Role of Individual Donor NAT in Further Improving Blood Safety

The importance of individual donor nucleic acid amplification testing (ID-NAT) in further ensuring the safety and availability of donations for transfusion of blood and plasma products as emphasized by Professor Jean-Pierre Allain, from the Division of Transfusion Medicine, Department of Haematology, University of Cambridge, United Kingdom.

Key Takeaways:

- Maximum analytical sensitivity is required for HIV, HCV, HBV NAT
- ID-NAT ensures that low virus levels are still detectable
- Total anti-HBc is not cost-effective for screening above prevalence of 10%
- Blood banks must ensure maximum blood safety, while balancing with blood availability
- ID-NAT together with serological assays HBsAg, anti-HIV and anti-HCV are presently necessary to ensure blood safety
- HBV recombinant vaccines of A2 genotype, especially at low anti-HBs titers, might not be adequately protective against genotypes D and C prevalent in India and elsewhere in Asia

Background

The early dynamics of viremia and infectious window periods (WP) of the human immunodeficiency (HIV), hepatitis C (HCV) and hepatitis B (HBV) viruses differ considerably and affect risk of viral transmission by transfusion of blood and plasma products.

The times at which the existing serology tests (anti-HIV, anti-HCV and HBsAg) become positive after initial viral contact also differ, at approximately 20 days for anti-HIV, 65 days for anti-HCV, and 36-55 days for HBsAg.[1]

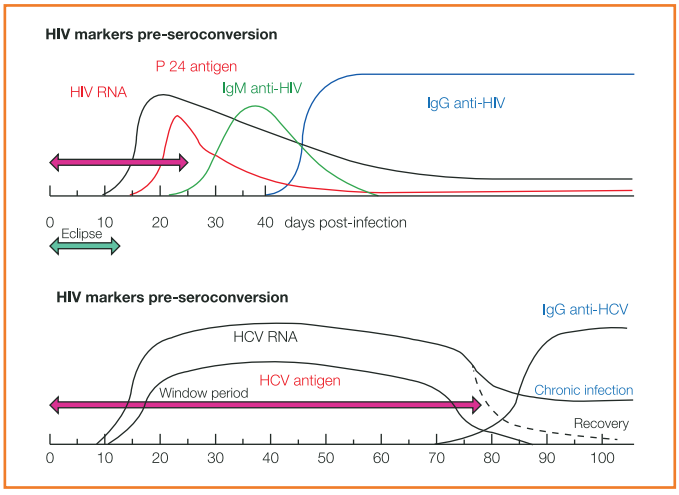

Figure 1. Evolution of markers for HIV and HCV before seroconversion

HIV, HCV

The evolution of HIV and HCV markers before seroconversion is shown in Figure 1. The eclipse period occurs when no viral marker is detectable and the blood is not infectious, while the WP is the time between initial viral contact and detection by either serology or nucleic acid testing (NAT) markers.

Many studies have investigated the impact of NAT on risk of HIV and HCV transmission. In two such series in the USA and France, [2,3] implementation of NAT in association with serology in plasma minipools of 16 to 24 reduced residual risk of HCV post-transfusion infection by approximately six-fold compared to serology alone, while that for HIV transmission was reduced by around 30%, with the difference in testing efficacy being due to the different lengths of WPs of the two viruses.

South Africa data on the efficacy of HIV NAT screening assays in repeat donations show that NAT in minipools (MP-NAT) detects slightly more of the WP than serology but misses so-called 'elite controllers', individuals who are anti-HIV positive with undetectable viral RNA.[4] Conversely, individual donor NAT (ID-NAT) is even more sensitive than MP- NAT, detecting more of the WP than both serology and MP-NAT and missing fewer elite controllers versus MP-NAT.

The South African investigators further examined the impact of MP-NAT versus ID-NAT by diluting 42 HIV RNA-positive WP samples, originally detected by ID-NAT, from 1:2 to 1:16. HIV detection clearly decreases considerably when samples were diluted. For example, at 1:8 dilutions, 24% of the original positives were missed.

Further evidence for requiring NAT is from a follow- up of 35 HCV NAT-positive donors by the American Red Cross (ARC). The ARC identified 35 HCV RNA yield cases, 33 of which seroconverted to anti- HCV.2 It is interesting to note that the remaining 2 non-seroconverted cases were individuals with chronic HCV infection who were unable to mount an immune response and produce antibodies to HCV (i.e. no anti-HCV).

While viral antigen tests are available for HIV and HCV, they offer no advantage over NAT for detection of either virus. HCV antigen covers approximately 90% of WP but HIV p24 antigen only 50% of WP.

In addition, the cost versus additional blood risks and availability challenges should be considered when deciding on MP or ID NAT. These additional issues include residual risk and impact on safety and timely availability of blood stocks.

"For HIV, ID-NAT is more sensitive than MP-NAT, detecting more of the WP than both serology and MP-NAT..."

HBV

HBV infection is of particular relevance in India, with an HBsAg prevalence of approximately 4.7% among general population.[15] Amongst first-time blood donors the prevalence of HBsAg is around 3% and of anti-HBc at 25%.[16]

Most individuals with competent immune systems recover from infection by developing antibodies to hepatitis B core antigen (anti-HBc) then antibodies to hepatitis B surface antigen (anti-HBs). Over time, anti-HBs decreases, sometimes to the point where only anti-HBc remains (Figure 2). Some cases carrying either both anti-HBs and anti-HBc or anti-HBc alone have detectable HBV DNA defining occult HBV infection or OBI.

However, in individuals with an immature or efficient immune system, chronic HBV infection develops where both HBsAg and HBV DNA remain detectable in the long term, with anti-HBc but no anti-HBs developing (Figure 2). Over time, HBsAg may also become undetectable, but HBV DNA and anti-HBc remain, which is considered a second type of OBI.

In early HBV infection in adults, following the eclipse period during which no marker is detectable, HBV DNA becomes detectable 15-20 days after contact, its concentration doubling every 2.6 days and HBsAg becoming detectable approximately 15 days later, with both peaking at around the same time. After this, both HBsAg and HBV DNA decrease in concentration but more rapidly for HBsAg as anti-HBs develops to form a complex with the antigen, giving rise to a second WP at around 75-85 days post-infection, when HBsAg is no longer detectable, anti-HBs not yet detectable, but HBV DNA is.[5-7]

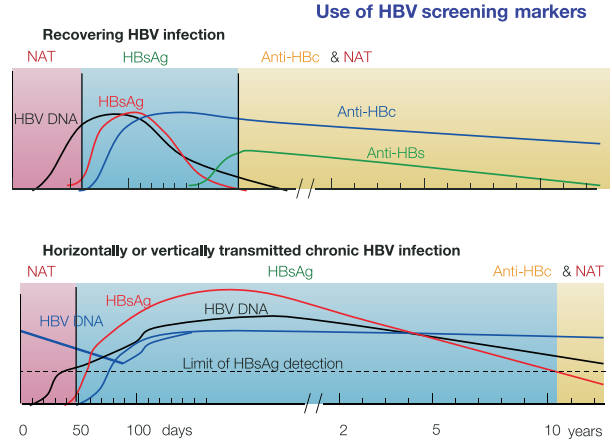

Figure 2. Profile of HBV DNA, Antigen and Antibody in Recovering and Chronic infection

HBV genotype and geographical differences

Asian data from blood centers in Taipei, Hong Kong, Kuala Lumpur, and Bangkok show that HBsAg concentrations are significantly higher in Thailand (genotype C) than in Taiwan (genotype B) ($P<0.0001$).

Significant differences are also seen between genotypes in terms of viral load. In asymptomatic HBsAg-positive donors, approximately 40% have a viral load $>10^5$ IU/mL for genotypes B and C, but <20% for genotypes A2, D and E, which explains why vertical HBV transmission is frequent in Asia, but rare in Africa. Similarly, very few HBsAg-positive samples have undetectable DNA in genotypes B and C but nearly 10% in genotypes D and E.

In an analysis of NAT screening data from 2.9 million individual blood donations in South Africa, an area of relatively high HBV prevalence, 85.6% of HBV-positive samples had both HBsAg and HBV DNA detected, 11.5% had only HBV DNA detectable, and 2.9% had HBsAg only.[8] Among the only HBV DNA detectable samples, one-third were acute WP infection and two-thirds were OBI.

Among five blood centers in Southeast Asia, the HBV DNA yield (HBV DNA-positive, HBsAg-negative) varied considerably between countries, e.g. from 1:980 in Taiwan to 1:18,060 in Singapore, depending on epidemiology and percentage of repeat donors.[9-11] Among these yield samples, 79% were OBIs and just 13% were WP infections.

"For HBV, ID-NAT identifies more potentially infectious HBV WP and OBI cases than MP-NAT"

In data from Southeast Asia and China, where the OBI frequency was 1:4,000, HBV DNA samples were quantified consistently <100 IU/mL and used to predict results. If instead of ID-NAT, screening had been done with an assay in minipools of 6 or 10 with an analytical 5 IU/mL sensitivity, 70.7% and 87.5% of samples respectively would have been undetected (Figure 3). Sensitivity is therefore of critical importance in screening donations for HBV DNA.

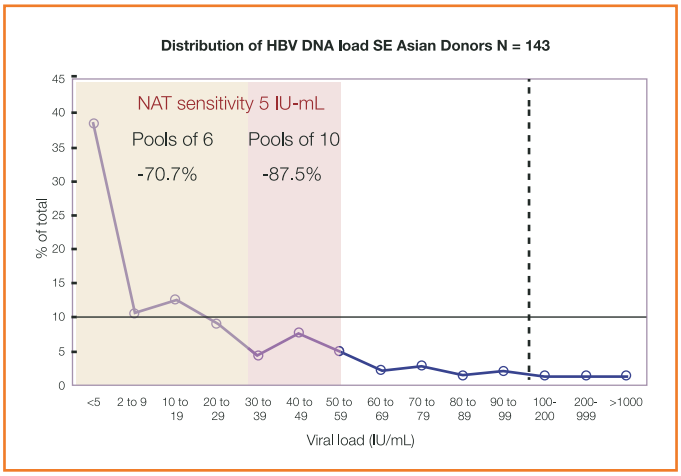

Figure 3. Distribution of HBV DNA load in Southeast Asian donors

HBV testing algorithms and limitations of anti-HBc

A sample algorithm for HBV DNA-positive yield case classification and follow-up is shown in Figure 4.[12] If an HBsAg-negative donation tests HBV DNA-positive and, an anti-HBc assay is positive, anti-HBs can classify OBIs as anti-HBs-positive (40%) or-negative (40%). If anti-HBc is negative, the sample can be in the WP if no anti-HBs is detected (10%), or OBI if the sample tests anti-HBs-positive (10%).

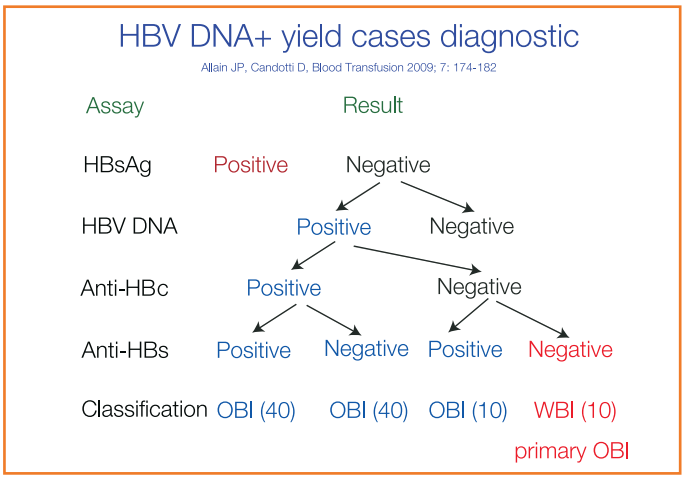

Figure 4. Classification algorithm for HBV DNA positive cases

Regarding the utility of NAT versus serology for HBV, HIV and HCV, serology detects samples undetected by NAT for all three viruses, at least with present assay sensitivity. However, these samples can still be infectious for both HBV and HIV, while NAT not only detects WP infections for the three viruses, but also OBIs for HBV.

Regarding the efficacy of HBV screening assays, ID- NAT identifies more potentially infectious HBV cases than MP-NAT in both the WP and OBIs.

Moreover, if anti-HBc is used as a serological marker for HBV infection, it only helps identify OBIs. However, screening do-nations for anti-HBc may not be cost-effective for populations with an in-country prevalence level of greater than 10% because of the cumulative cost of the units discarded and of replacing these discarded units to maintain the blood supply.

Viral infectivity, HBV vaccination efficiency and implications

Viral infectivity is maximal during the WP when DNA is high and neutralizing antibodies are absent, declining with increasing levels of neutralizing antibodies.

In a review comparing the infectivity of WP, OBI anti-HBc only and OBI with anti-HBs, the WP was highly infectious but varied according to viral load, at 63% when >100 IU/mL and 43% when <100 IU/mL.[13] Ten to 17% of anti-HBc only OBIs appeared infectious and infectivity was just 1.3% when anti-HBs was present, although these data were from relatively few samples.

To check the efficacy of HBsAg plus anti-HBc for HBV safety, HBV NAT screening was performed on 3.7 million ARC blood donations.[14] As expected from risk modeling, 1:712,000 donors were in the WP and all were infected with genotype A2.

"Six donors who had previously been vaccinated for HBV were unexpectedly found to be HBV DNA-positive"

Interestingly, however, six donors who had previously been vaccinated for HBV were unexpectedly found to be HBV DNA-positive, carrying various genotypes other than A2, the most common in the US. Five of these had detectable anti-HBs. Four of the six vaccinated donor's sexual partners were traced and all had chronic HBV infection with high viral loads (>2x10^6 IU/mL). The partners' viral genotypes and sequences were identical to those of the donors, confirming they had transmitted the infection to the donors.

Regarding the evolution of HBV markers in the vaccinated donors, HBV DNA reached a peak after 80 days, together with some HBsAg being detectable. This was followed by development of anti-HBc and an immune response to anti-HBs.

While this evolution indicates acute infection, compared to usual acute infections, the occurrence of the markers was delayed by 20 to 50 days for HBV DNA and HBsAg, and there were no clinical symptoms or ALT elevation. Although the acute infection was abortive, the vaccine antibodies did not prevent infection to non-A2 genotypes. This blood from vaccinated donors should therefore be deemed unsafe for transfusion.

These data have important implications, particularly for East Asia, since it means antibodies induced by the genotype A2 HBV vaccine may not be fully effective against other genotypes. Moreover, all infected individuals had anti-HBs titers <100 IU/L, suggesting that below 100 IU/L, vaccine anti-HBs appears protective against A2 infection but not other genotypes. Furthermore, for full vaccination protection in Asia, where non-A2 genotypes are prevalent, a minimum titer of 100 IU/L is fully protective that can be achieved by more frequent boosting. In addition, all donations from these 'infected yet vaccinated' donors are, depending on further scientific evidence to the contrary, at the moment are considered unsafe.

Q & A

Do we still need to perform HIV or HCV combination assays if HIV/HCV NAT is implemented?

HIV/HCV NAT completely overlaps with HIV or HCV antigen assays and, particularly when used in individual samples rather than pools detects virtually all infectious samples from the window period since there is no evidence that the eclipse period might be infectious.

Is HIV-2 important in routine NAT screening?

HIV-2 is less pathogenic than HIV-1 and is essentially restricted geographically to West Africa. However, in countries receiving substantial numbers of West African immigrants such as France and UK, it might occasionally be found outside of West Africa. To my knowledge, no cases of HIV-2 have been identified in Eastern Asia. In addition to being extremely rare, antiHIV covers detection of antibodies to HIV-2, reducing the potential utility of HIV-2 NAT to the window period. If the risk of finding HIV-2 in China is 1:500,000 HIV infections, and the window period 15 days out of 5 years of infection, the residual risk would be 500,000x122 or 1:61M which can be considered negligible.

Should we focus on HBV serology testing, especially anti-HBc, or NAT? In addition, should we stop doing anti-HBc if NAT is introduced?

Anti-HBc screening to improve HBV safety is currently only used in few countries where the prevalence of anti-HBc is <2% such as the USA, Canada or Germany. Where the prevalence is higher, the efficacy of anti-HBc has been considered too low, not costeffective and having too high impact on the blood supply to be implemented. This was the case in the UK (anti-HBc 0.4%), the Netherlands (anti-HBc 1%) or Mediterranean countries (anti-HBc 48%). All these countries have implemented HBV NAT. In high prevalence countries such as China or other Asian countries where anti-HBc is ranging between 20 and 60%, the impact of anti-HBc would be catastrophic for the blood supply, even if such an impact was limited by testing anti-HBc positive units for anti-HBs and using those anti-HBs positive units clinically. No more than 2% of anti-HBc positive units contain HBV DNA and might be infectious. Unpublished costeffectiveness data clearly indicates that above 10% anti-HBc, NAT screening is more costeffective than anti-HBc because of the very high cost of discarded units (>$50/unit) and the additional costs of replacing discarded units by increasing blood collection to maintain the blood supply.

How do we guard against HBV mutants in NAT testing?

HBV is a virus without proofreading during replication that mutates relatively rapidly. However, some regions of the genome are highly conserved and if the primers and probes utilized in NAT are targeting these regions, all mutants will be detected. At present, there is no evidence that any HBV mutant can be missed by commercially available NATs. In contrast, HBsAg is susceptible to missing variants of the major hydrophilic region of the S protein that is used to capture HBsAg in commercial assays. This situation is particularly frequent in OBIs as demonstrated in a recent article: El Chaar M, et al. Impact of Hepatitis B virus surface protein mutations on the diagnosis of occult Hepatitis B virus infection. Hepatology 2010; 52: 1600-10.

Given the data from the ARC, is there a risk from HBV-vaccinated blood donors?

Blood donors vaccinated to HBV present a massively reduced risk of HBV transmission to blood recipients compared to unvaccinated donors. In China, HBsAg positive chronically infected donors are approximately 8% and approximately 5% of these donors are NAT negative. In contrast, 69% are OBI (NAT positive/HBsAg negative). Unless both HBsAg and NAT are performed, these chronically infected donors present a considerable risk of transmitting HBV.

Vaccinated donors in the USA represent 45% of the donor population and when screening 3.7M donors with NAT or 1,665,000 vaccinated donors, 6 carried HBV DNA or 1:277,500. In addition, the presence of anti-HBs in these donors considerably reduces the risk of being infectious. As a result, vaccinated donors present an extremely low risk of transmitting HBV and blood donors should be encouraged to be vaccinated to HBV.

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