


# Effectiveness of blood donor screening by HIV, HCV, HBV–NAT assays, as well as HBsAg and anti–HBc immunoassays in Germany (2008–2015)

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## Vox Sanguinis

**Background and Objectives** In Germany, in addition to standard blood donor screening, further mandatory tests were introduced for HCV–RNA, HIV–1–RNA and for anti–HBc. Screening for HBV–DNA is optional. This study investigates the benefits of these additional tests for the detection of HIV, HCV, and HBV infections among German blood donors.

**Materials and Methods** From 2008 to 2015 we collected data on blood donations exclusively testing NAT positive (NAT yield) or reactive in only one of the screening assays. Assuming a Poisson distribution, we calculated NAT yield/reactive only rates on a per donation basis (number of yield/reactive only cases divided by the number of donations tested in the period under review) with 95% confidence intervals.

**Results** Responding establishments covered 95% of the donations. We identified 20 HIV–1–NAT, 61 HCV–NAT and 29 HBV–NAT yield cases among approximately 46 million blood donations tested corresponding to 0.43 HIV–1 NAT, 1.32 HCV–NAT, and 0.64 HBV–NAT yield cases per million blood donations tested. For one HBsAg reactive only case and 23 anti–HBc reactive only cases in repeat donors, infection was confirmed by ID–NAT which translates into 0.02 and 0.55 cases per million donations tested. During the 8-year-observation period, one HIV–1, no HCV and four HBV transmissions associated with donations in the viremic pre-seroconversion window period were reported.

**Conclusion** Annually, NAT screening alone detected 2.5 HIV–1, 7.6 HCV, and 3.6 HBV infectious donations; anti–HBc screening alone identified 2.9 infectious donations of repeat donors with occult HBV infection. Overall, the survey results support that the currently practiced donor HIV/HCV/HBV screening strategy in Germany does ensure a high standard of blood safety.

**Key words:** antibody to hepatitis B core antigen, blood safety, hemovigilance, Hepatitis B virus, hepatitis C virus, human immunodeficiency virus type 1, nucleic acid amplification technique.

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## Introduction

Benefits and costs of blood donor screening have been intensively and controversially debated in various publications [1, 2].

Basic European testing requirements for whole blood and plasma donations as laid down in Annex IV of 2002/

98/EC include screening of single blood donations for antibodies to human immunodeficiency virus Type 1/2 (anti-HIV-1/2), antibodies to hepatitis C virus (anti-HCV), and hepatitis B surface antigen (HBsAg) [3]. Along with an additional test for syphilis, these tests had been the only obligatory tests performed in Germany up to the late 1990s [4, 5]. After assessing hemovigilance data and the feasibility of nucleic acid amplification (NAT) testing, the national competent authority, the Paul-Ehrlich-Institut (PEI) implemented mandatory NAT screening for hepatitis C virus (HCV) in 1999 and for human immunodeficiency virus type 1 (HIV-1) in 2004, as well as mandatory screening for antibody to hepatitis B core antigen (anti-HBc) in 2006 as further risk-minimizing measures. Minimal sensitivity limits of 5000 IU HCV-RNA/ml and 10 000 IU HIV-1 RNA/ml were defined for the individual donation on the basis of virus replication rates in the early viremic period [6, 7] allowing NAT in minipools (MP). Hepatitis B virus (HBV) screening includes mandatory testing for HBsAg and anti-HBc. In most blood establishments (BE), it is supplemented by voluntary HBV-NAT. There is no required sensitivity for HBV-NAT assays, thus, test sensitivities related to the single donation vary both within BE over time and between BE. During the last two decades, several communauté européenne (CE) marked NAT assays have become available which compared to serological testing demonstrated a pronounced shortening of the viremic window period even when tested in MP [3].

In this survey, PEI asked German BE to report HIV-1, HCV and HBV NAT 'yield cases' and reactive only results for HBsAg and anti-HBc detected from 2008 to 2015.

This study aimed at investigating the benefits of using NAT for the detection of HIV-1, HCV, and HBV infections as well as the benefit of serological screening for anti-HBc introduced in 2006 among blood donors in Germany. We compared data on HIV-1- and HCV-NAT yield cases with those of Nübling *et al.* [8] published in 2009 in order to investigate whether the effectiveness of blood donor screening changed over time.

## Material and methods

According to the German Drug Law (AMG) [9], the PEI is authorized to obtain data from pharmaceutical manufacturers for blood safety surveillance reasons. We used this to obtain an update of the effectiveness of risk-minimizing measures taken beyond the blood donor screening tests currently required by the European legislation. In 2016, all BE holding a market authorization for blood components intended for transfusion had been invited to report cases of HIV-1, HCV, and HBV positive donations which were exclusively detected via the screening NAT assays in place

('NAT yield cases') as well as infectious donations which were exclusively detected by HBsAg or anti-HBc screening ('reactive only cases'). A 'NAT yield case' was defined as a donation which was serologically negative but repeatedly NAT positive. Donations were designated as HBsAg or anti-HBc 'reactive only cases' if they were repeatedly reactive, if tested negative in HBV-screening NAT tests, had no HBV vaccination history in case of HBsAg reactive only cases, and if supplementing HBV ID-NAT with at least a limit of detection (LoD) of 12 IU HBV-DNA/ml was positive, respectively.

Using an electronic Case Report Form, data were collected for the years 2008–2015 on the following items:

- (1) Number of donations collected and tested for HIV, HCV, and HBV in the period under review.
- (2) NAT: number of HIV-1-NAT, HCV-NAT, and HBV-NAT yield cases, additional information on the type of NAT screening assay, its LoD, the pool size, the viral load of the donation, the genotype of the respective virus, and in case of a HBV-NAT yield case also the type of HBsAg screening assay.
- (3) Anti-HBc: number of anti-HBc reactive only cases where the infection was confirmed by a positive result of supplemental ID-NAT testing, that is, occult HBV.
- (4) HBsAg: number of HBsAg reactive only cases, the type of HBsAg screening assay, available information on whether the donor was vaccinated against HBV prior to blood donation as well as the LoD of the confirming ID-NAT.

We requested only data on anti-HBc reactive cases from repeat donors in this survey as BE need not perform ID-NAT tests for anti-HBc repeatedly reactive first-time donors. A repeat donor is defined as a person who donated more than once within 365 days. The number of donations from repeat donors was estimated by subtracting the percentage of first time donors (7%, notified according to §22 TFG [10]) from the total number of donations reported by participating BE.

We obtained reports on suspected donor infections involving donor look-back procedures from BE as routine notifications according to AMG (§63i) [9]. In accordance with the rules laid down by the German Blood Advisory Committee [11] infectiousness is considered in case of positive results in ID-NAT tests with a 95% LoD of less than 100 IU/mL for HIV, 50 IU/mL for HCV and 12 IU/mL for HBV. The specificity of an anti-HBc reactive result is assumed on obtaining a reactive result in at least one out of two further anti-HBc assays. The specificity of HBsAg reactive results, according to the manufacturer's instructions, is validated by a positive neutralization assay or by a second reactive HBsAg assay. Reactive HIV-1/2 or HCV antibody assays are confirmed by positive immunoblots. Look-back procedures have to be initiated in case of

confirmed reactive, positive or indeterminate test results. These include testing of retained samples from all donations originating from the donor of the presumed infectious donation within defined periods (12 weeks for HIV and HCV, 16 weeks for HBV) prior to the last negative tested donation within the preceding 5 years. Furthermore, recipients of blood components derived from all donations for which an infection could not be ruled out have to be tested for the suspected virus.

### Statistical analyses

We calculated NAT yield rates on a per donation basis by dividing the number of NAT yield cases by the number of donations tested in the period under review and expressed this as number of NAT yield cases per million donations tested. To analyse a potential change over time as compared with data obtained within the scope of a previous study [8], we estimated NAT yield rate ratio(s) (YRR) by aggregating the number of donations tested as well as the counts of NAT yield cases for the period under review and analysing these using Poisson regression (log-linear regression of the counts of yield cases using the logarithm of donations tested as offset). For anti-HBc and HBsAg, a reactive only rate was calculated by dividing the number of reactive only cases by the number of donations tested in the period under review. The average number of infectious (HIV, HCV, and HBV positive) donations per year was estimated by dividing the number of NAT yield cases or reactive only cases notified in the period under review by the number of years under review ( $n = 8$ ). The rate of confirmed look-back procedures for HIV-1, HCV, and HBV was estimated by dividing the number of confirmed look-back procedures for HIV-1, HCV, and HBV by the number of donations from repeat donors in the period under review. We calculated the average number of confirmed look-back procedures for HIV-1, HCV and HBV per year by dividing the number of confirmed look-back procedures for HIV-1, HCV, and HBV in the period under review by the number of years under review ( $n = 8$ ). Under the assumption of a Poisson distribution, all 95% confidence intervals (CI) were estimated according to an algorithm described by Daly [12].

$P$ -values  $< 0.05$  indicate statistical significance. Statistical analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

### Ethics

According to the State Chamber of Medicine in Hessen, no Ethics Committee approval was required. All applicable data protection rules were respected.

## Results

Fifty-one of the 103 contacted BE agreed to participate in the survey (response rate 49.5%), and 49 of them (47.6%) provided the requested information, including eight major centres with more than one million donations each and 39 237 033 donations in total. Participating BE reported 46 151 554 donations which covered 94.6% of all German blood and plasma donations used for the manufacturing of blood components for transfusion ( $n = 48\,784\,544$ ) notified from 2008 to 2015 in accordance with legal requirements [8]. The majority of the 46 151 554 reported donations, were tested on a voluntary basis by HBV-NAT assays ( $n = 45\,270\,111$ , 98.1%), either by MP-NAT of up to 96 donations or by ID-NAT. Assuming that 93% of all donations in the reporting period originated from repeat donors, we estimated that 42 101 203 donations were from repeat donors (Table 1).

### HIV-1 NAT

In total, 20 HIV-1 NAT yield cases were reported (Table 2). For 14 of these 20 cases (70%), the viral load was determined. HIV-1 RNA concentrations varied between 100 and 1 000 000 IU/ml and, in eight out of the 14 yield cases (40%), viral loads were below 10 000 IU/ml per single donation. This result shows that a relatively high percentage of BE used screening assays with a sensitivity below the required detection limit for HIV-1 NAT (10 000 IU/ml) per single donation. The LoD of the screening NAT assays reported for the yield cases ranged from 61.25 to 2841 IU/ml per single donation depending on the applied test strategy. The MP size varied from 8 to 96 donations.

We estimated the HIV-1 NAT yield rate to be one HIV-1 NAT yield case in 2 307 578 donations tested or 0.43 HIV-1 yield cases per million blood donations tested. Compared with data reported by Nübling *et al.* [8] for the previous survey (observation period 1999–2007), the HIV-1 NAT yield rate decreased albeit not significantly from 0.64 to 0.43 per million donations tested. This corresponds to a non-significant decrease in yield cases by 33% (YRR: 0.67; 95% CI: 0.32–1.41;  $P = 0.2936$ ). Per year, on average, 2.5 HIV infectious donations were exclusively detected by HIV-1 NAT screening.

### HCV NAT

Sixty one yield cases were notified for the period under review (Table 2) with additional data on viral load given in 13 cases (21%). Only one case had a very low concentration of HCV-RNA (50 IU/ml). The viral concentration of the other 12 cases ranged from 12 283 to

**Table 1** Donations collected by participating BE

Survey denominators (2008–2015)	<i>n</i>	%
Number of donations collected by participating BE (denominator for HIV-1 NAT and HCV-NAT yield cases)	46 151 554	100
Number of HBV-NAT assay screened donations from first-time and repeat donors collected by participating BE (denominator for HBV-NAT yield and HBsAg reactive only cases)	45 270 111	98.1
Number of HBV-NAT assay screened donations from repeat donors collected by participating BE (denominator for anti-HBc reactive only cases, estimated as 93% of 45 270 111)	42 101 203	91.2

Anti-HBc, antibody to hepatitis B core antigen; BE, blood establishments; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV-1, human immunodeficiency virus Type 1, NAT, nucleic acid amplification technique.

100 000 000 IU/ml per single donation thus exceeding the required limit by a multiple. The LoD of the screening NAT assays reported for the yield cases varied from 5 to 1960 IU/ml per single donation depending on the test strategy applied. The MP size ranged from 10 to 96 donations.

The HCV-NAT yield rate was estimated as one HCV yield case in 756 583 donations tested or 1.32 HCV yield cases per million blood donations tested (Table 2). Compared with data from our previous survey [8], the HCV-NAT yield rate significantly dropped from 2.25 to 1.32 per million donations tested. This corresponds to a significant decrease in yield cases by 41% (YRR: 0.59; 95% CI: 0.42–0.81;  $P = 0.0012$ ).

Per year, 7.6 HCV infectious donations were exclusively detected by NAT screening.

### Hepatitis B virus

From 2008 through 2015, a total number of 45 270 111 donations were reported to be screened by HBV-NAT on a voluntary basis besides the obligatory screening for anti-HBc and HBsAg. During that period, 29 HBV-NAT yield cases were reported to the PEI (Table 2), a rate of one HBV-NAT yield case in 1 561 038 blood donations tested or 0.64 NAT yield cases per million blood donations tested. Per year, on average, 3.6 HBV infectious donations were detected by NAT screening alone. The LoD of the screening NAT assays reported for the yield cases varied from 1.4 to 582 IU/ml per single donation depending on the applied test strategy (ID-NAT or screening in MP of up to 96 donations). For the estimated 42 101 203 HBV-NAT screened donations of repeat donors, 23 occult HBV cases were identified (i.e. reactive only by anti-HBc and infection confirmed by supplemental HBV ID-NAT testing). This corresponds to one reactive only case in 1 830 487 blood donations tested or 0.55 anti-HBc occult HBV cases per million blood donations tested. Per year, on average, 2.9 donations of repeat donors with occult HBV infections (OBI) were identified by anti-HBc screening only.

Out of the 45 270 111 donations screened by HBV-NAT, a total of 261 were found to be exclusively HBsAg positive in screening tests. For 235, we found a negative result of supplemental ID-NAT testing and a history of hepatitis B vaccination (very shortly before blood donation). Another 23 reactive only cases were negative by HBV ID-NAT and a prior vaccination of the donors could not definitively be excluded. These latter may represent false positive HBsAg results as a positive result for HBsAg together with negative results for both HBV ID-NAT and anti-HBc seems to be implausible. One of the three remaining HBsAg reactive only cases was ID-NAT positive by supplemental testing and assessed as truly HBsAg only HBV infection only. No information on vaccination history was available for the other two HBsAg only reactive cases because they were first-time donors, no supplemental ID-NAT was performed. This translates into 0.02 HBsAg reactive only cases per million blood donations tested. On average, 1 HBV infectious donation per ten years was exclusively detected by HBsAg testing.

### Breakthrough transmissions

In the period under review, one HIV-1 and four HBV breakthrough transmissions following donations in the pre-seroconversion viraemic window period were reported (Table 2). Since the HIV-1 transmission in 2010 was ascribed to suboptimal amplification efficiency, the PEI introduced obligatory HIV-1 NAT dual-target assays as a risk-minimizing measure for blood donor screening in Germany. All four HBV transmissions were due to donations in an early viraemic window period. The viral loads estimated in retained donation samples were 15 IU/ml in one case, around the LoD of the ID-NAT used in the second case, and negative in ID-NAT in the other two cases. The sensitivities of the corresponding screening assays, calculated as LoD per single donation, were 2, 58 and 582 IU/ml. (in MP format). There were no reported HCV breakthrough transmissions.

**Table 2** HIV-1, HCV, HBV-NAT yield cases, anti-HBc and HBsAg reactive only cases (2008–15)

Screening assay <sup>a</sup>	Donations tested	Yield cases/Reactive only cases	Yield cases/Reactive only cases based on the number of donations tested	Yield rate/Rate of reactive only cases (cases per million donations tested) (95% CI)	Breakthrough transmissions
HIV-1 NAT	46 151 554	20	1:2 307 578	0.43 (0.26–0.67)	1
HCV-NAT	46 151 554	61	1:756 583	1.32 (1.01–1.70)	0
HBV-NAT	45 270 111 <sup>c</sup>	29 <sup>b</sup>	1:1 561 038	0.64 (0.43–0.92)	4
Anti-HBc	42 101 203 <sup>f</sup>	23 <sup>c</sup>	1:1 830 487	0.55 (0.35–0.82)	
HBsAg	45 270 111 <sup>c</sup>	1 <sup>d</sup>	1: 45 270 111	0.02 (0.001–0.12)	

95% CI, 95% confidence intervals; anti-HBc, antibody to hepatitis B core antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV-1, human immunodeficiency virus Type 1, NAT, nucleic acid amplification technique.

<sup>a</sup>NAT screening predominantly performed in minipools (MP).

<sup>b</sup>Screening: HBV-NAT positive, HBsAg negative, anti-HBc negative;

<sup>c</sup>Screening: anti-HBc repeatedly reactive, HBV-NAT negative, HBsAg negative; positive in supplemental HBV ID-NAT testing;

<sup>d</sup>Screening: HBsAg repeatedly reactive, HBV-NAT negative, anti-HBc negative; positive in supplemental HBV ID-NAT testing;

<sup>e</sup>Number of donations screened by HBV-NAT;

<sup>f</sup>Repeat donors of <sup>e</sup>estimated as 93% of 45 270 111.

### Look-back procedures for HIV, HCV, and HBV in repeat donors

In the 8 years covered by this survey, a total of 5397 look-back procedures were reported to the PEI according to § 63(i) AMG [9]. These look-back procedures initiated by reactive, positive or intermediate test results included 415 possible cases of HIV, 886 of HCV, and 4096 of HBV infections in repeat donors (Table 3). In 271 look-back procedures for HIV (65%) and in 241 for HCV (27%), donor infections were confirmed. In 447 look-back procedures for HBV (11%) we confirmed an infection or a history of vaccination or of natural HBV exposure. In the period under review, there were, on average, 34 confirmed cases of HIV, 30 for HCV, and 56 for HBV per year. The rates of confirmed cases per million donations of repeat donors were estimated to be 5.98 for HIV, 5.32 for HCV and 9.86 for HBV.

### Discussion

This is to our knowledge the most comprehensive analysis of the effectiveness of the additional blood donor screening assays introduced in Germany. It shows good compliance of the participating BE and an excellent coverage of blood donations in Germany from 2008 through 2015. Nevertheless, it is limited by missing data. Some items collected; for example, the viral loads of infectious donations were not suitable for analysis because of a large number of missing values. As the mandatory look-back procedure for suspected infections in donors does not require quantifying viral loads, this important information was not obtained for all cases.

To further prevent transfusion-transmitted viral infections, especially in the viraemic window period, NAT testing for HCV and HIV-1 became mandatory alongside anti-HCV and anti-HIV-1/2 immunoassays. With respect to HBV safety, anti-HBc testing became mandatory in addition to HBsAg testing to identify chronically infected low-level HBV donors. Most BE voluntarily do HBV-NAT screening mainly in MP. The objectives of the current survey were to quantify yield cases of NAT screening tests as well as cases reactive only with anti-HBc and/or HBsAg and to investigate the effectiveness of each test.

Compared with data reported by Nübling *et al* [8], the HIV-1 NAT yield rate decreased albeit not significantly from 0.64 to 0.43 per million donations tested. This reduction may be explained by improved serological testing and, in particular, increasing use of HIV antigen/antibody combi-tests with high sensitivity to HIV antigen, rather than by a decline in donor HIV-incidence. Of note, from 2008 through 2015, the number of HIV infections in German blood and plasma donors did not decrease over time [13, 14]. Nevertheless, there was only one case report of confirmed HIV transmission. This translates into one case in 45 million donations tested. These figures demonstrate that the HIV screening strategy practiced in Germany is effective.

The same applies to the risk of HCV transmission. Compared with data from our previous survey [8], the HCV-NAT yield rate significantly dropped from 2.25 to 1.32 per million donations tested. Notwithstanding the donor epidemiology regarding HIV, the RKI survey data [13, 14] showed a decline in HCV infections in the German donor population which may sufficiently explain the observed decrease in HCV-NAT yield rate. During the last decade, no

**Table 3** Look-back procedures for HIV, HCV, and HBV in repeat donors (2008–15)

2008–2015	Donations of repeat donors corresponding to 93% of all donations	Number of look-back procedures initiated by reactive, positive or intermediate test results	Cases with positive confirmation	Average number of confirmed cases per year (95% CI)	Rate of confirmed cases per million donations (95% CI)
HIV	45 339 375 <sup>a</sup>	415 (100%)	271 (65%)	33.88 (29.96–38.16)	5.98 (5.29–6.73)
HCV		886 (100%)	241 (27%)	30.13 (26.44–34.18)	5.32 (4.67–6.03)
HBV <sup>b</sup>		4096 (100%)	447 (11%)	55.88 (50.81–61.30)	9.86 (8.97–10.82)

95% CI, 95% confidence intervals; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

<sup>a</sup>Denominator: total number of donations ( $n = 48\,784\,544$ ) notified according to §21 TFG less 7% total number of first-time donations ( $n = 3\,445\,169$ ) notified according to §22 TFG.

<sup>b</sup>Cases confirmed by positive ID-NAT (infectious donations) and by confirmed reactive anti-HBc results (history of HBV contact).

further HCV transmission was reported indicating that the German HCV screening strategy is adequate and effective.

Regarding HBV, from 2008 through 2015, a total of four reported transmissions were confirmed which corresponds to one case per 11.3 million donations tested. As these transmissions were due to donations in an early viraemic window period, infectiousness could not be detected by anti-HBc testing. Within the same time period we found one case reactive only by HBsAg (anti-HBc/MP-NAT negative, ID-NAT positive), 23 OBI cases reactive only with anti-HBc (anti-HBc reactive only/ID-NAT positive), and 29 HBV-NAT yield cases. Based on these findings, HBV-NAT screening assays vary widely as to LoD but were found to be more effective than anti-HBc and HBsAg with rates of 0.64 HBV-NAT yield cases per million donations tested as compared with anti-HBc with 0.55 reactive only cases per million donations tested and HBsAg with 0.02 reactive only cases per million donations tested.

The much lower rate of confirmed look-back procedures for HBV (11%) as compared with HIV (65%) and HCV (27%) is because so many arose from the high percentage of look-back procedures initiated because of assumed specific anti-HBc reactivity (90%). In most cases, infectiousness was not confirmed by ID-NAT. Nor was a previous contact with HBV proven by detecting anti-HBs antibodies in the donation or in retained samples from previous donations.

Comparing our numbers of newly detected infections in blood donors, that is, NAT yields, with those of France [15] or UK [16], differences become evident. Laperche *et al.* [15] presented data of 40 million donations screened with nucleic acid testing in France between July 2001 and December 2015. The main benefit in terms of increased blood safety by NAT only was related to HBV with a yield rate of 0.88 cases per million donations, followed by HIV with 0.50 and HCV with 0.33 cases per million donations. Of note, HIV- and HBV-NAT yield rates of German blood donors are in the same order of magnitude as published for blood donors in France.

Soldan *et al.* [16] investigated the frequency of HBV, HCV and HIV infectious donations entering the UK blood supply during 1996–2003 after introduction of nucleic acid testing for HCV and HIV. During this period, estimated frequencies of infectious donations were 1.66, 0.80 and 0.14 per million for HBV, HCV and HIV, respectively. The most prominent figures are the high numbers of new HBV infections reported for UK, the high numbers of new HCV infections reported for Germany and the fairly low number of new HIV infections in UK donors.

Regarding the comparison with other countries, we cannot exclude bias due the fact that the yield rates were estimated based on donations tested in varying MP sizes in Germany.

The described test sensitivities argue against screening NAT performance as reason for the substantial differences between countries. While requiring higher test sensitivity for HCV-NAT screening [17], France had a much lower HCV-NAT yield rate as compared to Germany. Most likely, the differences in donor epidemiology itself, as observed and published by the ECDC, [18] will be the main reason. Thus, for example, the number of new infections within German repeat donors, reported by RKI, (2008–2010) [14] corresponds well to the HBV-NAT yield rate in our study despite the widespread differences in sensitivity of HBV-NAT used for donor screening.

Furthermore, the RKI data show that HBV prevalence in first-time donors ( $116\text{--}136$  per  $10^5$ ) is nearly twice as high as that for HCV ( $69\text{--}81/10^5$ ) whereas the rate of new HBV infections in repeat donors is decreasing ( $0.51\text{--}0.26/10^5$ ) and the rate of new HCV infections in repeat donors remains unchanged at a higher level ( $0.8\text{--}0.95/10^5$ ). This may be explained by the benefit of routine HBV vaccination in an increasingly younger donor population. In contrast, in absence of a vaccine against HCV the figures reflect the comparatively high HCV infection in the German population.

An international survey on NAT testing of blood donations (1999–2009) described the introduction of HIV, HCV

and HBV-NAT testing in Africa, Asia, Europe and America, test systems, pool sizes and NAT yield data [19]. Considerable differences between countries and continents were found. Rates of HIV-NAT only positive repeat donations ranged from 0.2 to 0.6 per million in Asia, Europe and North America, whereas 36.3 cases per million were found in Africa. HCV rates varied between 0.8 and 1.54 cases per million in Europe, Asia and Africa but amounted to 2.05 per million in North America. Regarding HBV, lower yield rates were found in North America and Europe (2.19 and 2.76 cases per million) and higher rates in Asia and Africa (22.1 and 35.3 cases per million). Since NAT testing had been introduced, a total of 81 HIV positive donations were detected in Africa, 73 in Europe, 45 in North America, and 44 in Asia. In Europe 206 HCV cases were exclusively identified by NAT testing in Europe, 299 cases in North America but only 4 in Africa. Finally, the number of NAT-HBV only positive donations was highest in Asia (1091), followed by Europe (550) and Africa (232).

These regional differences in NAT yield data have to be considered when assessing the effect of risk minimization measures and the benefit of donor screening. Borkent-Raven *et al.* [20] pointed out the high costs and the limited benefit associated with the additionally implemented donor screening by NAT. Therefore, these risk-minimizing measures seem to be unfavourable in low-incidence countries such as the Netherlands or Germany. At the same time, the authors emphasized the high safety standard and the confidence of the recipients assured by NAT donor screening. We expressly endorse this view and suppose that new cases of transfusion-associated hepatitis or HIV infection due to withdrawal of NAT testing would not be accepted by the German public.

Results of 22.4 million donations tested for HBsAg, anti-HBc and HBV-NAT were reported by the American Red Cross. [21]. From July 2011 to June 2015, a total of 29 NAT yield cases (1.29 cases per million donations) were identified, whereas only six HBsAg reactive only cases (0.26 cases per million donations) were found. The authors concluded that the frequency of HBV infection rates among blood donors continues to decline and elimination of HBsAg screening would have a negligible impact on recipient safety.

An analysis of 2.6 million Australian donors tested for HBV-NAT, anti-HBc and HBsAg between 2010 and 2012 demonstrated a substantially higher prevalence of occult hepatitis B infections (OBI) compared to acute serologic window period HBV infections [22].

The follow-up testing of OBI cases showed intermittent detection of HBV-DNA and emphasized the importance of anti-HBc for a sufficient HBV donor screening.

A multi-regional study investigated the clinical sensitivity of hepatitis B surface antigen and HBV-DNA

including 10.9 million donations from South Africa, the Mediterranean, North and Central Europe and South East Asia [23]. NAT yield rate for occult HBV infections varied from 1: 3900 to 1: 59 000 donations. HBsAg testing (chemiluminescence immunoassay) detected 97.0% of infections in first-time donors, 62.7% in lapsed donors, and 41.0% in repeat donors, whereas NAT testing (Ultri Plus assay) detected 93.1%, 95.0%, and 98.3% of infections in these groups, respectively. The authors concluded that ID-NAT and serology are complementary in detecting HBV infection in first-time donors and confirmed the superiority of HBV-NAT over HBsAg detection in repeat donors.

Our results are generally consistent with these studies. Considering the very low rate of donations reactive only for HBsAg (one case per 45 million donations) and the much higher HBV-NAT yield rate (one case per 1.6 million donations) as well as OBI detected by anti-HBc only (one case per 1.8 million donations), a combination of screening for HBV-NAT to catch early window period infections together with screening for anti-HBc to catch OBI cases with a very low viral load seems to be a more effective screening strategy than combined testing for HBsAg and anti-HBc. Whereas HBV-NAT assays proved to have a high specificity, the implementation of anti-HBc donor testing led to a great number of look-back procedures. In most of these cases, HBV seroconversion without infectiousness was found.

## Conclusions

The latest survey data on blood donor screening in Germany are in accordance with the results of other European and US studies and confirm low numbers of yield cases of NAT screening mainly performed in minipools. Based on data for more than 40 million donations, it was demonstrated that the anti-HBc screening is effective and allows detection of occult HBV infections in donors. To identify the best test strategy to further prevent transfusion-transmitted HBV infections in Germany, would require a more detailed analysis of the HBV figures from this survey. In conclusion, the currently practiced donor screening strategy does ensure a high standard of blood safety.

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## Conflict of Interest

The authors declare no conflict of interests.

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