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Impact of PCR-based Multiplex Minipool NAT on Donor Blood Screening at a Tertiary Care Hospital Blood Bank in North India

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ABSTRACT

Background: Serononreactive samples of donated blood may harbour infections which can be identified by nucleic acid amplification testing (NAT). We evaluated a minipool NAT-polymerase chain reaction (PCR) test to screen for transfusion- transmittable infections in serononreactive blood donations.

Materials and Methods: This retrospective observational study was performed in a tertiary care hospital blood bank in New Delhi, India. We analysed the results of serological testing and NAT performed over 5 years (2015-2019). All donated samples were first serologically tested for Hepatitis B and C viruses (HBV and HCV) and HIV through chemiluminescence. Serononreactive samples were next subjected to NAT using cobas TaqScreen MPX Test, version 2.0 in minipools of 6 samples. Minipools showing NAT-reactivity were resolved by testing single members of the pool to identify the reactive sample.

Results: NAT was performed on 155,211 serononreactive samples, and 97 (6.25%) of the samples were NAT reactive, giving a NAT yield rate of 1:1,600. The NAT yields for HBV, HCV, and HIV were 87, 9 and 1 respectively. The NAT yield rates were 1:1,784 for HBV, 1:17,246 for HCV and 1:155,211 for HIV.

Conclusions: Through NAT, we could identify 97 infections which were missed by serological testing. Routine implementation of NAT screening in blood banks in India can improve blood safety.

Keywords: Blood donor screening; Hepatitis B Virus; Hepatitis C Virus; NAT yield; Nucleic Acid Amplification Testing; Transfusion-transmitted infections

INTRODUCTION

The prevalence of transfusion-transmitted infections (TTIs), especially those of viral origins including Hepatitis B virus (HBV), Hepatitis C virus (HCV), and HIV continues to remain high in India. As per recent estimates in India, the seroprevalence in the general population ranges between 2%-8% for HBV [1], 0.5%-1.5% for HCV [2], and 0.17%-0.29% for HIV [3]. In order to render safer blood for transfusions, serological testing of all blood donation samples for HBV, HCV, HIV, malaria, and syphilis has been made mandatory in Indian blood banks. However, it has been repeatedly observed that some serononreactive samples harbour HBV, HCV, and HIV, as detected by nucleic acid amplification testing (NAT) screening [4].

NAT has a prominent place in transfusion medicine as a complementary screening test to serologic testing of donated blood as it detects early infection before seroconversion, as well as chronic occult infections, thereby improving blood safety [5]. NAT is technically demanding and requires dedicated infrastructure, consumables, equipment, and expertise [6]. However, availability of automated NAT can overcome some of the challenges like need for special lab infrastructure, manual intervention time, contamination and errors. The cobas TaqScreen MPX

Test, version 2.0 (MPX2) is a highly sensitive multiplex test that can simultaneously detect multiple viruses in the same reaction tube, and is performed on the automated cobas s 201 system [5].

In this retrospective study, we report the results of screening of serononreactive samples using the MPX2 assay over a period of five years from a blood bank in North India which has implemented NAT as a part of routine blood screening procedures.

MATERIALS AND METHODS

This retrospective observational study was performed in the blood bank of Max Superspeciality Hospitals, New Delhi, India, which is a state-of-the-art blood bank that presently processes over 40,000 units per year. This study was performed on the blood donations received from blood banks of Max group of hospitals across North India. After initial screening of all the donors through a comprehensive questionnaire, thorough history, and physical examination, blood was collected through recommended aseptic precautions and processed as per NACO (National AIDS Control Organization) guidelines [7]. After blood collection, samples of the collected blood were subjected to serological testing, and units associated with seroreactive samples were discarded. The serononreactive samples were then subjected to NAT. In this study, we analysed the serological and NAT data collected from 1st January 2015 to 31st December 2019.

The initial serological screening of all samples was performed for HBsAg, anti-HCV, anti-HIV-1, and anti-HIV-2, by chemiluminescence assays (VITROS ECiQ Immunodiagnostic Systems, Ortho Clinical Diagnostics), in addition to screening for malaria (Rapid malaria antigen test) and syphilis (Rapid plasma reagin or chemiluminescence assay on Vitros ECiQ Immunodiagnostic Systems, Ortho Clinical Diagnostics). All assays were performed according to the manufacturers' instructions. The serononreactive samples were next subjected to NAT testing, using the cobas TaqScreen MPX Test, version 2.0 (MPX2) on the cobas s 201 platform (Roche Molecular Systems, Branchburg, NJ, USA).

The MPX2 is a qualitative multiplex polymerase chain reaction (PCR) test for simultaneous detection of HBV-DNA, HCV-RNA, and HIV-RNA (HIV-1 Groups M and O RNA, HIV-2 RNA), along with an internal quality control, in a single test in human plasma [8]. The entire NAT procedure is automated, and utilises Hamilton Microlab Starlet IVD pipettor sample pooler, automated sample preparation using the cobas AmpliPrep instrument, and automated amplification and detection using the cobas TaqMan analyzer. In addition, the system also consists of an internal control for monitoring test performance in each individual test, and the AmpErase enzyme to reduce potential contamination by previously amplified material [8].

Minipools of six serononreactive samples (MP6) were created and NAT testing was performed. Minipools showing NAT-reactivity were resolved by performing NAT on all six single units for identifying the reactive sample.

All data was collected in accordance with the principles of ethical conduct of human research as enshrined in the Helsinki Declaration of 1957 and subsequent relevant amendments. Since this was a retrospective analysis of routinely collected data, special informed consent was not applicable, apart from the informed consent routinely obtained from all donors which also includes consent for HIV testing. Results are presented descriptively, and no statistical analysis was performed. All data leading to the conclusions of the present study are available with the corresponding author on reasonable request.

RESULTS

Over the duration of the study period, 188,803 samples underwent serological testing in our centre out of which 182,031 (96.41%) were from male donors and 6,772 (3.59%) were from female donors. Voluntary donors comprised of 13.00% (n=24,537), and the remaining 87.00% (n=164,266) were replacement donors. Of all samples tested for serology, 3,923 (2.08%) samples were seroreactive. The proportion of seroreactive samples was highest for HCV (1390 samples; 35.43% of all seroreactive samples), followed by HBV (n=1278; 32.58%) and HIV (n=453; 11.55%). The results of serological testing are provided in **table 1.**

Out of the 184,880 serononreactive samples, 155,211 samples underwent NAT testing. The remaining serononreactive samples were used to prepare single donor platelet concentrates and random donor platelet concentrates and per blood bank protocol, not

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subjected to NAT testing. A total of 97 (0.062%) of the 155,211 serononreactive samples were reactive with NAT, giving a NAT yield rate of 1:1,600. Most of the NAT yields were for HBV (87/97, 89.69%), followed by HCV (9/97, 9.28%) and HIV (1/97, 1.03%). The NAT yields and NAT yield rates are presented in **table 2.**

DISCUSSION

In this study we have presented the NAT yields of HBV, HCV, and HIV from a tertiary care hospital blood bank in north India over a period of 5 years. The major findings of our study are that using NAT we were able to detect infections that are missed by serology in as many as 97 samples of 155,211 declared to be serononreactive. The most common infection that we found in the serononreactive samples through NAT was HBV infection. Since each blood unit is split up into three different units to be used for three separate transfusions, by performing NAT, we were able to prevent the potential transmission of these viral infections in as many as 291 transfusions, thereby demonstrating the significance of NAT as an essential screening test for blood banks in India to enhance blood safety. This is particularly important given the high prevalence of TTIs in India. Thus, our findings further support various studies showing importance of NAT screening in identifying infections that are missed by serological testing [9-14].

Various studies have previously described NAT yield rates in India. A 2017 systematic review pooled the results of various studies reporting NAT yields from across different regions in India [15]. Considering a total of 12 studies and NAT performed in 389,387 serononreactive samples, the pooled analysis reported a NAT yield of 1:1361. The pooled NAT yield rates of HBV, HCV, and HIV were found to be 1:1761, 1:5484, and 1:66,000 respectively, which are similar to our study except that we found lower HCV NAT yield rate [15]. Minor regional variations notwithstanding, in most of the studies it is observed that the NAT yield rates are higher for HBV when compared to HIV and HCV [9-14].

With a seroprevalence of 2-8% among the general population and 0.75-2.61% among blood donors, HBV is the most frequent TTI in India [4]. HBV is a DNA virus which has an average doubling time of around 2.6 days, which is much slower than the doubling rates of HIV (0.83 days) and HCV (0.45 days) [16]. Further, the diagnostic window period for serological assays is longer for HBV , compared with HIV and HCV [16]. Since HBV has a high prevalence in India, a number of infections would be in the window period or resolving phase, when the levels of viral nucleic acids is very low. The chronic occult HBV infections which are not detected by HBsAg testing are a major transfusion risk. In India, due to the high prevalence of HBV, proportions of occult infections maybe higher than window period infections. Such occult HBV infections are generally associated with low levels of circulating HBV DNA. For the above reasons, NAT with high sensitivity of detection for HBV is essential [5]. Thus, use of a highly sensitive NAT in our centre led to higher NAT yield of HBV when compared with HCV though the serology yields were comparable. These results indicate that window period and occult HBV infections that are missed by serology screening could be identified by use of a highly sensitive MPX2 MP-NAT assay as was used in our study.

The seroprevalence of HBV, HCV, and HIV has been observed to be higher among replacement donors compared to voluntary donors in India, despite the presence of multiple interlocking steps to reduce the TTIs, such as donor exclusion based on interview, mandatory serological tests, repeat donation records, and discouragement of cash incentives for blood donation [15,17]. Considering the fact that replacement donors can outnumber voluntary donors as observed in our study, it is essential to have a stringent screening process to enhance blood safety and to prevent TTIs. Using a highly sensitive NAT technique is critical in enhancing blood safety by identifying infections missed by serology screening. However, as of 2017, only 2.27% of all the public and private blood banks in India (58/2550) were doing NAT testing of any format in addition to routine serological screening, however, adaption of NAT seems to have increased since then [15]. Considering the high prevalence of viral infections in India, the high NAT yields observed in different Indian studies and the reduction of morbidity and mortality and saving of treatment costs by prevention of TTIs, the benefits of NAT testing can potentially outweigh the costs of NAT testing.

In our study, we have utilised the latest generation MPX MP-NAT with high sensitivity, which is based

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on PCR technology [5]. The MPX2 PCR-NAT has the ability to detect and amplify more than one target sequence by using multiple pairs of primers and probes in the same reaction tube. The MPX2 used in the present study is unique in that it utilises four unique fluorescent dyes, one each for the amplified HBV, HCV and HIV targets, and one for the internal control. Since each dye is to be measured at a different wavelength, this system can simultaneously detect and identify the virus present in the sample in real-time, thereby mitigating the need for a separate discriminatory testing [5]. This, and the fact that the system employs minipools of 6 donation samples, results in short turn-around time per batch, which reduces the cost of each test. Being a completely automated technique, this system is not only simple enough to be handled entirely by technicians, but also has a very low chance of contamination. The AmpErase enzyme incorporated in the system reduces potential contamination and false positive results by removal of previously amplified material [8]. All these features enhance the specificity of the test by reducing false positives. Use of minipools which are further confirmed by single unit testing improves the specificity of the test [5].

Our study is not without limitations. Not all serononreactive samples were subjected to NAT testing. Since the study was planned in a retrospective manner, follow up of donors to check for seroconversion and viral load estimation of the NAT yields could not be performed. A follow-up studies would have helped to characterise the stage of infection in donors, and understand the sensitivity of detection of these infections according to viral loads.

To conclude, our data shows that a cobas TaqScreen MPX2 assay used on minipools can identify infections in serononreactive samples with a high NAT yield. NAT has an essential and invaluable place in the blood banks in India for complementing serological screening of donated blood samples. The MPX2 used in our study is a highly-sensitive and specific test performed on an automated, user-friendly platform, which can lower the risk of window-period transmission of TTIs, thereby enhancing the safety of blood transfusions in India. With only miniscule number of blood banks in India performing NAT routinely, the time has come for a more widespread adoption of NAT in blood banks across the country.

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TABLES

Table 1: Serology details of all samples (n = 188,803)

Screened target	Seroreactives [N, (%)]
HBV	1,278 (0.68%)
HCV	1,390 (0.74%)
HIV	453 (0.24%)
Syphilis	800 (0.42%)
Malaria	2 (0.001%)
Total seroreactives	**3,923 (2.08%)**

Note: HBV: Hepatitis B virus; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus

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Table 2: Details of NAT on serononreactive samples (n = 155,211)

Screened target	NAT yield (NAT yield rate)
Overall	97 (1:1,600)
HBV	87 (1:1,784)
HCV	9 (1:17,246)
HIV	1 (1:155,211)

Note: NAT: Nucleic acid amplification testing; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus

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