# One window-period donation in two years of individual donor-nucleic acid test screening for hepatitis B, hepatitis C and human immunodeficiency virus

José Eduardo Levi
Ricardo Antonio D'Almeida Pereira
Márcia Bernardino de Carvalho Polite
Mariza Aparecida Mota
Silvia Patricia Nunez
João Renato Rebello Pinho
José Mauro Kutner

Hospital Israelita Albert Einstein - HIAE, São Paulo, SP, Brazil

***Objective:*** *To describe general data on nucleic acid/serology testing and report the first hepatitis B-nucleic acid testing yield case of an immunized donor in Brazil.*

***Methods:*** *A total of 24,441 donations collected in 2010 and 2011 were submitted to individual nucleic acid testing for hepatitis B, hepatitis C and human immunodeficiency virus using the TaqMan® MPX kit (Roche) on the Cobas s201 platform, in addition to routine screening for serological markers. Nucleic acid testing-reactive donations were further evaluated by real-time polymerase chain reaction using Cobas AmpliPrep/ Cobas TaqMan hepatitis B virus, hepatitis C virus and human immunodeficiency virus tests.*

***Results:*** *Thirty-two donations were reactive by nucleic acid testing, 31 were also serologically reactive and one first-time donor was identified as having hepatitis B in the window period. Follow-up samples showed increasing titers of anti-HBs rising from 19 UI/mL in the index donation to 109 IU/mL seven months later attributable to his vaccination history. Curiously, this donor was never reactive for HbsAg nor for anti-HBc. In the yield donation, he was concomitantly reactive for syphilis (enzyme immunoassay and fluorescent treponemal antibody-absorption; venereal disease research laboratory non-reactive). Overall, six donors (0.02%) were characterized as occult hepatitis B. A total of 35% of the confirmed (recombinant immunoblot assay positive) hepatitis C donations were nucleic acid testing non-reactive and no human immunodeficiency virus "elite controller" was identified.*

***Conclusion:*** *The yield rate (1:24,441; 95% confidence interval: 1:9,537 - 1:89,717) contrasts to the North American rate (1:410,540 donations) and strongly advocates the adoption of nucleic acid testing for hepatitis B in Brazil despite the increasing rate of anti-HBs reactive subjects due to the successful immunization program.*

***Keywords:*** *Hepatitis C virus; HIV; Hepatitis B virus; Blood donors; Hepatitis B/diagnosis; Hepatitis C/ diagnosis; Reverse transcriptase polymerase chain reaction*

## Introduction

Brazil collects and transfuses approximately 4 million blood units annually[1]. The list of agents for which testing is mandatory in Brazil is possibly the most extensive in the world. This reflects the importance given to blood safety in the context of public health, but also the historical and geographical characteristics of the country, where globally emerging diseases, such as human immunodeficiency virus (HIV), co-exist with "old" parasitic illnesses like malaria. Currently, legislation requires antibody testing for syphilis, hepatitis B (anti-HBc and HBsAg), hepatitis C, HIV-1 and -2, Human T-lymphotropic virus (HTLV) Types 1 and 2 and Chagas disease. In the endemic regions of the country, a laboratorial pre-donation malaria test is also required. With great regional differences, approximately 4% of the blood collected in Brazil is discarded due to reactivity to one or more markers[1].

About ten years ago, molecular methods achieved a reasonable level of throughput and automation, permitting their introduction as an additional tool in blood screening, aiming chiefly to avoid window-period donations from reaching the blood supply[2,3]. The first agent to be targeted by nucleic acid tests (NATs) was the hepatitis C virus (HCV) due to the long period, of approximately 80 days[4], observed between exposure and antibody development. HIV NAT followed, as the methodology for HCV NAT was easily adapted to embrace a second RNA target. More recently, new generation NATs including hepatitis B virus (HBV)-DNA detection were introduced worldwide.

An international survey[5] reported the NAT yield among 330 million donations, showing an overall rate of 1:446,428 for HCV; 1:1,111,111 for HIV and 1:66,137 for HBV.

In Brazil, up to 2011, only a few blood banks, corresponding to less than 5% of the national blood supply, introduced NAT screening voluntarily. More recently, an HCV/ HIV NAT kit was developed by Fiocruz/Biomanguinhos (Rio de Janeiro, Brazil). This test is already in use in some large public blood banks and projected to be extended to all public blood banks by the end of 2012 when the test may become mandatory. A compilation of the results of Brazilian centers using distinct NAT platforms reported a yield of 1:67,265 for HBV-DNA, and 1:204,951 and 1:271,387 for HIV-RNA and HCV-RNA, respectively[6]. Noticeably, the NAT yield of HIV-RNA is higher than for HCV-RNA

Conflict-of-interest disclosure:
The authors declare no competing financial interest

Submitted: 6/27/2012
Accepted: 8/20/2012

**Corresponding author:**
Jose Eduardo Levi
Departamento de Hemoterapia e Terapia Celular do HIAE
Av. Albert Einstein, 627 - Bloco A - 4º andar - Morumbi
05652-900 São Paulo, SP, Brazil
dudilevi@usp.br

www.rbhh.org or www.scielo.br/rbhh

DOI: 10.5581/1516-8484.20130039

whereas the prevalence of HCV among blood donors is higher than that of HIV. This inverse correlation may be partially attributed to HIV test seekers, presenting an important source of risk for blood recipients[7]. Although the experience with HBV NAT is limited, it is clear that the yield for this virus will be higher than for HIV. Reasons for this are the prevalence and incidence of HBV in the Brazilian population and the insufficient coverage of HBV immunization in donors. The Brazilian immunization program is less than 20 years old, so, it will take years until all blood donors are immunized.

In this article, we describe our experience with a NAT system covering all three viruses and report our first HBV yield case.

## Methods

Blood donations: A total of 24,441 voluntary blood donations were collected in 2010 and 2011 at the Hospital Israelita Albert Einstein (HIAE) blood bank. A 5-mL tube of blood was drawn in $K_2$-ethylenediaminetetraacetic acid (EDTA) specifically for NAT. Approximately 50% of these donations came from repeat donors.

Serology: Serological methods comprised two tests for HIV-1 and HIV-2; one enzyme immunoassay (Biomeriuex Vironostika® HIV Uni-Form II plus O) and one chemiluminescent microparticle immunoassay (CMIA - Ortho Clinical Diagnostics), two tests for HBV; anti-HBc and HBsAg also by CMIA (Ortho Clinical Diagnostics) and one anti-HCV CMIA (Ortho Clinical Diagnostics) test, in addition to the other mandatory tests for syphilis, Chagas disease and HTLV 1 and 2.

Confirmatory testing: Donations presenting a repeatedly reactive (rr) antibody result were submitted to confirmatory testing by Western blot (Cambridge Biotech HIV-1 WB Kit, Maxim Biomedical Inc., USA) when anti-HIV rr and recombinant immunoblot assay (RIBA) (CHIRON RIBA® HCV 3.0 SIA, Ortho Clinical Diagnostics, USA) when anti-HCV rr, which were interpreted according to the manufacturer instructions.

NAT: Plasma from individual donations was submitted to the Cobas TaqScreen MPX (Roche Molecular Systems) HBV/HCV/HIV tests on the Cobas s201 platform. Reactive donations were further evaluated by real-time polymerase chain reaction (PCR) using Cobas AmpliPrep/Cobas TaqMan HBV, HCV and HIV tests.

HBV Viral load: HBV Viral load was evaluated by the Abbot Real Time M2000RT test (Abbott Molecular Inc., Des Plaines, IL, USA).

HBV sequencing: HBV DNA was amplified with primers spanning the *pol*/*HBsAg* gene and sequenced by the standard Sanger dideoxy method (BigDye v 3.1, Applied Biosystems, USA) and run in an ABI 3100 sequencer. Sequences were aligned to HBV wild-type prototype sequences and mutations/polymorphisms were identified by a user-customized software. HBV genotyping was performed by comparing HBsAg sequences to a database containing HBV isolates from all assigned genotypes and genotype-specific polymorphisms[8].

## Results

NAT and serology: In the last two years (2010 and 2011) the prevalence of infectious markers among the screened blood donors remained stable. Overall, there were 275 (1.12%) donations discarded due to the presence of anti-HBc, by far the most prevalent marker (Table 1). In contrast, 29 donations contained HBsAg (0.13%), 19 of which had HBsAg as the only marker and ten together with anti-HBc. One-hundred and two donations were found anti-HCV reactive (0.43%) with 20 (20%) also displaying a reactive immunoblot (RIBA), while 28 and 53 had indeterminate and negative results, respectively (Table 2). Seventy donations were reactive to either one or both anti-HIV tests but only seven were confirmed by Western blot (Table 3). Overall there were 32 NAT reactive HbsAg donations, 31 concomitantly reactive by antibody tests and one HBV NAT yield. Among the 20 RIBA-confirmed HCV reactive donations there were seven in which HCV RNA was not detected (35% clearance rate) whereas all seven HIV Western blot reactive donations were also RNA positive and thus no HIV "elite controller"[9] was identified. Six donors (0.02%) were characterized as occult hepatitis B[10] as shown in Table 4.

Table 1 - Blood donation discard rate due to positivity to infectious markers in decreasing prevalence (n = 24,441 donations)

| Marker/Test | Discard rate (%) |
|---|---|
| anti-HBc | 1.12 |
| Syphilis | 0.75 |
| anti-HCV | 0.43 |
| anti-HIV 1 and 2 | 0.29 |
| Chagas | 0.17 |
| NAT HBV/HCV/HIV | 0.14 |
| HBsAg | 0.13 |
| anti-HTLV 1 and 2 | 0.04 |

Table 2 - RIBA results of anti-HCV reactive donors

| | RIBA* | | |
|---|---|---|---|
| | Positive | Indeterminate | Non-reactive |
| anti- HCV positive by NAT | 20 | 28 | 53 |
| Positive | 13 | 0 | 0 |
| Negative | 7 | 28 | 53 |

102 donations were anti-HCV positive by NAT

* RIBA was not performed for one donor

Table 3 - Western blot results of anti-HIV reactive donors

| | Western blot | | |
|---|---|---|---|
| | Positive | Indeterminate | Non-reactive |
| anti-HIV positive by NAT | 7 | 9 | 54 |
| Positive | 7 | 0 | 0 |
| Negative | 0 | 9 | 54 |

70 donations were anti-HIV positive by NAT

Table 4 - HBV reactive donations in 2010 and 2011 distributed according to all possible combinations of the three markers (anti-HBc, HBsAg and HBV-DNA)

| Combinations of HBV test results | | | n |
|---|---|---|---|
| Anti-HBc RT | HBsAg NRT | HBV DNA NRT | 259 |
| Anti-HBc RT | HBsAg RT | HBV DNA RT | 8 |
| Anti-HBc NRT | HBsAg RT | HBV DNA NRT | 19 |
| Anti-HBc RT | HBsAg RT | HBV DNA NRT | 2 |
| Anti-HBc NRT | HBsAg RT | HBV DNA RT | 0 |
| Anti-HBc NRT | HBsAg NRT | HBV DNA RT | 1 |
| Anti-HBc RT | HBsAg NRT | HBV DNA RT* | 6 |

| Marker | Prevalence % |
|---|---|
| Anti-HBc | 1.13 |
| HBsAg | 0.12 |
| HBV-DNA | 0.06 |

* This pattern corresponds to the definition of Occult Hepatitis B Infection
RT: reactive; NRT: non-reactive

NAT HBV yield case: One first-time donor was identified in the HBV-window period. This index donation was shown to contain 18 IU/mL of HBV-DNA and the isolate was ascribed as genotype A2. Sequencing of the HBsAg open reading frame depicted genotype-specific polymorphisms and one missense substitution, threonine to asparagine (T131N) at position 131 in the major hydrophilic region corresponding to the "a" determinant (aa 124-147). Follow-up samples showed increasing titers of anti-HBs, rising from 19 UI/mL in the index donation to 109 IU/mL seven months later, attributable to his vaccination history. Curiously, in the four follow-up samples obtained, this donor was never reactive for HBsAg or anti-HBc, as shown in Table 5. In the yield donation he was concomitantly reactive for syphilis [EIA and FTA-Abs reactive and venereal disease research laboratory (VDRL) non-reactive].

## Discussion

This report describes the experience of our blood screening laboratory, adopting all mandatory tests in Brazil in addition to NAT, so far not required by the Brazilian regulatory guidelines. This experience is quite unique as it applies a number of tests that are not currently adopted by many other countries, as some would be epidemiologically meaningless such as HTLV in low endemicity countries and Chagas disease out of the Americas, its geographical area of natural occurrence. Moreover, NAT in our service is always performed on individual donations giving an accurate assessment of the prevalence of so-called occult hepatitis B cases. The Cobas® TaqScreen MPX Test has a 95% limit of detection (LOD) of 3.8 IU/mL for HBV and thus is able to detect carriers with a viremia below the common thresholds of quantitative molecular assays.

The donor identified in the window period reported that he did not complete the recommended three doses of HBsAg immunization, receiving just the first shot. Partial immunity could have contributed to the establishment of HBV infection, even though the viremia was low, and may also have induced the emergence of an HBsAg mutation (T131N) found in other HBV A2 isolates[11]. In the United States, the majority of HBV NAT yield donors were indeed vaccine escapes, but probably due to infection by non-vaccine genotypes (non-A HBV genotypes)[12]. Our case was phylogenetically classified as HBV A2, so the failure in preventing HBV infection cannot be attributed to heterotypic non-protective anti-HBs. Several mutations in the HBsAg lead to vaccine escape and false-negative HBsAg results[13]. In general, these mutations occur in the "a" domain comprising the major hydrophilic region, located between amino acids 127 and 141. The substitution of the amino acid threonine by an asparagine is not expected to radically alter the biochemical and antigenic properties of HBsAg as both are polar and have neutral side chains. Since

Table 5 - Laboratorial data from the nucleic acid testing hepatitis B virus window period donation and follow-up samples

| | | Nucleic acid testing | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Syphilis | | | Cobas TaqScreen | | Cobas TaqMan Viral Load | | Hepatitis B markers | | | | | | Hepatic enzymes | | |
| Sample ID | Date | EIE | FTA-ABS | VDRL | MPX v 1.0 | MPX v 2.0 | | | anti-HBc | HBsAg | Anti-HBs | Anti-HBs QT | Anti-HBe | Ag HBe | GGT | TGO | TGP |
| B300111217036 | May 12, 2011 | **POS** | **POS** | NEG | **POS** | **HBV POS** | Undetectable below 20 IU/mL | | **NEG** | **NEG** | **POS** | **19.3** UI/mL | **NEG** | **NEG** | 15 U/L | 15 U/L | 29 U/L |
| B300111217292 | May 18, 2011 | **POS** | ND | ND | **POS** | **ND** | **ND** | **ND** | **NEG** | **NEG** | **POS** | **17.8** UI/mL | **NEG** | **NEG** | 15 U/L | 32 U/L | 41 U/L |
| B300111218043 | June 1, 2011 | ND | ND | ND | **POS** | **ND** | **ND** | **ND** | **NEG** | **NEG** | **POS** | **31.6** UI/mL | ND | ND | ND | ND | ND |
| B300112228330 | January 12, 2012 | ND | ND | ND | **NEG** | **ND** | **ND** | **ND** | **NEG** | **NEG** | **POS** | **109.0** UI/mL | ND | ND | ND | ND | ND |

VDRL: venereal disease research laboratory; HBV: hepatitis B virus; POS: positive; NEG: negative; ND: Not done

HBsAg was also undetectable by other assays (data not shown) we believe that it was really absent or at a very low amount in the index donation, thus, lack of detection is probably unrelated to the T131N mutation. In a thorough evaluation of the performance of HBsAg assays, this variant (T131N) was detectable by all and no reduction in analytical sensitivity was associated to it[11].

Particular epidemiological features can be observed among Brazilian blood donors. While the seroprevalence and the rate of NAT yield cases for HCV are quite similar to those verified in the United States[12] (0.3% and 1:250,000, respectively), much higher prevalence and yields are found for HIV in Brazilian donors (0.3% and 1:205,000)[1,6] in comparison to the United States (0.007% and 1:1,847,429)[12]. Another important aspect refers to HBV epidemiology. Brazil is still considered a country of medium endemicity[14] with areas of high[15] and very high[16] prevalence. The HBV immunization program is now fourteen years old, so it will take time until this benefit becomes evident among blood donors by decreasing the anti-HBc prevalence. Currently, anti-HBc is responsible for most of the discarded blood, by far. A large fraction of these donations have anti-HBc as the only marker. Other countries of high endemicity, such as Italy and Greece, do not test for anti-HBc, as the transmission risk posed by anti-HBc only donors is negligible, while the associated discard rate would jeopardize their blood supply. However, our data show a prevalence of 0.02% of occult hepatitis B thus favoring the maintenance of this marker, at least until NAT HBV on single donations is universally adopted. Furthermore, the high rate of HBV window-period yield donations, 1:24,441 (95% confidence interval: 1:9,537-1:89,717) in this report and 1:67,265 in a compilation of Brazilian centers[6], contrasts to the North American rate (1:410,540 donations)[12], and strongly advocates NAT-HBV adoption in Brazil, despite of the increasing rate of anti-HBs reactive subjects due to the successful immunization program.

## Conclusions

NAT in Brazil is depicting a high yield for HIV and HBV whereas for HCV it is similar to the rates observed in the United States and Europe. The Brazilian situation for HIV is peculiar in presenting a high incidence of seroconverters and NAT only donors, posing a high-risk for its transfusional transmission in centers not adopting NAT. HBV NAT displays the highest yield, recommending, when feasible, the universal adoption of NAT for all three markers. The rate of occult hepatitis B (0.02%) reinforces the need to keep the current anti-HBc/HBsAg algorithm for blood screening in Brazil.

## Acknowledgments

We would like to thank Dr Roberta Sitnik from the LATE, Hospital Israelita Albert Einstein, São Paulo, Brazil for helpful discussions on HBsAg sequence interpretation and Dr Ester Sabino (Infectious Diseases Dept, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil) and Dr Susan L. Stramer (American Red Cross, Gaithersburg, MD, USA) for reviewing this manuscript.

## References

1. Agência Nacional de Vigilância Sanitária- ANVISA. Regulamentar, controlar e fiscalizar os produtos e serviços que envolvam riscos à Saúde Pública, considerando o sangue como bem e produto submetido ao controle e fiscalização sanitária. Bol Anual Prod Hemoterápica [Internet]. 2001;n. 1. [cited 2012 Nov 21]. Available from: http://portal.anvisa.gov.br/wps/wcm/connect/5818d500491bf162bd86bd466b74119d/boletim_producao_hemoterapica.pdf?MOD=AJPERES
2. Stramer SL, Glynn SA, Kleinman SH, Strong DM, Caglioti S, Wright DJ, Dodd RY, Busch MP; National Heart, Lung, and Blood Institute Nucleic Acid Test Study Group. Detection of HIV-1 and HCV infections among antibody-negative blood donors by nucleic acid-amplification testing. N Engl J Med. 2004;351(8):760-8. Comment in: N Engl J Med. 2004;351(21):2232-5; author reply 2232-5; N Engl J Med. 2004;351(8):819-22.
3. Laperche S. Blood safety and nucleic acid testing in Europe. Euro Surveill. 2005;10(2):3-4.
4. Schreiber GB, Busch MP, Kleinman SH, Korelitz JJ. The risk of transfusion-viral infections. The Retrovirus Epidemiology Donor Study. N Engl J Med. 1996:334(26):1685–90. Comment in: N Engl J Med. 1996;335(21):1609-10; N Engl J Med. 1996;334(26):1734-5.
5. Roth WK, Busch MP, Schuller A, Ismay S, Cheng A, Seed CR, Jungbauer, et al. International survey on NAT testing of blood donations: expanding implementation and yield from 1999 to 2009. Vox Sang. 2012;102(1):82-90.
6. Levi JE, Pereira RA, Pondé RA, Petry A, Benati FJ, Melo CP, Wendel S. Experiência multicêntrica brasileira com triagem molecular de doadores de sangue. Rev Bras Hematol Hemoter. 2011;33(Supl 2): Abstract 968
7. Gonçalez T, Sabino E, Sales N, Chen YH, Chamone D, Busch M, et al. Human immunodeficiency virus test-seeking blood donors in a large blood bank in São Paulo, Brazil. Transfusion. 2010;50(8):1806-14.
8. Sitnik R, Pinho JR, Bertolini DA, Bernardini AP, Da Silva LC, Carrilho FJ. Hepatitis B virus genotypes and precore and core mutants in Brazilian patients. J Clin Microbiol. 2004;42(6):2455-60.
9. Okulicza JF, Lambotte O. Epidemiology and clinical characteristics of elite controllers. Curr Opin HIV AIDS. 2011;6(3):163-8.
10. Gerlich WH, Bremer C, Saniewski M, Schüttler CG, Wend UC, Willems WR, et al. Occult hepatitis B virus infection: detection and significance. Dig Dis. 2010;28(1):116-25.
11. Scheiblauer H, El-Nageh M, Diaz S, Nick S, Zeichhardt H, Grunert HP, et al. Performance evaluation of 70 hepatitis B virus (HBV) surface antigen (HBsAg) assays from around the world by a geographically diverse panel with an array of HBV genotypes and HBsAg subtypes.Vox Sang. 2010;98(3 Pt 2):403-14.Erratum: Vox Sang. 2010. 98(4):581.
12. Stramer SL, Wend U, Candotti D, Foster GA, Hollinger FB, Dodd RY, et al. Nucleic acid testing to detect HBV infection in blood donors. N Engl J Med. 2011;364(3):236-47.
13. Qiang Ma Q, Wang Y. comprehensive analysis of the prevalence of hepatitis b virus escape mutations in the major hydrophilic region of surface antigen. J Med Virol. 2012;84(2):198-206.
14. Nascimento MC, Mayaud P, Sabino EC, Torres KL, Franceschi S. Prevalence of hepatitis B and C serological markers among first-time blood donors in Brazil: a multi-center serosurvey. J Med Virol. 2008;80(1):53-7.
15. Viana S, Paraná R, Moreira RC, Compri AP, Macedo V. High prevalence of hepatitis B virus and hepatitis D virus in the western Brazilian Amazon. Am J Trop Med Hyg. 2005;73(4):808-14.
16. Bertolini DA, Pinho JR, Saraceni CP, Moreira RC, Granato CF, Carrilho FJ. Prevalence of serological markers of hepatitis B virus in pregnant women from Paraná State, Brazil. Braz J Med Biol Res. 2006;39(8):1083-90.