The Brazilian experience of nucleic acid testing to detect human immunodeficiency virus, hepatitis C virus, and hepatitis B virus infections in blood donors

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BACKGROUND: The history of the development and implementation of the Brazilian nucleic acid testing (NAT) platform to detect and discriminate among human immunodeficiency virus (HIV), hepatitis C virus (HCV), and hepatitis B virus (HBV) infections in blood donors is described here. The results for the sensitivity, reproducibility, and NAT yield of the platform since program implementation are provided.

STUDY DESIGN AND METHODS: The Brazilian NAT HIV, HCV, and HBV kit was developed and evaluated with regard to analytical sensitivity, specificity, intralot and interlot reproducibility, interfering substances, and genotype and diagnostic sensitivity. Additionally, a sample of identified NAT-yield cases was characterized with regard to viral load.

RESULTS: The 95% limits of detection for HIV, HCV, and HBV were 68.02, 102.35, and 9.08 IU/mL, respectively. All replicates were detected with reproducibility assays between the acceptable values. A total of 13,610,536 blood donors was screened from 2010 to 2016, and 63 HIV-yield cases and 28 HCV-yield cases were detected. Among 5,795,424 blood donors screened for HBV from 2014 to 2016, 42 yield cases were found.

CONCLUSION: The Brazilian NAT HIV, HCV, and HBV kit is an automated NAT system suitable for routine blood donor screening in a completely traceable process. The analytical sensitivity as well as the diagnostic sensitivity fulfilled all requirements set by the health ministry for blood donor screening. A significant number of transmission cases were prevented by the implementation of this important program.

Blood transfusion is an essential component of health care that saves millions of lives worldwide each year. According to the World Health Organization (WHO), 108 million blood donations are collected worldwide. The risk of transmission of serious infections, including human immunodeficiency virus (HIV) and hepatitis, through unsafe blood and chronic blood shortages brought global attention to the importance of blood safety and availability.[1]

Although more sensitive serologic tests have shortened the preseroconversion window period (WP), they still are not able to identify many newly infected donors. These infections in the undetected WP are responsible for most of the transfusion transmission of these viruses. Therefore, it is important to use nucleic acid testing (NAT) in conjunction with serologic testing to decrease the residual risk of these viral transmissions.

NAT has been introduced in several countries around the world to screen blood donations. The aim of NAT is to

ABBREVIATIONS: ANVISA = national agency of sanitary surveillance; HEMOSC = center of hematology and hemotherapy of santa catarina state; IC = internal control; ICQ = internal quality control; LOD(s) = limit(s) of detection; NIBSC = national institute for biological standards and control; SUS = brazilian public health service; WHO = world health organization; WP = window period.

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identify nucleic acids from HIV RNA, hepatitis C virus (HCV) RNA, and hepatitis B virus (HBV) DNA transmitted by transfusion, allowing the early detection of infection during the preseroconversion WP.[2,3] Due to their high sensitivity and specificity, NAT significantly reduces the risk of transfusion-transmitted infections by reducing the diagnostic WP, detecting immunovariant viruses, and identifying occult or persistent viral carriage.[4] Currently, NAT screening of blood donations is either done on individual donations (individual donor testing) or on minipools consisting of equal volumes of donor samples, ranging in number from 6 to 96 samples (minipool testing).[5]

The Brazilian NAT was born from a demand by the Ministry of Health, which is committed to promoting people's access to safe hematologic and hemotherapeutic health care. In Brazil, blood donation is voluntary and noncompensated, and the SUS (Brazilian Public Health Service) is responsible for more than 60% of the blood donations.[6] In this article, we describe our experience with the Brazilian NAT platform in detecting and discriminating among HIV, HCV, and HBV infections in blood donors. Here, we provide the results for the sensitivity, reproducibility, and NAT yield since the beginning of the program's implementation.

MATERIALS AND METHODS

The Brazilian NAT HIV, HCV, and HBV platform

The Brazilian NAT platform consists of automated sample pooling of blood donations using an automated workstation (Janus, PerkinElmer), automated nucleic acid isolation using a molecular biology workstation (BioRobot MDx, Qiagen), and an automated amplification on a real-time PCR system (Applied Biosystems 7500, Thermo Scientific). The HIV, HCV, and HBV kit (Bio-Manguinhos) is a real-time nucleic acid amplification multiplex test for use on the Brazilian NAT platform, and it detects and discriminates among HIV, HCV, and HBV infections in human plasma. Our primers and MGB probes for the reverse transcription-PCR step target the C-terminal nucleotide sequence of integrase in HIV (VIC), the 5′UTR of HCV (FAM), and the S region of HBV (FAM). All steps in sample preparation and the amplification or detection process are monitored by the inclusion of an internal control (IC) in each individual test. Positive controls supplied by the manufacturer are run with every batch. A batch is tracked from sample pooling to results using a computerized system that links each pool to each PCR result. A batch consists of two plates (on Plate 1, HIV and HCV targets can be amplified and detected, while on Plate 2, the target is HBV), two multiple positive controls (Control AB and Control CD) tested in duplicate, and a maximum of 92 reactions to be tested individually or in pools of six (552 samples maximum). Target-specific fluorescent dyes are used in each plate for detection of the amplified nucleic acids. In a typical routine, the HIV, HCV, and HBV kit is run in parallel with the serology test, and the results are compared side by side. The HIV, HCV, and HBV kit is run with pools of six samples, and if a pool tests positive for any molecular target, the individual samples in the pool are tested.

Additionally, an internal quality control (ICQ) from the blood banks is added to each run. The ICQ is a sample known to be positive for HIV, HCV, and HBV. Performing the ICQ is mandatory according to resolution RDC 34/2014 published by ANVISA (Brazilian National Agency of Sanitary Surveillance), which regulates the validation of diagnostic tests in medicine.[7]

Reference blood samples

Usually, blood banks send blood bags that were positive in triage tests and surpluses of negative plasma to Bio-Manguinhos (Fiocruz). Those samples have been subjected to broad serologic and molecular characterization to be included in an internal reference panel and were used in this study. In Brazil, the following serologic tests are mandatory: syphilis, HBV, HCV, HIV-1 and -2, human T lymphotropic virus (HTLV) Types I and II, and Chagas disease.[8]

IC

The IC is a virus-like particle that mimics the HIV virus and is protected by a patent (PI0600715-5). It is biosecure because it does not have the envelope proteins from the HIV virus that are responsible for interacting with CD4 cells. Thus, this virus-like particle has no ability to replicate. The IC aims to control all steps and reactions, including nucleic acid isolation, without interacting with the virus present in the plasma.

Analytical sensitivity

The analytical sensitivity was investigated with serial half-log dilutions of the WHO International Standards, National Institute for Biological Standards and Control (NIBSC) for HIV-1 (97/650), HCV (06/102), and HBV (00/588). Additionally, the HBV seroconversion panel PHM936 (Seracare) was used. All panels were used according to the supplier's instructions.

Each dilution was tested in eight replicates with the NAT HIV, HCV, and HBV kit on three separate days to give a total of 24 tests for each dilution of each virus. The analytical sensitivity of the assay was calculated by determining the 95% limit of detection (LOD) and 50% LOD with a PROBIT analysis.

Interfering substances

Evaluation of the robustness of the Brazilian NAT HIV, HCV, and HBV kit in the presence of interfering substances was done with the commercial kit inhibition panel

TABLE 1. Concentration ranges of each virus used to evaluate the reproducibility of the Brazilian NAT HIV, HCV, and HBV kit

Viruses	> LOD range	LOD range	< LOD range
HIV	3.42×10^2-3.40×10^3	1.55×10^2-2.20×10^2	7.75×10^1-1.10×10^2
HCV	5.72×10^2-1.80×10^3	2.86×10^2-3.00×10^2	1.43×10^2-1.50×10^2
HBV	9.00×10^1-1.02×10^2	4.50×10^1-5.08×10^1	2.25×10^1-2.54×10^1

(OptiChallenge, Acrometrix). Negative human plasma as well as plasma samples spiked with HIV, HCV, and HBV were tested in the presence of EDTA plasma, hemolyzed plasma, heparin plasma, triglycerides, and bilirubin. Eight replicates of each interfering substance were tested individually.

Additionally, samples tested positive by serological and/or molecular assays to HTLV, dengue, chikungunya, Zika, malaria, syphilis, and Chagas diseases were also tested for potential interference. At least 10 samples of each interfering substance were tested in triplicate.

Specificity

A total of 5520 samples were selected from our blood banks that were truly seronegative for Chagas, syphilis, HIV-1/2, HCV, HBV, and HTLV-I/II. All those samples were tested again with a commercial HCV, HIV, and HBV virus load assay to ensure that they were not in a seroconversion WP. Afterward, the truly negative samples were tested with the Brazilian NAT HIV, HCV, and HBV kit. Different lots were used to perform the test according to the manufacturer's instructions. Additionally, the commercial kit inhibition panel (OptiChallenge, Acrometrix) was used to test for possible false positives.

Reproducibility

The reproducibility of the Brazilian NAT HIV, HCV, and HBV kit was evaluated by testing control samples prepared from known, characterized positive samples. The viral concentrations of the samples were prepared in three different ranges named >LOD (above the LOD), LOD, and <LOD (below the LOD). The concentration ranges of each virus are shown in Table 1. Sixty-two lots produced between July 2014 and August 2015 were evaluated. For each lot, the three ranges were tested in eight replicates.

Genotype inclusivity studies

The viral genotype detection capacity of the Brazilian NAT HIV, HVC, and HBV platform was determined by testing NIBSC genotype panels for HCV (02/202), which is composed of the six major genotypes (1-6); an HIV-1 panel (01/466), which is composed of 11 samples representing HIV-1 genotypes (A, B, C, D, AE, F, G, AA-GH, Group N, and Group O); and the Acrometrix HBV genotype panel composed of the eight genotypes (A-G). Four independent replicates were tested for each genotype isolate in four separate runs.

Clinical sensitivity

The 95% LOD and the 50% LOD were determined by using dilutions of pooled clinical samples that were positive for HIV, HCV, or HBV. Serial half-log dilutions were prepared for pooled negative samples. A total of 24 HIV replicates, 32 HCV replicates, and 52 HBV replicates were tested at each dilution level. The data were then used to calculate an LOD using a PROBIT (IBM SPSS Statistics 20.0) analysis. The LOD values are included in our product insert.

Computerized network system

The computer systems of all the equipment for each Brazilian NAT platform are connected via a mini-network line. The barcode on the primary tubes, secondary tubes, nucleic acid isolation plates, and optical plates guarantee specimen traceability.

The NAT software analyzes if at least one positive and one negative control are between the acceptable values to validate the assay. After validation, the software analyzes each well individually. When positive results are observed in a pool of six plasma samples, those samples are retested individually in single assays. A positive result is released only when the sample has been tested in a single assay. If the reaction wells are negative for the targets, the IC validation is performed. When the IC is positive between the acceptable Ct values, the result is not detectable for the tested targets. When the IC is not validated, this reaction must be repeated from the beginning.

Program implementation

In the first phase, one NAT platform was installed in the Center of Hematology and Hemotherapy of Santa Catarina State (HEMOSC), and 5000 blood samples were tested with the NAT HIV and HCV kit (Bio-Manguinhos). A second phase was designed including four blood banks: HEMOSC, the Center of Hematology and Hemotherapy of Pernambuco, the Center of Hematology and Hemotherapy of Rio de Janeiro, and the Center of Hematology and Hemotherapy of São Paulo (Fundação Pró-Sangue). During this stage, the NAT platform was installed in each

Fig. 1. Brazilian map indicating where the NAT platform is present and when it was set up.

blood bank, and 200,000 blood samples were tested with the NAT HIV and HCV kit (Bio-Manguinhos).

The third phase occurred gradually from 2011 to 2013, after the registration of the Brazilian NAT HIV, HCV, and HBV kit with the regulatory agency ANVISA. The third phase involved the installation of 14 NAT platforms in reference centers located in Rio de Janeiro, São Paulo, Minas Gerais, Pernambuco, Bahia, Paraná, Santa Catarina, Ceará, Mato Grosso do Sul, Amazonas, Pará, and Distrito Federal (Fig. 1). Currently, each reference center has at least two high-throughput platforms working in parallel to provide equipment backup.

NAT yield

The yield was defined as a NAT-positive test result that was negative in serologic testing. For quantification of the viral load in NAT-only positive blood donations, the following assays were used: the COBAS TaqMan HBV test, the COBAS TaqMan HCV v2.0, and the COBAS TaqMan HIV v2.0. Each test was performed according to the manufacturer's instructions. All quantitative test results for HCV RNA and HBV DNA are expressed by the assay in IU/mL, and HIV RNA values are usually expressed in copies/mL, which were converted to IU/mL.

TABLE 2. Analytical sensitivity of the Brazilian NAT HIV, HCV, and HBV kit

	Detection probabilities	
Panel tested	95% LOD (IU/mL)	50% LOD (IU/mL)
HIV WHO (NIBSC 97/650)	95.86	46.77
HCV WHO (NIBSC 06/102)	44.09	7.11
HBV WHO (NIBSC 00/588)	*	*
HBV Seracare (PHM936)	4.86	2.72

*It was not possible to calculate the PROBIT analysis of the HBV NIBSC panel because all replicates were detected.

RESULTS

The International Panel of the NIBSC/WHO was used to calculate the analytical sensitivity of the Brazilian NAT HIV, HCV, and HBV kit. In addition, the assessed results with 95% positivity detection limits were 95.86 IU/mL for HIV, 44.09 IU/mL for HCV, and 4.86 IU/mL for HBV. The results are summarized in Table 2.

For an analysis of specificity, a total of 5520 samples tested negative when screened with the Brazilian NAT HIV, HCV, and HBV kit. All samples had already tested negative for the three viruses by serology and/or molecular assays, but they could have been positive for other

pathologies such as HTLV, dengue, chikungunya, Zika, malaria, syphilis, or Chagas diseases. No false positives were detected when testing negative plasma samples that occasionally contained interfering substances such as EDTA, hemolysis, heparin, triglycerides, or bilirubin.

No inhibition was observed for HIV, HCV, HBV, or the IC PCR amplification reaction when tested with the interfering panel or with samples positive for other pathogens such as HTLV, dengue, chikungunya, Zika, malaria, syphilis, or Chagas diseases. All replicates tested were reactive, and the Ct values were within the acceptable range.

Reproducibility was evaluated in 62 kit lots and with different operators. The Brazilian NAT HIV, HCV, and HBV kit detected all 496 replicates from each range of the three viruses. In the genotype inclusivity study, the Brazilian NAT HIV, HCV, and HBV kit was able to detect all the panel members of HIV, HCV, and HBV (Table 3).

All spiked samples were detected, and all the spiked viruses were identified. Here, we have demonstrated that the Brazilian NAT HIV, HCV, and HBV kit is able to detect all the genotypes of HIV, HBV, and HCV.

TABLE 3. Ct values, mean, and SD for 496 replicates evaluated in 62 lots*

	HIV	HCV	HBV
> LOD range	31.75 (±0.55)	31.38 (±1.27)	33.66 (±0.69)
LOD range	35.09 (±1.34)	33.4 (±1.4)	34.7 (±0.74)
< LOD range	36.64 (±1.81)	34.6 (±1.5)	36.29 (±1.23)

*Data are reported as mean (±SD)

TABLE 4. Diagnostic sensitivity of the Brazilian NAT HIV, HCV, and HBV kit

Positive clinical samples tested	95% LOD (IU/mL)	50% LOD (IU/mL)
HIV	68.02	10.23
HCV	102.35	29.36
HBV	9.08	2.37

In other to challenge the Brazilian NAT HIV, HCV, and HBV kit with virus isolates circulating among Brazilian blood donors and to calculate the clinical sensitivity, we performed an LOD estimation using HIV, HCV, and HBV field samples. Table 4 summarizes the results of the 95% LOD and 50% LOD determinations of the Brazilian NAT HIV, HCV, and HBV kit using positive clinical samples. The results show a 95% LOD of 68.02 IU/mL for HIV, 102.35 IU/mL for HCV, and 9.08 IU/mL for HBV.

The first phase of Brazilian NAT implementation was conducted in 2008, and 5392 blood bags were tested with no NAT-positive HIV and/or HCV samples identified. The second phase, conducted in 2009 to 2010, tested 219,791 blood bags and detected two bags NAT positive for HIV. From 2011 on, the Brazilian NAT HIV and HCV platform was implemented all over the country. Figure 1 shows on a map where the Brazilian NAT platform was installed and where each phase of program implementation was performed. Additionally, the map shows the national coverage of NAT indicated by NAT platform implementation.

A total of 13,610,536 blood samples were screened from 2008 to 2016 by the Brazilian NAT HIV, HCV, and HBV platform, yielding 63 and 28 cases that were negative according to serologic tests but NAT positive for HIV and HCV, respectively. For the HBV NAT, 5,795,424 blood samples were screened, yielding 42 samples within the immunologic window (Table 5). We observed 0.04% of invalid

TABLE 6. Geographic distribution of blood bags that are NAT positive for HIV, HCV, and HBV and NAT-yield rates per million

Geographic region	NAT positive					
	HIV (yield)		HCV (yield)		HBV (yield)	
South	14	(5.87)	8	(3.35)	2	(1.52)
Southeast	21	(3.29)	13	(2.04)	12	(4.24)
Midwest	6	(5.05)	4	(3.37)	5	(7.68)
Northeast	11	(4.01)	3	(1.09)	17	(12.11)
North	11	(12.10)	0		6	(14.14)
Total	63		28		42	

TABLE 5. Results of the tested blood bags and NAT-positive detection of HIV, HCV, and HBV, every year since the pilot study

Year	Blood bags screened for HIV and HCV	NAT-positive		Blood bags screened to HBV	NAT-positive
		HIV	HCV		HBV
2008 (pilot study)	5,392	0	0	NT	
2009/2010 (multicentric)	219,791	2	0	NT	
2011	471,360	0	1	NT	
2012	1,292,558	3	1	NT	
2013	2,511,092	14	4	NT	
2014	2,939,086	12	5	43,442	0
2015	3,015,848	13	6	2,596,573	18
2016	3,155,409	19	11	3,155,409	24
Total	13,610,536	63	28	5,795,424	42

NT = not tested.

runs throughout the 8 years of Brazilian NAT implementation, based on customer calls to the NAT hotline. Overall, NAT-yield rates per million donations were 4.62 for HIV, 2.05 for HCV, and 7.24 for HBV. Table 6 presents the geographic distribution of yield cases throughout the South, Southeast, Midwest, North, and Northeast Brazilian regions.

A total of 27 HIV-, 10 HCV-, and 19 HBV-yield cases were recovered and characterized with regard to the viral RNA or DNA quantification. Figure 2 shows the viral load of each yield case. Unfortunately, not all yield samples were subjected to quantification due to an insufficient amount of material to be tested or because they were not sent to the laboratory to be characterized.

DISCUSSION

Transfusion-transmitted infections represent an ongoing challenge in transfusion medicine. Studies developed by the Ministry of Health and ANVISA in 2003 have shown the need for public investment in developing a national product. The development of the Brazilian NAT platform started with a consortium from the Institute of Technology in Immunobiology Bio-Manguinhos/Fiocruz, Federal University of Rio de Janeiro and the Institute of Molecular Biology from Paraná, coordinated by the health ministry. The Institute of Technology in Immunobiology Bio-Manguinhos is the unit of the Oswaldo Cruz Foundation (Fiocruz) responsible for technology development and production of vaccines, reagents, and biopharmaceuticals targeted primarily to meet the demands of national public health.

Once the NAT HIV and HCV kit was developed and the equipment was installed in the research laboratory facilities, the Brazilian NAT platform was validated in the first phase conducted in HEMOSC. During this period, it was possible to implement improvements to the kit and platform, and as a result, the certification of good manufacturing practices was received from ANVISA.

This first phase resulted in improvements to the Brazilian NAT HIV and HCV kit that made the implementation of the second phase possible. In December 2010, with partial results from the multicentric study, the Brazilian NAT HIV and HCV kit developed by Bio-Manguinhos/Fiocruz had its registration approved by ANVISA (number 80142170025). By that time, the Brazilian NAT HIV and HCV kit was the only multiplex discriminatory kit available with ANVISA registration. Other international NAT kits were able to detect HIV and HCV in one reaction, but they could not discriminate between these viruses, making a second reaction necessary to distinguish between them.

The development of the Brazilian NAT HIV and HCV kit brought several benefits to Brazil. Among these benefits were the implementation of politics focused on research and development, the nationalization of technology and supplies, technologic advancement, facilities certified by good manufacturing practice, investments in training competencies, the development of skilled labor, and the reduction of international need regarding diagnostic tests and supplies.

In November 2013, the Ministry of Health made mandatory, by the ministry ordinance "Portaria Ministerial 2.712/2013," NAT screening for HIV and HCV in every blood donation collected by public and private blood banks in Brazil.[7] Currently, the Brazilian NAT platform is a national network, screening 100% of the donated blood in SUS. In less than 48 hours, the blood from the entire country is transported to one of those reference centers, NAT is performed, and the result is available. This complete barcoded-controlled process for blood screening by NAT with an automated robot system represents the current state-of-the-art practice, excludes manual failures, and improves performance. A multicentric manager software named GSM-NAT (Gerenciador do Sistema Multicêntrico NAT) automatically manages the sending of samples, which are collected and held by hemotherapy services for the NAT reference center and allows the online viewing of released NAT results in all NAT reference centers. The current NAT platform is available free of charge by the Ministry of Health in Brazil, and customer service is available 7 days a week to help solve any problem.

In Brazil, unlike NAT, serology testing is performed by the hemotherapy service in an independent way, with each blood bank choosing which serology kit they use. The list of agents for which serologic testing is mandatory in Brazil includes syphilis, HBV, HCV, HIV-1 and -2, HTLV-I and -II, and Chagas disease. In addition to this, in the endemic malarial regions of the country, a laboratorial predonation malaria test is also required.

The detection of HBV DNA was not introduced at the beginning of Brazilian NAT development. However, an update to our product was needed, since new-generation NAT kits including HBV DNA detection were introduced worldwide. In November 2014, the Brazilian NAT HIV and HCV kit had its ANVISA registration changed to include HBV. Currently the Brazilian NAT kit detects and discriminates among HIV, HCV, and HBV.

This extensive study of the automated Brazilian NAT platform has provided a large body of performance evaluation data, which contributed to our understanding of the impact of this new technology on blood safety in Brazil. The clinical sensitivity calculated by PROBIT analysis for Brazilian NAT, considering 95% detection limits, is in accordance with test specifications and the limits announced by the Brazilian Ministry of Health for NAT for blood donations. However, if we compare the 95% LOD from the Brazilian NAT with two other commercial NAT platforms, such as the Procleix Ultrio Plus Assay and the

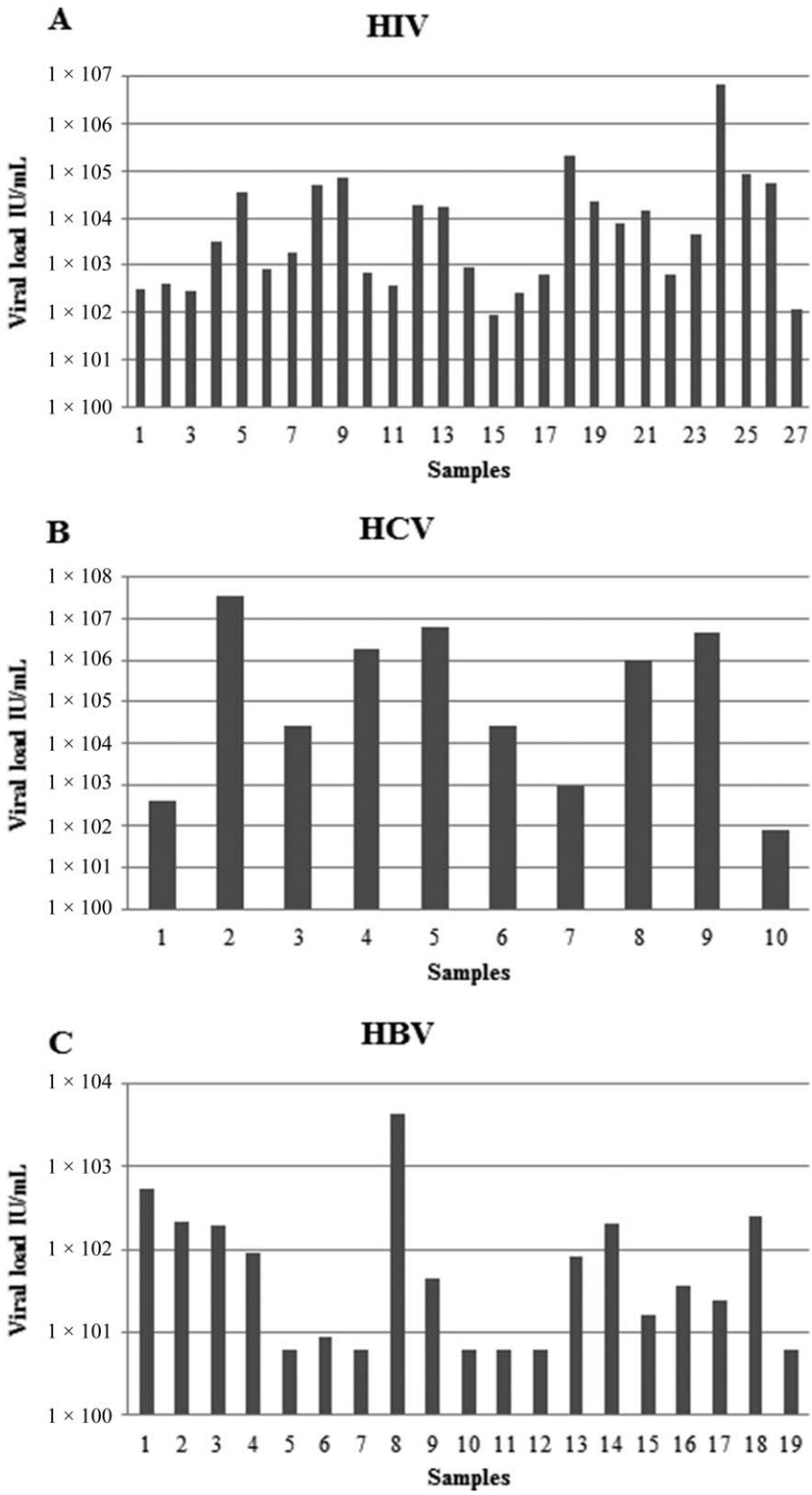

Fig. 2. Viral load in yield cases detected by the Brazilian NAT HIV, HCV, and HBV kit.

cobas TaqScreen MPX test, our results are approximately 2.0× to 2.5× less sensitive for HIV, 10× less sensitive for HCV, and 2× to 3× less sensitive for HBV.

International requirements for sensitivity limits vary depending on the country. For example, in Germany, the Paul Ehrlich Institute requires the test to be able to detect

5000 IU/mL HCV RNA and 10,000 IU/mL HIV-1 RNA in a single donation.[9,10] HBV NAT with a sensitivity limit of 25 IU/mL for individual blood donations is mandatory in Switzerland.[11] The US Food and Drug Administration requires a 95% detection limit of at least 100 copies/mL for both HCV and HIV NATs, with a sensitivity of at least 5000 copies/mL when sample pooling is performed[10] and a minimum sensitivity of 100 IU/mL for HBV DNA detection.[12] The sensitivity limits of the Brazilian NAT HIV, HCV, and HBV kit have been shown to be in agreement with the seroconversion window profile, with regard to the analysis of viral loads from yield cases by quantitative NAT.

The Brazilian NAT HIV, HCV, and HBV kit showed good specificity. No false positives were detected when testing 5520 truly negative samples for HIV, HCV, and HBV. The robustness of the test was demonstrated by the reproducibility study, both interlot and intralot. Sixty-two lots of the kit, produced over 1 year, were evaluated, and all 496 replicates of each range of the three viruses were detected. Here, we have used virus samples from all over the country. It is worth noting that Brazil is 8,516,000 km^2 and is the world's fifth-largest country, both by geographical area and by population.[13] We believe that we have covered the diversity of circulating HIV, HCV, and HBV in Brazil. Thirty-four lots were evaluated with a specific sample of each virus—HIV560, HCV4640, and HBV791—and all 272 replicates of each range of the three viruses were detected. This study presents many NAT lots and replicates used to detect HIV, HCV, and HBV in blood donors. Previous studies have reported eight to 32 replicates and a maximum of two lots evaluated.[5,9,14]

The results of this study represent a broader and more reliable assessment of safety measures for Brazilian blood supplies. Here, we provide direct evidence of the benefits of HIV, HCV, and HBV NAT. Since the Brazilian NAT HIV and HCV kit was registered by ANVISA in 2010, a total of 13,610,536 blood bags have been screened. After the introduction of the HBV target into the Brazilian NAT HIV, HCV, and HBV kit, 5,795,424 blood bags have already been screened for HBV. As HBV NAT is not yet mandatory, some services do not routinely perform the assay. Overall, NAT-yield rates per million donations were 4.62, 2.05, and 7.24 for HIV, HCV, and HBV, respectively.

In fact, the HIV NAT yield of 4.62:1,000,000 in Brazil from 2010 to 2016 was similar to the 4.38:1,000,000 previously reported by Andrea and coworkers,[15] in Brazil, between May 2011 and July 2013, using the Brazilian NAT HIV and HCV kit. If compared to other world regions, our HIV NAT yield of 4.62:1,000,000 is higher than the 0.5:1,000,000 reported for the period 1999 to 2008 in the United States[16] and Central and Northern Europe[17] and the 2.5:1,000,000 reported in Mediterranean Europe, regardless the lower LOD of our HIV assays when compared to commercial tests utilized in the United States and Europe. However, our yield is almost the same as the 4.8:1,000,000 reported in Southeast Asia.[17] On the other hand, the HCV NAT yield of 2.05:1,000,000 in Brazil from 2010 to 2016 was lower than the 3.7:1,000,000 reported in the United States,[16] the 2.5:1,000,000 reported in Italy,[18] the 2.38:1,000,000 reported in Spain,[19] the 2.25:1,000,000 reported in Germany,[20] and the 3.7:1,000,000 reported in South Africa.[21] However, it was higher than the 0.5:1,000,000 reported in Canada.[22] These numbers must be considered carefully since in Brazil, serology testing for HCV is done with a fourth-generation assay, including the detection of anti-HCV and HCV surface antigens, while in the United States and Europe, the third-generation assay is used, which only detects anti-HCV. The fourth-generation HCV serology assay can reduce the immunologic window, and consequently, the HCV NAT yield is lower, and the positive serology testing is higher. Another plausible explanation could be the lower LOD of the Brazilian HCV NAT when compared with the tests utilized in the United States and Europe.

Interestingly, the HBV NAT yield of 7.24:1,000,000 in Brazil from 2014 to 2016 was lower than the 43.2:1,000,000 reported in Africa and the 17.4:1,000,000 reported in the Asia-Pacific region and higher than the 2.97:1,000,000 reported in Europe and the 3.1:1,000,000 reported in North America.[3] Notably, Brazilian blood banks adopted fourth-generation serology tests to detect the hepatitis B surface antigen and antibodies to the hepatitis B core antigen.

Analyzing the geographic distribution of NAT-yield rates per million for HIV, HCV, and HBV, we found that the North region had higher yield rates for HIV (12.10) and HBV (14.14). HCV had higher yield rates in the Midwest (3.37) and South regions (3.35). A lower yield rate for HIV was found in the Southeast region (3.29), for HCV in the Northeast region (1.09) and for HBV in the South region (1.52).

The Brazilian NAT platform (Bio-Manguinhos) can be considered a milestone in the diagnosis and laboratory screening of infectious diseases in Brazil, due to the increase in the safety of transfused blood and because of the technologic development. Technologic advances obtained with NAT deployment may be applied to other segments in health care services linked to SUS and aimed at improving the health of the population and focused on preventive actions in the diagnostic area. The continuous improvement program from Bio-Manguinhos, aimed at increasing the safety of blood transfusions, is already working on adding new targets to the Brazilian NAT platform, such as malaria, dengue, chikungunya, and Zika. In addition, a more modern platform with magnetic bead extraction is also being developed and standardized. This new technology will further increase the sensitivity of the kit.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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