Original Article

NATSpert ID TripleH: A Novel Individual Donor Multiplex Nucleic Acid Amplification Test to Reduce Risk of Transfusion-Transmitted Infections: Two-Year Experience of a Blood Bank in Central India

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ABSTRACT

Background: In India serological screening is mandatory for all donated blood for hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) while Nucleic Acid Amplification Testing (NAT) is not a mandatory screening test. There is a risk of transfusion-transmitted infections (TTI) during window period using serology. **Aims and Objectives:** The aim of this study was to analyse NAT screening using an indigenously developed, Indian manufactured Individual Donor NAT (ID-NAT) and compare it with serology. **Materials and Methods:** All blood donations between June 2017-March 2019 were screened serologically for HBV, HCV and HIV at an Indian blood bank. Blood donations also underwent ID-NAT screening using the NATSpert ID TripleH detection assay based on real time PCR. The results were analysed to identify yield cases. **Results:** In the study, 30,772 blood donor samples were screened serologically out of which 214 were reactive. 30,558 serologically non-reactive blood donations and 77 randomly selected, serologically reactive blood donations were screened using NATSpert. Out of 30,635, 85 donor samples were reactive on the NATSpert which included 77 serology positive and 8 NAT yield cases. The NAT yield found was 2 each for HBV/HIV and 4 of HCV. **Conclusion:** The NATSpert ID TripleH offers a statistically significant advantage over EIA in ability to detect TTI in blood donors ($P < 0.05$, Fishers Exact Test). The NAT yield of 1:3829 was in line with other Indian studies. NATSpert assay will provide a significant improvement in blood safety and offer a cost benefit compared to the imported products.

Keywords: *Blood safety, individual donor multiplex nucleic acid amplification test, nucleic acid amplification acid test, transfusion-transmitted infections*

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Introduction

A well-organized blood transfusion service (BTS) in the country is a vital component of any healthcare delivery system. An integrated strategy for blood safety is required for elimination of transfusion-transmitted infections (TTIs) and for provision of safe and adequate blood to the population. Government of India adopted the National Blood Policy in April 2002 which aims to develop a nationwide system to ensure easy access to blood and blood components collected from a voluntary nonremunerated regular blood donors in a well-equipped premises, which is free from TTIs.[1] One major objective of the National Blood Policy is to make latest technology available for operating the BTSs and ensure its functioning in an updated manner.

The technology to detect TTI has evolved rapidly over the past 2–3 decades beginning with less sensitive immunochromatographic rapid tests or less-specific tests based on surrogate markers to the sensitive serological tests based on specific antibodies (and then antigens) utilizing either enzyme-linked

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immunosorbent assay (ELISA) or chemiluminescent immunoassay (CMIA).[2] Despite the use of new generation of serological tests, a significant risk of TTI still remains as these tests are not able to detect a newly infected blood donor in the window period, that is, the time after infection and before seroconversion, during which markers of infection (antigen and antibodies) are absent or too scarce to be detected.

Since 1990s, usage of nucleic acid amplification acid test (NAT) in blood banks has expanded rapidly to help detect HIV, hepatitis B virus (HBV), and hepatitis C virus (HCV) infections in the window period. The NAT detects the presence of nucleic acid of the target virus, by amplifying it manifold, and a sensitive detection system confirms its absence or presence. Germany was the first country to introduce NAT screening in 1997 on a routine basis. Today, NAT is a mandatory screening test for donated blood in most of the developed and developing countries.[3,4]

NAT donors screening is not mandatory in India as of now, and a review published in 2017 stated that NAT screening is carried out in only 2% blood banks and covers only 7% of all collected blood units in India[5] in spite of the fact that the seroprevalence of infections in India is much higher than in the developed world. Major barriers of implementing routine NAT in India are its high cost and lack of technical expertise in most of the blood centers.[6]

There are many Indian and international papers published on the use of NAT, suggesting the importance and utility of NAT in blood donor screening vis-a-vis EIA.[7-9] In the Indian studies carried out using NAT kits manufactured in Europe or USA, the NAT yield for various viruses varied from 1:476 to 1:4403.[5]

Aims and objectives

The objective of this study was to analyze the results of a comparative study carried out over 2 years, where in an indigenously developed NAT kit NATSpert ID TripleH, based on hydrolysis probe, chemistry was compared with a combination of CMIA and ELISA for screening of TTI in blood donors in a blood bank in central India. The aim of our study was to ascertain the NAT yield, its benefit in increasing blood safety, and its cost-effectiveness.

Materials and Methods

The study was carried out from June 2017 to March 2019 at a Blood Bank in Central India, which is a NABH-accredited standalone blood bank. The ethical clearance for the study was taken from the board of the trust that runs the blood bank, and blood donors were informed, and consent was taken for testing donated blood by screening tests required to ensure blood safety. All blood units collected, either at the blood bank or blood donation camps, during the study period were donated by voluntary nonremunerated blood donors or nonrenumerated replacement blood donors. As per the policy of the blood center, the donors underwent a stringent predonation history taking and screening according to the Indian regulatory guidelines before they were cleared to donate blood. The serological testing was performed in the blood bank whereas a separate NAT laboratory was set up in the same building for the individual donor multiplex NAT (ID NAT) screening.

Serological testing

Venous blood samples were collected in 6-mL vacutainer tubes from all blood donors, and serological testing was carried out on Abbott ARCHITECT system (Abbott Diagnostics, USA), using the ARCHITECT HIV Ag/Ab Combo assay, a CMIA-based test for the simultaneous qualitative detection of HIV p24 antigen and antibodies to Type 1 and/or Type 2 (HIV-1/HIV-2) in human serum/plasma. For screening HBV, the ARCHITECT hepatitis B surface antigen (HBsAg) assay was used for the qualitative detection of HBsAg while HCV was screened using Qualisa (ELISA) kits for detection of antibodies to HCV in human serum or plasma.

Samples were run in batches in line with the protocol given by the manufacturer with positive and negative controls run simultaneously to validate the results.

Nucleic acid amplification acid test screening

The NAT screening was carried out using NatSpert ID TripleH Detection test, an ID-NAT (Mylab Discovery Solutions, India). Plasma, separated from venous blood samples, was stored at 2–8°C when tested within 8 h or stored at −20°C when testing was to be carried out later. Viral nucleic acid was isolated from plasma samples using paramagnetic bead technology with a sample volume of 1 ml and elution volume of 90 µl. As per the manufacturer's protocol, internal control (IC) was added to the plasma before extraction to control the efficacy of extraction and absence of inhibitory compounds. After the extraction of viral nucleic acid, multiplex one-step real-time reverse transcription polymerase chain reaction (RT-PCR) based on hydrolysis probe chemistry was carried for the amplification, detection, and discrimination of HIV RNA, HCV RNA, and HBV DNA. The NATSpert ID TripleH Detection test targets the amplified conserved regions of HIV-1M/N, HIV-1 O, HIV-2, HCV,[1-6] HBV (A-H), and IC using specific primers and detection by multidye probe . All three HIV targets (HIV-M/N, HIV-1 O, and HIV-2) are identified using the same dye and thus are not discriminated from each other.

The amplification and reading was carried out on the QuantStudio 5 Real-Time PCR System (ThermoFisher Scientific, USA). The PCR conditions were 50°C for 15 min for RT, 95°C for 20 s to inactivate the RT enzyme, followed by 50 cycles of 5 s at 95°C and 30 s at 60°C. The PCR software was used to determine the cycle threshold values.

Statistical analysis

This prospective cross-sectional, blinded study was analyzed using Fisher exact test to find any statistically significant difference in ability to detect TTI using NAT as compared to EIA.

Results

32,981 blood donors came to the blood bank or blood donation camps during the study period, of which 30,772 donors were found eligible for blood donation and 2209 (6.7%) blood donors were deferred/rejected as per standard criteria. Of these donors, 29,080 donors were male.

Seroprevalence of transfusion-transmitted infections

After serologic screening, 214 (0.695%) donors of 30,772 were found to be reactive. Of these 214 seroreactive donors, 123 (0.4%) were reactive for hepatitis B surface antigen, 30 (0.097%) showed presence of antibody to anti-HCV, and 61 (0.2%) were found to be reactive for HIV p24 antigen or antibodies to HIV Type 1 and/or Type 2.

Nucleic acid amplification acid test testing

All the serologically nonreactive blood donor samples and 77 randomly selected and blinded serologically reactive blood donor samples, totaling to 30,635, were screened on the NATSpert ID TripleH assay. Of the 77 serologically reactive samples, 40 were HbsAg reactive, 23 were reactive for HIV Ag/Ab Combo, and 14 were anti-HCV reactive. On NAT screening, 85 donor samples were found to be reactive. All the 77 serology reactive samples were reactive for the same infection on NAT screening. Significantly, there were 8 reactive results on NAT screening that were nonreactive on serologic testing (NAT yield of 1: 3829), of which 4 were HCV reactive and 2 each were HBV and HIV reactive [Table 1 and Figures 1, 2].

On statistical analysis using Fisher's exact test, the NATSpert ID TripleH assay was found to offer a statistically significant advantage over EIA in ability to detect TTI in blood donors ($P < 0.05$, Fisher's exact test) [Table 2].

The researchers wanted to test all the NAT reactive/serology nonreactive samples using a quantitative RT-PCR based kit from another manufacturer. Of 8 samples, 2 were not stored at optimum temperature for molecular testing while 1 sample was insufficient for further testing. All the 5 remaining samples were processed further for quantitative testing and found to be positive.

Table 1: Total number of seroreactive and individual donor multiplex nucleic acid amplification test reactive samples

Total number of donations	30,635			
TTI test	Total	HBV, *n* (%)	HCV, *n* (%)	HIV, *n* (%)
Seroreactive samples	77	40 (0.13)	14 (0.45)	23 (0.075)
ID NAT reactive samples	85	42 (0.14)	18 (0.058)	25 (0.082)
NAT yield	8	2 (0.006)	4 (0.013)	2 (0.006)

HBV: Hepatitis B virus, HCV: Hepatitis C virus, HIV: Human immunodeficiency virus, TTI: Transfusion-transmitted infections, ID NAT: Individual donor multiplex nucleic acid amplification test

Table 2: Fisher exact test 2×2 table

EIA (serology)	NATSpert ID	
	Positive	Negative
Positive	77	0
Negative	8	30550

$P<0.05$, Fisher's exact test. EIA: Enzyme immunoassay

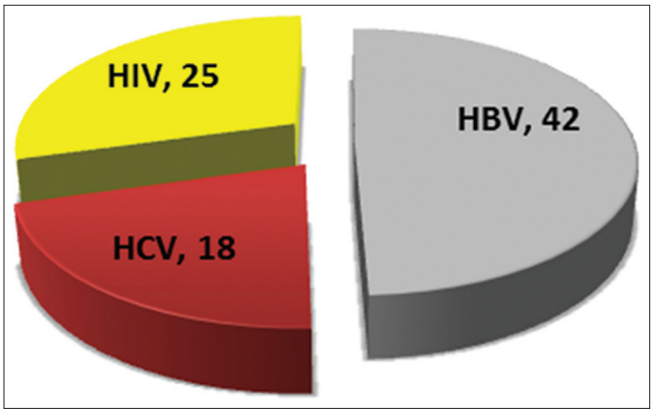

Figure 1: Individual donor multiplex nucleic acid amplification test reactive samples of 30,635

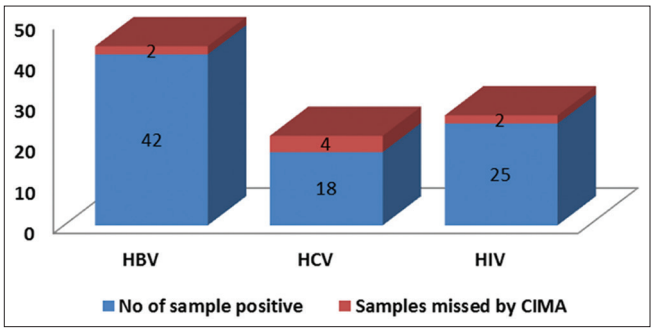

Figure 2: Number of samples missed by chemiluminescent immunoassay

Discussion

In this study, we compared the results of screening a population of Indian blood donors using an indigenously developed and manufactured-NAT assay with the results obtained from a combination of CMIA for HIV and HBV and ELISA for HCV.

Our study showed that 85 blood donor samples of 30,635 were reactive on NAT screening whereas only 77 of these were reactive on serological screening. Thus, 8 whole blood units, that would have been transfused to potentially 24 recipients (as each whole blood unit is made into 3 components), were detected and taken out of the transfusion cycle due to NAT screening over 21 months.

For combined TTI, NAT yield in our study was 1:3829 which is in line with previous published studies from other institutes of India where in the NAT yield varied from 1:476 to 1:4403.[5] For individual infection, NAT yield showed HIV in 1:15,317, HBV 1:15,317, and HCV in 1:7658.

It is important to note that most of the studies from India comparing NAT and EIA have compared NAT to a 3rd-generation ELISA[7,10] whereas this study compared the results to 4th-generation CMIA except for HCV. It is natural for the NAT yield to be higher when compared to 3rd-generation antibody; only ELISA as the period between getting infected and appearance of an antibody is much longer when compared to the period between getting infected and detection of the antigen (pathogen itself).

This study has potential limitations. The authors would have preferred to repeat the serological tests of the NAT yield blood samples on different manufacturer's kits and check for the presence of HCV antigens serologically for the HCV yield samples. Wherever feasible, the NAT yield samples could also be repeated on another make of NAT kit. These aspects can be included for any future studies of a similar nature.

In the past decade, there has been a continuous improvement in transfusion safety, especially with regard to TTI. Many factors have contributed to this including stringent donor selection criteria, donor self-awareness, improved sensitivity of the screening tests, better training of the blood bank staff, and implementation of NAT testing as a routine donor screening.

Of all the factors mentioned above, the last one, that is implementation of NAT, has been a matter of great interest the world over. In spite of being aware of its importance, very few blood banks in India (~2%) have adopted NAT technology.[5]

It is even more important that an advanced screening test for TTI such as NAT is universally implemented in India because of:

- Higher prevalence of infections in blood donors in India as compared to developed countries[11]
- Many blood donors are first-time donors rather than repeated voluntary blood donors, and it is well-known that higher rates of TTIs are observed among 1st-time donors and the prevalence decreases in repeated donors[12]
- Higher probability of professional/paid blood donors.

The current study was based on a NAT system using 1 mL each of IDs sample commonly referred to as the ID-NAT system. ID-NAT system has several advantages over pooling of sample for NAT, the most important being better sensitivity. The extraction system of most NAT systems uses 1 mL of plasma for extraction of nucleic acids. If a pool of 6–8 donors is used, then just about 150–200 µl of one donor plasma is used in a the pool. This means that if the load of viruses is very small, then there are chances that viral count may be lower than the detection limit of the PCR assay. Most importantly, even these very small number of virus particles are capable of transmitting infection to the recipient. As per the manufacturer, the performance characteristics of the NATSpert ID TripleH Detection assay have been determined using NIBSC WHO standards for infectious Diseases.[13-15] The 95% probability of detecting each of the three viruses was 17.5 IU/ml for HIV-1 M, 18.5 IU/ml for HIV-1 O, 8.17 IU/ml for HIV-2, 7 IU/ml for HCV, and 2 IU/ml for HBV.

ELISA and CMIA have been used to screen blood donors for TTI in India for close to 25 years now, and it is mandatory to use either of this technology as per the laws in India. However, the use of NAT for screening blood donors for TTI is not mandatory. As per the discussion with concerned administrators of other blood banks, we also understood that the use of NAT is limited to very few blood banks in India so far due to the high cost of the hardware and the screening test itself. The availability of this important screening test from an Indian manufacturer with CDSCO validated and approved kits can play a big role in making NAT a universally used screening test to provide safer blood in blood banks across India.

Conclusion

The NATSpert ID from Mylab Discovery Solutions Pvt Ltd. provided an advantage over EIA in ability to detect TTI in blood donors. This new NAT assay can contribute to the cause of blood safety in India as it also offers a cost benefit compared to the imported NAT screening kits currently available.

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Conflicts of interest

Wankhede GR, Desai S, Dakhave M are associated with Mylab Discovery Solutions Pvt. Ltd., Pune, India.

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