

Original Article

Impact of nucleic acid testing in double screening of blood donations in Northern India: A single-center experience

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ABSTRACT

Objectives: The aim of our study is to assess the ability of nucleic acid testing (NAT) to detect hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) from donor blood samples which were declared sero-non-reactive.

Materials and Methods: The whole blood donations were collected over the 6-year period from 2017 to 2023 which were initially screened for hepatitis B surface antigen, anti-HCV, anti-HIV-1/2, and p24 antigen and syphilis by electrochemiluminescence assays by Roche Diagnostics, Germany and malaria by Pan/Pf rapid test. Donations were declared nonreactive by these tests which were further subjected to NAT testing using minipool method. It was done in pool of 6 using cobas Taq Screen MPX version 2.0 on the cobas s201 platform. The NAT yields were quantitated for viral loads and followed up by serology.

Statistical analysis: The data obtained was entered in Microsoft Excel and analyzed using descriptive statistics using the Statistical Package for the Social Science 21 version.

Results: Out of 152,575 donations, 149,304 were sero-non-reactive and screened by NAT. Out of 82, 45 were reactive for HBV deoxyribonucleic acid and 37 for HCV ribonucleic acid. The NAT yield was 1:1831 overall, 1:3337 for HBV and 1:4059 for HCV. Viral load was quantitated in 65/82 NAT yields (35 HBV and 30 HCV). The viral loads of HBV samples were <20 IU/mL for 19/35 samples and the HCV sample viral loads ranged from 25.3 to 9.3×10^6 IU/mL. Eleven NAT-yield donors (4 HBV, 7 HCV) reported for follow-up showed sero-conversion between 90 and 210 days after NAT screening.

Conclusions: This study confirmed the benefit of a highly sensitive minipool NAT (MP-NAT) in interdicting infectious donations undetected by conventional screening and illustrates the detection of donations with low viral loads using the MP-NAT polymerase chain reaction.

Keywords: Blood safety, Transfusion-transmitted infections, Nucleic acid testing, Minipool-nucleic acid testing

INTRODUCTION

India has an annual requirement of 12 million units for transfusion.^[1] Due to the high prevalence of infections in the donor and general population in India, especially with respect to the three major transfusion-transmitted infections (TTIs) of viral origin, namely, hepatitis B virus (HBV) hepatitis C virus (HCV) and human immunodeficiency virus (HIV),^[2] serological screening is mandatory for the three viral TTIs in blood banks in India.^[3] Even though the use of advanced

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serological techniques improves the sensitivity of detection of TTIs, complete reliance on serology-only screening techniques still leads to missing of some of the donations harboring infections.^[4,5] The conventional serological screening assays have wide “window period” of detection and may also miss occult hepatitis B infections (OBI), immune-silent carriers, and immunologically variant viruses.^[6] Consequently, the Drugs and Cosmetics Act and the National AIDS Control Organization (NACO) allow for the introduction of better techniques to improve blood safety.^[7,8]

The national seroprevalence of hepatitis B and hepatitis C is 0.95% (0.89–1.01) and 0.32% (0.28–0.36), respectively.^[9] Moreover, the distribution of the disease burden among the states is unequal. The seroprevalence of HCV in Punjab has been found to be about 3.29% in the general population.^[10] Punjab, at 1.35%, also has the highest seropositivity for hepatitis C among blood donors, much higher than the national average of 0.34%. In fact, unlike the rest of the country, the seropositivity of hepatitis C is higher than that of hepatitis B (0.65%) in blood donors in Punjab.^[11,12] The heightened prevalence of HCV in Punjab can be traced to a combination of factors including insecure medical practices, unsafe therapeutic injections and surgeries, intravenous drug use, blood transfusions, hazardous dental procedures, and risky sexual practices.^[13]

Molecular screening of HBV, HCV, and HIV by nucleic acid testing (NAT), in addition to serology, can improve the sensitivity of screening. It offers the potential of reduction of transmission of infections through transfusion.^[14] A donated blood sample in which serological assay failed to detect the infection, but NAT detects a viral genome is referred to as a NAT yield.^[15,16] The use of automated systems offers additional benefits of simplification of requirements of facilities in the blood center. The introduction of technological advances and automated molecular screening systems requires an investigation of their potential benefit.

This study reports the 6 year experience of implementing minipool NAT (MP-NAT) in a tertiary care hospital in North India. The data include the incremental yield obtained by employing a highly sensitive NAT in minipool format to seronegative blood donations and subsequent quantitation of viral targets.

MATERIALS AND METHODS

Study setting

Blood donations were received in Department of Immuno-Hematology and Blood Transfusion, Dayanand Medical College and Hospital, Ludhiana, Punjab, India, from April 2017 to March 2023 were included in the study. All blood donations were taken after routine consent as per NACO guidelines.

Serological screening

All the collected donations underwent initial screening by routine serology. Serological screening was done for hepatitis B surface antigen (HBsAg) (Elecsys HBsAg-II), anti-HCV (Elecsys Anti-HCV-II), anti-HIV-1, anti-HIV-2, and HIV-p24 antigen (Elecsys HIV-combi PT) and syphilis (Elecsys Syphilis) by electrochemiluminescence technology on cobas e411 system (Roche Diagnostics, Germany). Screening for malaria was done by Pan/Pf rapid test (Malascan Plus, Viola Diagnostic System, India). All assays were performed as per the manufacturers' instructions.

Molecular screening

Only donations that showed non-reactivity to screening for all serology targets were further tested by NAT. NAT was performed using the cobas® TaqScreen MPX Test version 2.0 (MPX2; Roche Molecular Systems, Branchburg, NJ, USA) on the cobas s201 platform (Roche Instrument Center, Rotkreuz, Switzerland). The system includes an automated sample pooling on the Hamilton MICROLAB® STARlet IVD Pipettor, automated sample preparation on the cobas Ampli Prep (CAP) instrument, and automated amplification and detection using the cobas TaqMan (CTM) analyzer. The MPX2 is a multiplex, multidye test employing polymerase chain reaction (PCR), for the simultaneous detection and identification of HBV, HCV, and HIV through detection of the targets HBV deoxyribonucleic acid (DNA), HCV ribonucleic acid (RNA), HIV-1 group M, HIV-1 group O, and HIV-2RNA.

Screening was performed on minipools of 6 samples. Reactive minipools were resolved by individually testing all 6 units of the minipool to identify the reactive donations and the virus. NAT was performed following the manufacturer's instructions for the assay on an automated platform. The screening algorithm is summarized in Figure 1. The 95% and 50% detection limits for MPX2 are shown in Table 1.^[17-20]

Viral nucleic acid quantitation

The NAT yield donations were further evaluated for quantification of viral load using retained samples from frozen storage (−40°C). Samples were sent in the same order in which they were collected, as resources allowed. Samples were stored for a maximum of 3 months. Testing was performed using real time PCR by the CTM HBV test v2.0 and HCV test v2.0 run on a CTM 48 Analyzer (Roche Molecular Systems, Branchburg, USA). Table 1 shows the limits of detection and quantification for the viral load assays.

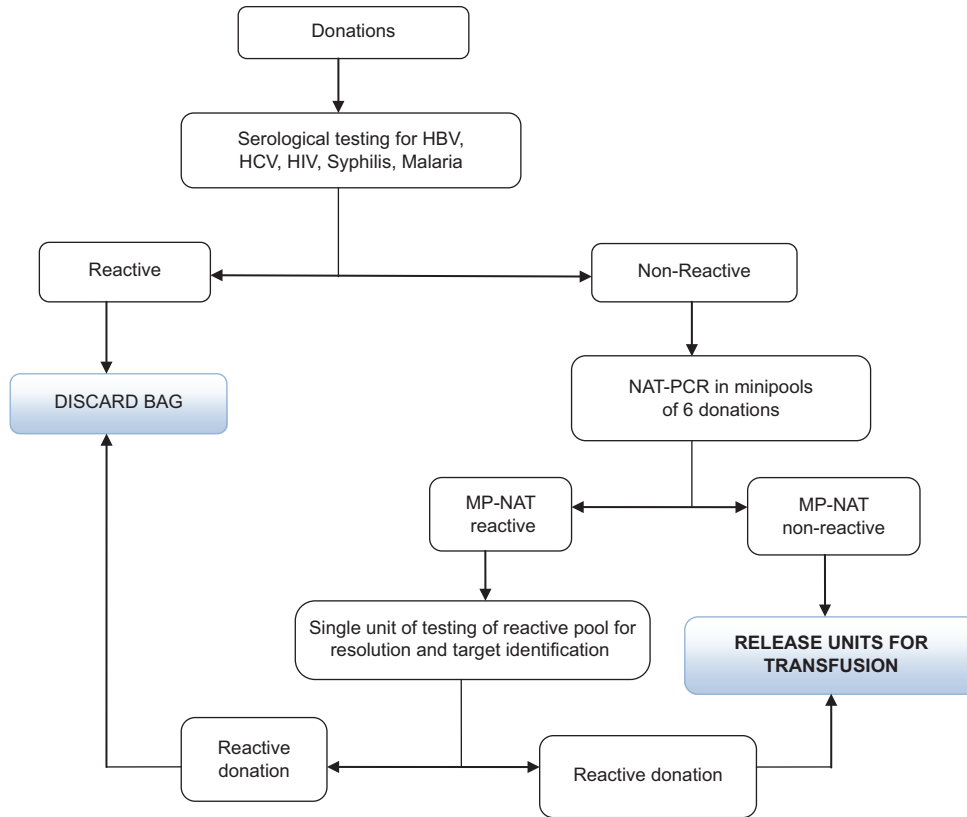


Figure 1: Flow chart of minipool-nucleic acid testing algorithm. HBV: Hepatitis B, HCV: Hepatitis C, HIV: Human Immuno deficiency Virus, NAT-PCR: Nucleic Acid Testing-Polymerase chain reaction, MP- NAT: Minipool-Nucleic Acid Testing

Table 1: Detection limits of the molecular assays.

Target	cobas® TaqScreen MPX Test, v2.0 donor screening assay		cobas® AmpliPrep/cobas TaqMan viral load assays	
	95% LOD* ^[16]	50% LOD* ^[17]	LLOQ ^[18,19]	95% LOD ^[18,19]
HIV-1 Group M*	50.3 IU/mL	9.4 IU/mL	-	-
HCV	6.8 IU/mL	1.3 IU/mL	15 IU/mL	11 IU/mL
HBV	2.3 IU/mL	0.46 IU/mL	20 IU/mL	8.2 IU/mL

LOD: Limit of detection, LLOQ: Lower limit of quantification, HIV: Human immunodeficiency virus, HBV: Hepatitis B virus, HCV: Hepatitis C virus. * LODs for HIV-1 Group O and HIV-2 are not shown here

Follow-up testing of NAT yields

Donors of NAT-reactive donations were asked to return for follow-up serology at 90 days after NAT screening. Serological testing was performed with the same assays used for the initial serology screening on those reporting for follow-up.

Quality assurance

Our blood center is participating in External Quality Assessment Scheme, an external quality assurance program.

Statistical analysis

The data so obtained were entered into Microsoft Excel and analyzed using descriptive statistics. All statistical calculations were done using the Statistical Package for the Social Science 21 version statistical program for Microsoft Windows.

RESULTS

Serology

A total of 152,575 blood donations were screened by serology between April 2017 and March 2023. Of these, 4481 (2.93%)

were seroreactive, as shown in Table 2. The remaining 148,094 donations were “serononreactive.” The highest seroreactivity was observed for HCV (2213/4481, 49.3%), followed by syphilis (1210/4481, 27.0%), HBV (867/4481, 19.3%), and HIV (191/4481, 4.26%).

Molecular screening

149,304 sero-non-reactive donations excluding syphilis were screened for viral nucleic acids, out of which 82 (0.05%) donations were found to be NAT reactive (NAT yields). The NAT yields by year are given in Table 3.

Viral load

Out of the total 82 NAT yield donations, we could evaluate viral loads for 65 donations. Of these, 35 were HBV reactive, and 30 were HCV reactive on NAT, as shown in Table 4. Results recorded as <20 IU/mL were reported as such by the assay, meaning that HBV DNA was detected but could not be quantified as the load was below the lower limit of quantitation of the assay.

Follow-up testing

Eleven of the 82 NAT reactive donors reported for follow-up. The donors reported between 3 and 7 months (90–210 days) after NAT screening though they were called at 90 days. All 11 of these donors showed sero-conversion for the respective

NAT reactive virus with follow-up ECLIA screening: four donors with HBV, and seven donors with HCV.

DISCUSSION

The data presented in this study demonstrate the ability of NAT applied in minipools to detect viral infections that are missed by serology. The data on viral load quantification and follow-up testing further indicate the potential high risk of transmission through the blood donations declared serononreactive, that were averted by NAT testing. Our study showed a high seroreactivity of 1.45% among all donors with HCV. Studies in the past have reported HCV seroprevalence among blood donors in Punjab to be around 1.38%,^[21] and 1.45%.^[22] As per the NACO/National Blood Transfusion Council (NBTC) report in 2016, the anti-HCV reactivity among donors in the blood center in Punjab was 1.35% in 2016.^[11] This high seroreactivity for HCV in Punjab, where our blood center is located, is a reflection of the high seroprevalence of HCV (3.2%) in the general population of Punjab.^[13] The higher seroreactivity observed for HCV in our study could be due to the use of a highly sensitive serological assay using the advanced ECLIA technology. Interestingly, the second highest seroreactivity in our study was observed for syphilis at 0.80%. Although blood center in Punjab has a high seroreactivity of 0.49% for syphilis,^[11] the rate observed in our study is higher which could be again due to the use of a highly sensitive chemiluminescence based assay as against the rapid plasma reagin (RPR) test commonly employed in blood banks in the state. The seroreactivity for HBV and HIV was similar to other blood banks in the state.^[11]

During the study, NAT in minipools was able to detect 45 HBV and 37 HCV infections from the donations which were “sero-non-reactive.” The NAT yields were thus 1:3337 for HBV, 1:4059 for HCV, and 1:1831 overall, as shown in Table 3.

The combined NAT yields reported from Indian tertiary care settings and stand-alone blood center are quite varied, and range between 1:476 and 1:4403 as per a 2017 review of 11 Indian studies.^[23] A few studies have reported higher

Table 2: Serology screening results.

Screening target	Screen reactive by serology (%)
Total samples	1,52,575 (100)
HBV surface antigen	867 (0.56)
HCV antibody	2213 (1.45)
HIV antigen/antibody	191 (0.13)
Syphilis	1210 (0.80)
Malaria	0 (0)
Total	4481 (2.93)

HIV: Human immunodeficiency virus, HBV: Hepatitis B virus, HCV: Hepatitis C virus

Table 3: NAT yield results by year.

Time period	NAT tested	Number of NAT-positive donations			NAT yield per donations tested		
		HBV	HCV	Total	HBV	HCV	Total
April 2017–Mar 2018	25,023	11	12	23	1:2275	1:2085	1:1088
April 2018–Mar 2019	24,316	7	10	17	1:3474	1:2432	1:1430
April 2019–Mar 2020	25,780	4	5	9	1:6445	1:5156	1:2864
April 2020–Mar 2021	22,003	10	2	12	1:2200	1:11001	1:1833
April 2021–Mar 2022	24,861	8	5	13	1:3163	1:5061	1:1946
April 2022–Mar 2023	27,321	5	3	8	1:1553	1:9256	1:3471
Total	149,304	45	37	82	1:3337	1:4059	1:1831

NAT: Nucleic acid testing, HBV: Hepatitis B virus, HCV: Hepatitis C virus

Table 4: Details of NAT yield and viral loads.

S. No.	NAT yield virus	Viral load (IU/mL)	S. No.	NAT yield virus	Viral load (IU/mL)
1	HBV	<20	36	HCV	25.3
2	HBV	<20	37	HCV	1.25×10 ³
3	HBV	<20	38	HCV	7.49×10 ³
4	HBV	<20	39	HCV	1.05×10 ⁴
5	HBV	<20	40	HCV	3.49×10 ⁴
6	HBV	<20	41	HCV	7.03×10 ⁴
7	HBV	<20	42	HCV	7.51×10 ⁴
8	HBV	<20	43	HCV	9.01×10 ⁴
9	HBV	<20	44	HCV	1.01×10 ⁵
10	HBV	<20	45	HCV	3.02×10 ⁵
11	HBV	<20	46	HCV	4.82×10 ⁵
12	HBV	<20	47	HCV	4.93×10 ⁵
13	HBV	<20	48	HCV	5.72×10 ⁵
14	HBV	<20	49	HCV	5.72×10 ⁵
15	HBV	<20	50	HCV	2.2×10 ⁶
16	HBV	<20	51	HCV	2.29×10 ⁶
17	HBV	1.07×10 ³	52	HCV	2.59×10 ⁶
18	HBV	1.5×10 ³	53	HCV	4.69×10 ⁶
19	HBV	6.3×10 ⁴	54	HCV	4.7×10 ⁶
20	HBV	TND	55	HCV	7.04×10 ⁶
21	HBV	<20	56	HCV	9.3×10 ⁶
22	HBV	5.3×10 ³	57	HCV	TND
23	HBV	6.4×10 ⁴	58	HCV	1.03×10 ³
24	HBV	7.3×10 ³	59	HCV	6.2×10 ³
25	HBV	4.02×10 ³	60	HCV	5.03×10 ³
26	HBV	<20	61	HCV	7.2×10 ⁴
27	HBV	7.06×10 ³	62	HCV	6.2×10 ⁴
28	HBV	9.03×10 ³	63	HCV	3.2×10 ³
29	HBV	8.5×10 ⁴	64	HCV	4.0×10 ³
30	HBV	<20	65	HCV	8.0×10 ⁴
31	HBV	1.09×10 ³			
32	HBV	2.2×10 ³			
33	HBV	5.5×10 ³			
34	HBV	2.2×10 ³			
35	HBV	5.6×10 ³			

NAT: Nucleic acid testing, HBV: Hepatitis B virus, HCV: Hepatitis C virus, TND: Target not detected

NAT yields of 1:354^[24] and 1:974^[25] both the investigators used enzyme-linked immunosorbent assay (ELISA)-based serological testing. Chandra *et al.* also demonstrated a decrease in NAT yield by employing chemiluminescence on donations previously tested using ELISA.^[24] Our study reported a higher overall NAT yield (1:1831) than most recent studies, and this could be due to differing prevalence of TTIs. Thus, a high NAT yield for HBV and a higher than usual HCV NAT yield have pushed the combined NAT yield higher in our study. Of note, the NAT yield in this study is comparable to or higher than other studies despite the fact that many other studies have employed individual donation NAT (ID-NAT), whereas we have used MP-NAT.

A high NAT reactivity was observed in our study over and above a high seroreactivity obtained with an advanced

serological technology like ECLIA. This indicates a significant benefit of NAT in helping to minimize the risk of infectious donations entering the blood supply. Since each blood bag may be separated into multiple components for transfusion, each NAT yield has the potential to prevent transmission of TTIs to multiple transfusion recipients.

A noteworthy finding of our study is the result of the viral load quantitation in NAT yield samples. However, the initial plan was to quantify all NAT yields; viral load testing could only be done on 65/82 NAT yields due to inadequate sampling and clotting in a few samples. However, some interesting insights were revealed from this testing. The HCV NAT yields in our study showed very high viral loads ranging from 25.3 to 9.3 × 10⁶. The high HCV viral loads observed in our study are consistent with the rapid

doubling time of HCV in early infection. This demonstrates that donations with high viral titers of HCV, having a high potential for transmission, may be missed by serological assays detecting antibodies to HCV, further underlining the importance of narrowing the window period (WP) of detection through detection of the viral genome by NAT. In our study, the viral loads of 19/35 tested HBV NAT yields were below 20 IU/mL. These HBV infections with low viral loads that were missed by serology but picked up by NAT could be either OBI, recent HBV infections at a stage before the appearance of detectable levels of HBsAg, or resolving HBV infection at a stage where HBsAg has declined to below detectable levels but circulating HBV DNA is not yet cleared. There was one case each of HBV and HCV where NAT yield was positive, but the viral load was undetectable. One possible reason for this is that the sensitivities of the HBV and HCV quantitative molecular assays are lower than those for the MPX2 blood screening assay. In either of these cases, the viral load could possibly be below the limit of detection of the quantitative assays; however, the impact of sampling effects also cannot be completely ruled out.^[26] The differences between the viral loads of HCV and HBV echo the differences in the biology of the two viruses.

Out of 82, 11 NAT reactive donors reported back after follow-up and were found to be positive for serology. According to NBTC guidelines,^[27] all initial seroreactive and NAT positive donors should be recalled, given post donation counseling and repeat sample is to be taken for testing. 11 NAT initial reactive donors developed seropositivity (four donors with HBV and seven donors with HCV) and were counseled. The eventual appearance of HBsAg in the four HBV NAT reactive donors and anti-HCV in the seven HCV NAT reactive donors suggests that these were serological WP donations. In our study, all 11 donors with NAT-positive donations seroconverted, thereby affirming the value of NAT in averting certain TTI transmission. A similar finding was reported by Mishra *et al.* in 2017, where out of 51 seronegative samples showing NAT yield, seropositivity was observed within 6 months – 1 year for 38 donors (31/44 HBV, 2/2 HCV, and 5/5 HIV seroconversions).^[28] In India, where the HBV endemicity is high, the frequency of OBI is often higher than acute infection. In 2014, Doda *et al.* evaluated the HBV antibody profiles of HBsAg negative, HBV DNA positive donations; out of 18 samples profiled, 12 showed antibodies against HBc (HBV core antigen), signifying OBI, whereas the remaining 6 had no detectable serological markers, indicating that these donors were in the WP of HBV infection.^[29] The HCV NAT yields are majorly expected to be from the serological WP for HCV infection. While occult Hepatitis C infection is also described in the literature, the information about this entity is rather limited, and much research is needed in this area to understand the

clinical implications of this condition.^[30] A complete follow-up of all NAT yield donations would be a step toward a more comprehensive understanding of the stage of infection, the clinical implications of such donations, and mitigation of the risk.

Before the implementation of MP-NAT PCR in our set-up, we had been performing ID-NAT using the transcription-mediated amplification-based Procleix Ultrio Plus® (Novartis Diagnostic, USA) on the Procleix Panther system. The purpose of the current study was also to evaluate the overall ease of implementation of the fully automated NAT-PCR system. The cobas MPX2test is a multiplex PCR test that has the ability to simultaneously detect a reactive donation and identify the target virus (HBV, HCV, or HIV) with specific dyes in different channels. This eliminates the need for discriminatory testing in reactive donation samples for separately identifying the target virus and helps the blood bank plan further actions with certainty. Difficulty in confirming reactive donations obtained through ID-NAT on the Ultrio Plus assay has been reported, including recently in India, where only 22%^[31] and 41%^[32] of initially reactive samples could be confirmed by repeat testing and viral target discrimination. In our previously published study of the ID-NAT assay, too, only about 83% of the initially reactive results were confirmed.^[15] The algorithm that we used previously for the ID-NAT assay is given in Figure 2.^[15] This discrepancy and non-confirmation lead to uncertainty on the cause of initial reactivity (non-specific amplification, sample contamination, low viral load, etc.) and the viral target, leading to subsequent difficulties in donor recall and counseling and unnecessary deferrals and repeat tests.^[18] In comparison, we found the screening algorithm of the MP-NAT PCR assay to be simpler. This algorithm uses NAT for testing only the sero-nonreactive donations; this reduces the number of tests to be carried out on reactive donor samples. Further, since the screening is done in minipools of multiple donations (in our case, six donations), there are additional benefits of fewer number of tests, higher specificity, and lower overall cost. Moreover, PCR is a time-tested technology. A few additional benefits that we found as a user of the MP-NAT technology are minimal maintenance and hands-on time (since the system is completely automated), availability of ready-to-use reagents, no requirement of test calibrations, provision of internal control for monitoring test performance in each individual test, and incorporation of the AmpErase enzyme to reduce potential contamination by previously amplified material.

The data generated by this study and their interpretation are reinforced by the size of the study, the additional quantitation data available, and follow-up serology. This large study demonstrated the utility of the highly sensitive cobas Taq Screen MPX2 to screen pooled samples and

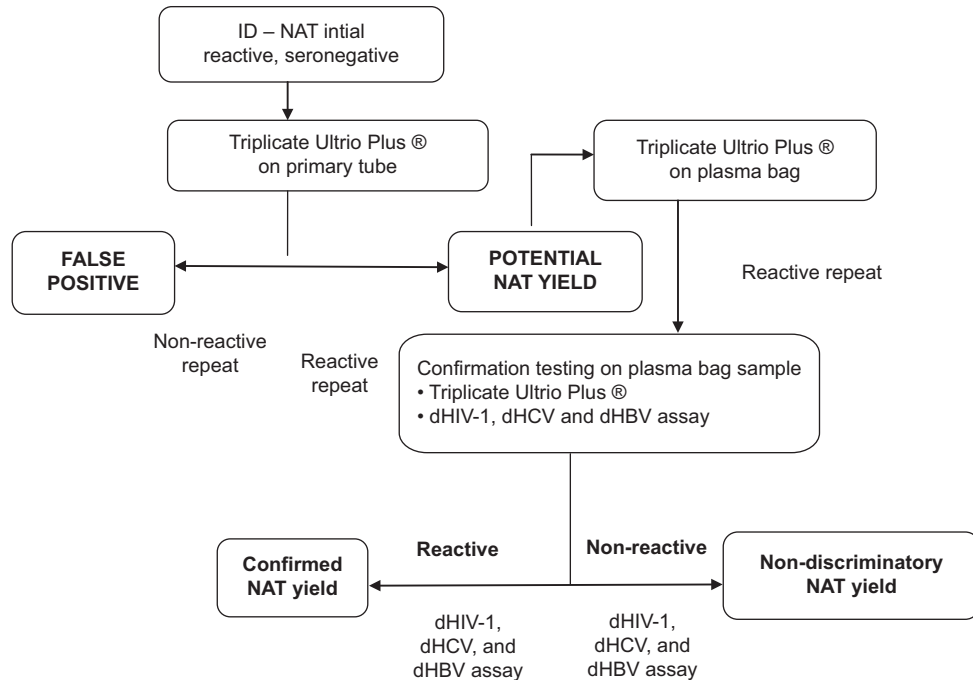


Figure 2: Screening algorithm for individual donation-nucleic acid testing used by our blood bank previously. ID-NAT: Individual nucleic testing, dHIV: Discriminatory Human Immuno Deficiency Virus, dHCV: Discriminatory Hepatitis C, dHBV: Discriminatory Hepatitis B

obtain high NAT yields. It also identified the viral loads and stage of infection of these donations, which can be used to derive the risk to blood safety and public and individual health. The investigation also illustrated the high convenience and ease of using automation and minipool testing. However, there are some limitations in the present study. The use of additional serological markers like antibody against Hepatitis C core antigen (Hbc) in follow-up testing would have helped characterize OBIs. The use of a higher sensitivity quantitative molecular assay would have enabled us to demonstrate that the blood screening assay detects samples with much lower viral loads as has been shown by other authors.^[24] Finally, the cost-effectiveness of the incorporation of MP-NAT, in addition to serological testing and comparison to ID-NAT, was not assessed; further studies are required in this direction.

CONCLUSIONS

The present study has reconfirmed the high burden of TTIs in blood donations in India despite mandatory serological screening and has indicated that the routine application of MP-NAT screening in Indian blood centers would be a move one step closer to safer blood. Cardinal features required for adoption and smooth running in blood centers of varied sizes and donor infection prevalence include reliability, ensured by the use of a highly sensitive assay, ease of operation, ensured

by the use of a fully automated system, and an uncomplicated testing algorithm. MP-NAT performed using the cobas MPX2 suitably fulfills both these conditions.

Ethical approval

The research/study was approved by the Institutional Ethics Committee, number IEC-2023-880 dated 10/07/2023.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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