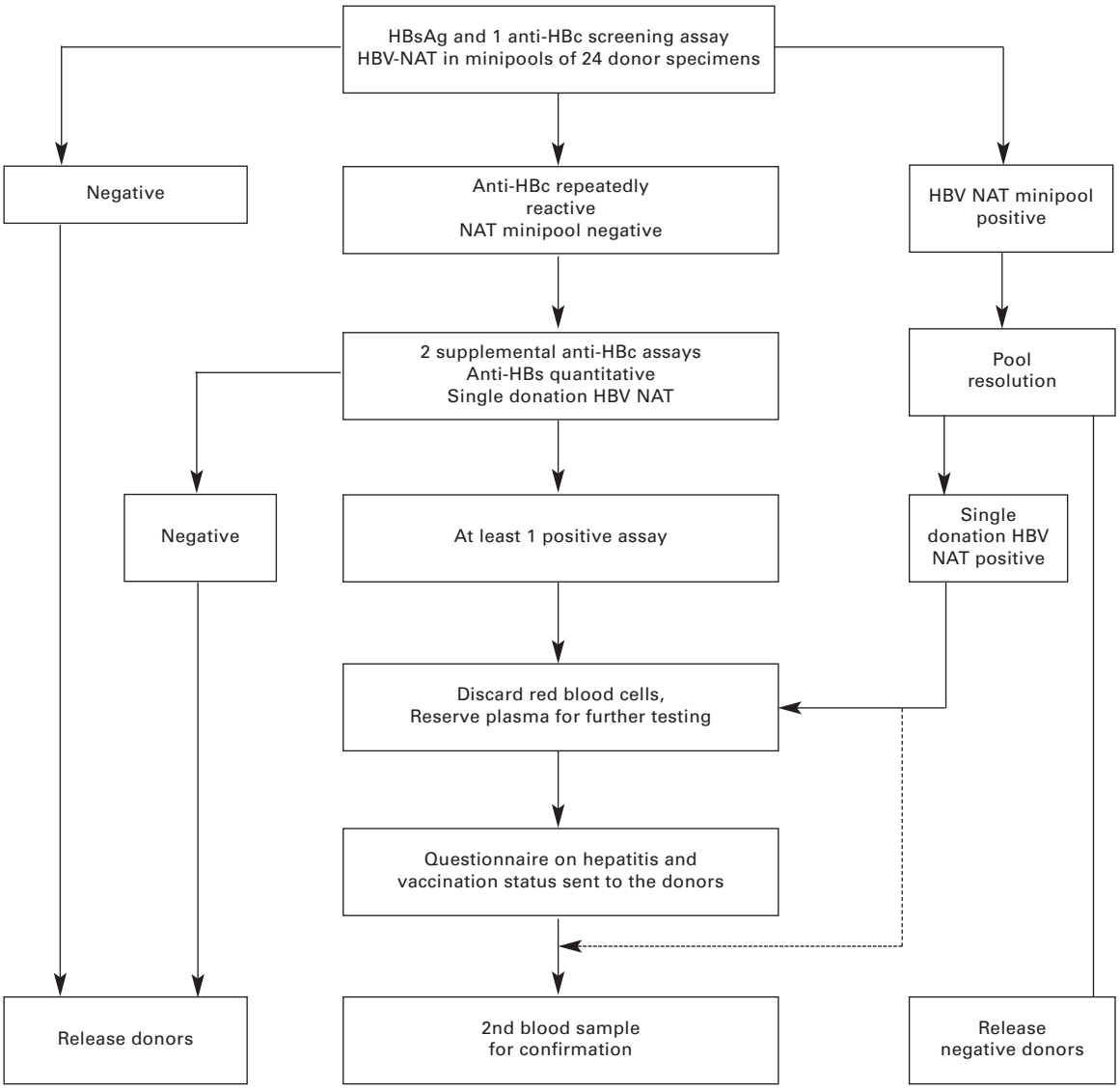
The repeatedly reactive anti-HBc donations from the BTS BE were tested by two other independent anti-HBc assays, the Monolisa anti-HBc Plus and the Core Axsym (Abbott, Delkenheim, Germany). In addition, the quantitative anti-HBs assay AUSAB (Abbott, Delkenheim, Germany) was performed and individual donation samples were tested by the Cobas Ampliscreen HBV PCR test (Roche, Rotkreuz, Switzerland) at a sensitivity level of twelve copies (2.4 IU/ml). Anti-HBc screening repeatedly reactive donations and repeatedly reactive with at least one of both supplemental anti-HBc assays were considered anti-HBc "confirmed" positive. Anti-HBs concentrations of less than 10 IU/ml were considered negative.

A questionnaire was sent to the 262 donors, who were repeatedly reactive in anti-HBc screening, asking whether they had been aware of ever suffering from hepatitis or whether they were vaccinated for HBV. An overview of the complete testing algorithm for the Bernese donor population is shown in figure 1.

For the anti-HBc repeatedly reactive donations from the BTS VD with one or two anti-HBc assays, the quantitative anti-HBs AUSAB assay was performed. Anti-HBc repeatedly reactive donations with both assays were considered anti-HBc "confirmed" positive. Anti-HBs concentrations of less than 10 IU/ml were considered negative.

Figure 1

Test algorithm for the study at the BTS BE.



Results

BTS BE: All test results are summarised in table 1. From the 18143 consecutive donations collected at the BTS BE, 17593 (97.0%) were screened negative for HBsAg and anti-HBc, and with HBV NAT in minipools of 24. Only 1/891 (0.1%) tested NAT minipools was positive. 550 donations were repeatedly reactive in screening with the Enzygnost anti-HBc assay. 549 (3.0%) were HBsAg negative. Of these 549 donations, 287 (52.3%) were negative with all supplemental tests conducted (ie 2 anti-HBc and 1 anti-HBs assays, as well as HBV NAT in individual donations). 262 were anti-HBc repeatedly reactive in anti-HBc screening. 51 of the 262 were negative with the two supplemental anti-HBc assays but positive for anti-HBs. Finally, only 211 (38.4%) HBsAg negative donations, which were repeatedly reactive in anti-HBc screening, were also reactive with at least one of two supplemental anti-HBc assays. One HBsAg and HBV NAT confirmed positive donation was repeatedly reactive with three anti-HBc assays and positive for anti-HBs with a concentration of 10 IU/ml (table 1). A blood sample from the same donor drawn one month later revealed a viral load of 5582 geq/ml.

The prevalence of the anti-HBc "confirmed" positive donations (reactive with at least one of the two additional anti-HBc assays) in the screened donor population of the BTS BE was 1.2%.

A comparison of the cut-off levels from the screening anti-HBc assay shows that there was a clear separation between low-level and high-level reactors. High-level reactors (mean value 0.100 S/Co) were "confirmed" with both the additional anti-HBc and an anti-HBs assay. Low level reactors (mean value 0.778 S/Co) were only reactive with the screening anti-HBc assay but negative with the two additional anti-HBc assays and negative with the anti-HBs assay.

Of the 550 anti-HBc repeatedly reactive donations in screening, 324 were anti-HBs negative and 226 were positive. Of these latter, 32 had anti-HBs concentrations below 100 IU/ml, 88 had concentrations between 100 and 1000 IU/ml and 106 had concentrations greater than 1000 IU/ml (table 2).

The response rate to the questionnaires circulated to the donors was 93.4% (243 out of 263). 225 donors said they had never had hepatitis, 10 stated they had had hepatitis and 8 did not know. All 10 donors who said they had had hepatitis were reactive with all 3 anti-HBc assays and anti-HBs positive. 65 of the 243 donors were vaccinated and 178 were not. Interestingly, of the 51 anti-HBc repeatedly reactive donations in screening, which were anti-HBs positive but negative

Table 1

Testing results of the 18143 donations from BTS BE; anti-HBc testing was performed with 3 different assays (1) anti-HBc Enzygnost from Dade Behring, (2) anti-HBc Monolisa from Biorad and (3) anti-HBc from Abbott.

HBsAg	Anti-HBc (1)	Anti-HBc (2)	Anti-HBc (3)	Anti-HBs	NAT in minipools of 24	NAT in single donation	Status of vaccination yes	Status of vaccination no	No answer	Number	
–	–	nd	nd	nd	–	nd	nd	nd	nd	17593	
–	+	–	–	–	–	–	nd	nd	nd	287	549*
–	+	–	–	+	–	–	38	8	5	51	262*
–	+	+	–	–	–	–	1	23	1	25	211*
–	+	+	–	+	–	–	3	1	0	4	
–	+	–	+	–	–	–	0	3	1	4	
–	+	–	+	+	–	–	1	2	0	3	
–	+	+	+	–	–	–	0	8	0	8	
–	+	+	+	+	–	–	22	132	13	167	
+	+	+	+	+	+	+	0	1	0	1	

nd: not done
* numbers: see text of result section

Table 2

Concentrations of anti-HBs compared to the results of anti-HBc assays for donations from BTS BE.

Anti-HBs concentration	3 out of 3 anti-HBc assays repeated reactive	Only 2 out of 3 anti-HBc assays repeated reactive	Only 1 out of 3 anti-HBc assays repeated reactive
Negative	8	29	287
<100 IU/ml	17	1	14
100–1000 IU/ml	64	3	21
>1000 IU/ml	87	3	16

Table 3

Testing results of the 4186 donations from BTS VD; anti-HBc testing was performed with 2 different assays, (1) anti-HBc Enzygnost from Dade Behring and (2) anti-HBc Monolisa from Biorad.

HBsAg	Anti-HBc (1)	Anti-HBc (2)	Anti-HBs	Number
–	–	–	nd	4014
–	+	–	–	71
–	+	–	+	15
–	–	+	–	12
–	–	+	+	1
–	+	+	–	15
–	+	+	+	58

nd: not done

with the two supplemental anti-HBc assays, 38 were from vaccinated donors (all of them had anti-HBs concentrations >100 IU/ml), and 8 were from non-vaccinated donors (2 having concentrations >1000 IU/ml, 4 >500 IU/ml and 2 <100 IU/ml respectively). Five donors did not return the questionnaire. Of the 7 donors whose donations were screened anti-HBc repeatedly reactive and were also reactive with one of the two additional anti-HBc assays as well as positive for anti-HBs, 4 were vaccinated and 3 were not.

BTS VD: Of the 4186 consecutive donations which were screened for HBsAg and with two anti-HBc assays, 4014 (95.9%) were negative for HBsAg and anti-HBc with both assays (table 3). 172 (4.1%) were repeatedly reactive with at least one anti-HBc screening assay. Of these 172 donations, 86 were repeatedly reactive with the Enzygnost anti-HBc assay only, 13 with the Monolisa anti-HBc assay only, and 73 with both anti-HBc assays. Thus, the prevalence of the anti-HBc "confirmed" positive donations (both anti-HBc assays reactive) in the screened donor population of the BTS VD was 1.7%.

Of the 86 donations repeatedly reactive with the Enzygnost assay only, 16 were positive for anti-HBs. Of the 13 donations repeatedly reactive with the Monolisa assay only, 1 was positive for anti-HBs. 58 of the 73 donations repeatedly reactive with both assays were positive for anti-HBs (table 3), and the majority (51 out of 58) had anti-HBs concentrations greater than 100 IU/ml. In contrast, if only one anti-HBc assay was repeatedly reactive, the majority of the donations (84 out of 99) had concentrations below 100 IU/ml (table 4). No data on hepatitis history and vaccination status were obtained from the corresponding set of donors.

Discussion

General

The HBsAg test is at present the only mandatory HBV screening tool for blood donations in Switzerland. However, donations from seroconverting donors and from chronic HBV carriers with low HBsAg levels, and from donors infected with rarely occurring mutant HBsAg HBV strains, may be not detected by the currently implemented HBsAg assays and therefore represent the most frequent residual risks for HBV transmission to recipients of blood products. Hence additional HBV markers must be evaluated to reduce these risks.

In the early phase of HBV infection (window phase), comparison of the sensitivity of NAT in minipools with HBsAg assays shows discrepant results. Different studies have shown that NAT in minipools was more sensitive than HBsAg assays [34], whereas others have shown that more recently-developed sensitive HBsAg assays were comparable in sensitivity to pooled-sample NAT [35]. Using the most sensitive amplification assays it appears that most HBsAg positive samples in apparently healthy individuals contain HBV DNA [24]. Moreover, HBV NAT screening of 3.6 million blood donations (minipools of 96 donations) in central Europe identified only 2 HBV DNA positive donors from HBsAg negative seroconverters [33]. In our study, out of 18143 consecutive donations individually screened with the HBsAg Enzygnost Integral 5.0 assay and by HBV NAT in minipools of 24 donations, only 1 donation was HBV NAT as well as HBsAg positive.

In the phase preceding the appearance of neutralising anti-HBs antibodies, HBsAg tests become negative but anti-HBc antibodies are detectable as a marker of HBV infection [36]. During this phase a low level of HBV DNA is often reported [31, 33, 37]. Further long-term persistent and intermittent viraemia in isolated anti-HBc positive individuals is not infrequent [19, 38, 39]. Previous studies have shown that HBV DNA may be detected in HBsAg negative, anti-HBc reactive blood donations within the range of 0% to 5% [40, 41]. In our study we found no individual donation to be positive for HBV

Table 4

Concentrations of anti-HBs compared to the results of anti-HBc assays for donations from BTS VD.

Anti-HBs concentration	Both anti-HBc assays repeated reactive	First anti-HBc assay repeated reactive and second anti-HBc negative	Second anti-HBc assay repeated reactive and first anti-HBc negative
Negative	15	71	12
<100 IU/ml	7	1	0
100–1000 IU/ml	18	8	1
>1000 IU/ml	33	6	0

First anti-HBc assay: Enzygnost Dade Behring; Second anti-HBc assay: Monolisa Biorad

DNA in "anti-HBc alone" positive donations, or in anti-HBc plus anti-HBs positive donations. However, other published studies on post-transfusion hepatitis have shown that donations reactive for "anti-HBc alone" or for anti-HBc plus anti-HBs have transmitted HBV infection to transfusion recipients [2, 6, 36, 42]. Estimating the frequency of this event from contemporary data has proven extremely difficult. It was estimated that the risk of HBV transmission through HBsAg negative and anti-HBc positive donations was approximately 1 in 50000 donations [37, 43, 44]. From 3.6 million German blood donations screened by HBV NAT in minipools of 96, four donations were HBV DNA positive in minipools and these turned out to be also anti-HBc reactive [33]. If HBV NAT screening in minipools were introduced, probably one infectious donation per 900000 donations would detected and thus a potential transfusion-transmitted infection avoided. In donors with chronically low level viraemia it seems unlikely that NAT in minipools is sensitive enough to detect the majority of potentially infectious donations from anti-HBc positive donors [37]. Thus, chronically HBV infected donors could be eliminated more effectively only with individual donation NAT [33].

We considered donations which were repeatedly reactive with at least two different anti-HBc assays as "confirmed" positive to calculate the prevalence of anti-HBc "confirmed" positive donations. Due to protection rights or pending patents the specification of the HBc antigens used in the 3 different anti-HBc assays were not known. It is thus possible that the same HBc antigens were present in 2 or perhaps all of the 3 anti-HBc assays used. This fact compromises the prediction that confirmation with a second or even third anti-HBc increases specificity.

This prevalence was 1.2% in the donors of BTS BE (being partially preselected by previous anti-HBc and anti-HBs testing) and 1.7% in the donors of BTS VD (not being pre-selected by preceding anti-HBc and anti-HBs testing). For comparison, studies performed in Europe and the United States, both areas with low HBV endemy, revealed that 0.35 to 8.71% of the population had serological signs of a previous HBV infection [43, 45–52]. Henning and co-authors showed that 1.52% of 14251 volunteer first-time German donors were positive by two different anti-HBc assays [53]. There are several reasons which may explain the differences in anti-HBc prevalence, such as use of a preselected donor population, different screening and confirmation algorithms, different anti-HBc assays and regional differences in the prevalence of HBV infection.

The specificity of the anti-HBc assays used is an important point to be considered. In our study "confirmation" testing using two alternative anti-HBc assays reduced the number of reactive donations by 61.5% and 57.6% for BTS BE and for BTS VD respectively. Our findings agree with two other studies in blood donors, which showed that respectively 32% and 58% of samples reactive with an initial anti-HBc assay could not be confirmed with two additional assays [37, 43]. This finding highlights the low specificity of the current anti-HBc assays. Thus it is difficult to evaluate precisely the exact rate of "false" positive reactions with the different available assays. The American Red Cross estimates that over 200000 donors were deferred for isolated anti-HBc reactivity from April 1991 to the end of 2003. In the USA, approximately 500000 donors were deferred for isolated anti-HBc reactivity and it has been estimated that 65% of these deferrals were due to false positive results [26]. However, anti-HBc testing may reduce the residual risk of transfusion transmitted HBV infection by deferring potential HBV carriers from the donor population. The Paul Ehrlich Institute (PEI) reported that 7 out of 18 cases of proven HBV transmission by blood components reported to this institute could have been prevented by anti-HBc testing [54]. In reaching a decision one must weigh the deferral rate of approximately 1–2% of anti-HBc "true" positive blood donors against the security gain, to obtain a significant decrease of the theoretical residual risk of HBV down to the level of HIV and HCV.

Although we observed generally higher concentrations of anti-HBs in all the anti-HBc "confirmed" donations, we also found anti-HBc positive donations in the "non confirmed" donations from non-vaccinated donors with anti-HBs concentrations >100 IU/ml.

In conclusion, the prevalence of anti-HBc "confirmed" positive donations in the two Swiss blood donor populations studied was low (<2%) and we found only one HBV NAT positive (HBsAg positive) donation among more than 18000. Concerning blood product safety, an increase in the deferral rate of less than 2% of anti-HBc positive, potentially infectious donors, may in our opinion make routine anti-HBc testing of blood donations cost effective. However, this can only be demonstrated if future anti-HBc screening data is related or compared to the avoidance of HBV transfusion-transmitted infections. But there is still a need for more specific assays if unacceptably high deferral rates of "false" positive donors are to be avoided. On the other hand, the introduction of HBV NAT in minipools provides minimal benefit due to the inadequate sensitivity of the assay. The value of individual NAT, alone or in conjunction with anti-HBc, as an additional screening assay in the Swiss donor population requires further evaluation.

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Correspondence:
C. Niederhauser
Regional Blood Transfusion Service Berne
Murtenstrasse 133
CH-3008 Berne
Switzerland
E-Mail: christoph.niederhauser@bsd-be.ch

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