Original Article

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Nucleic Acid Test versus Enzyme Linked Immunosorbent Assay for Screening of Human Immunodeficiency Virus in Donated Blood Units: A Comparative Study at a Blood Centre in Western Rajasthan, India

Transfusion Medicine Section

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ABSTRACT

Introduction: With a population of 1.3 billion people and a frequency of Human Immunodeficiency Virus (HIV) (0.17-0.29%), Hepatitis B Virus (HBV) (3-4%), and Hepatitis C Virus (HCV) (0.09-15%) in the general population, maintaining blood safety is a difficult undertaking in India. The fourth generation Enzyme Linked Immunosorbent Assay (ELISA), which can be used in place of Nucleic Acid Amplification Test (NAT) testing in situations with limited resources, has been recommended as the minimal HIV test to increase the safety of blood transfusions.

Aim: To compare the fourth generation ELISA with NAT in the screening of transfusion transmissible HIV infection in blood donors.

Materials and Methods: A cross-sectional study was carried out at the Blood Centre, Department of Immunohaematology and Transfusion Medicine, Sardar Patel Medical College and Associated Groups of Hospitals, in Bikaner, Rajasthan, India. A total of 2000 voluntary and replacement blood donors were recruited consecutively between January 2020 to December 2021 (two years), and their samples were screened using fourth generation ELISA. All of these samples were sent to AIIMS in Jodhpur for NAT to identify HIV RNA, HCV RNA and HBV DNA.

Results: In this study, when the fourth generation ELISA negative samples were subjected to NAT, no sample was found to be reactive for HIV in NAT, i.e. there was no NAT yield for HIV.

Conclusion: As a bare minimum, the fourth generation ELISA test should be used for blood donor screening and can be considered a cost-effective and reliable test in a resource limited setting. However, additional tests can be advocated for an additional layer of blood safety.

Keywords: Amplification test, Blood donors, Safety

INTRODUCTION

In developing nations, Transfusion-Transmitted Infections (TTIs) tend to be a huge concern attributed with the transfusion of blood and its components. HIV, HCV and HBV all have high prevalence rates in India, with prevalence rates of 0.17-0.29%, 0.09-15% and 3-4% respectively and on the other hand, according to National Acquired Immunodeficiency Syndrome (AIDS) Control Organisation (NACO), seropositivity for HIV, HCV and HBV within Indian blood donors is 0.12%, 0.30% and 0.92% respectively [1,2]. These obscure TTIs are caused at diverse stages of infection. Some are Window Period (WP) donors at an early stage of infection. As per the report of NACO, adult HIV prevalence in the age group of 15-49 years was around 0.17-0.29% in 2020 [3]. HIV can be spread sexually, through blood transfusions, by sharing intravenous needles and through breastfeeding and delivery from a mother to foetus. Currently, HIV isolates are divided into two categories: HIV-type 1 (HIV-1) and HIV-type 2. (HIV-2). HIV-1 is the primary cause of AIDS worldwide, although HIV-2 is more common in specific parts of Western and Central Africa. HIV is a genetically connected member of the *Retroviridae* family's *Lentivirus* genus [4].

Outside of the bloodstream or lymphatic tissue, HIV cannot survive. HIV transmission is influenced by the host's susceptibility, the concentration of the virus in infected bodily fluid and the isolate's biologic characteristics [5,6]. In transfusion medicine, HIV infection has always been a substantial trouble. Since, it was revealed that HIV infection is a TTI, it became imperative to test all contemplating blood donations for HIV before transfusion to warrant safety of all blood and blood products to the recipients [7]. HIV transmission through this very effective channel decreased as a result of the introduction of mandated pretransfusion screening for TTIs. Successfully identifying such individuals during a short window (the time when the infection is present but antibodies are not yet detectable) may necessitate a frequent screening program [8]. Screening tests are used to determine whether or not a specimen contains anti-HIV antibodies. According to reports, ELISAs are preferred for detecting HIV antibodies/antigens due to their high sensitivity and HIV-1 and HIV-2 antibodies, as well as HIV-1 p24 antigen, which can be identified many days before antibody, have recently been available in fourth generation combination ELISAs [9-12].

The NAT is an isothermal transcription based amplification system. It is designed to detect Ribonucleic Acid (RNA) and Deoxyribonucleic Acid (DNA) targets. A constant temperature is maintained throughout the amplification reaction, allowing each step to proceed as soon as an intermediate is created. NAT is not yet obligatory in India for blood donor screening. The risk of enzyme immunoassay negative, NAT reactive donations in Indian blood donors has rarely been reported. In order to determine whether or not the fourth generation ELISA use for HIV screening is adequate, this investigation was carried out. Therefore, only seronegative samples were selected and was sent to Jodhpur for NAT screening to see, if any negative results turned out to be positive in NAT or not. The State Government of Rajasthan outsources the testing of donated units by NAT in thalassaemia and sickle cell anaemia patients, bearing all costs. Although, NAT blood donation screening is not required in India, it is regularly done in some Indian cities [13-15]. The purpose of the present study was to compare the fourth generation ELISA with NAT in the screening of transfusion transmissible HIV infection in blood doors.

MATERIALS AND METHODS

A blood centre-based cross-sectional study was performed from January 2020 to December 2021 (two years) at the Department of Immnohaematology and Transfusion Medicine, Sardar Patel Medical College and Associated Groups of Hospitals, Bikaner, Rajasthan, India. Whole blood from 2000 blood donors was collected. They donated blood either in the blood centre or in the camps organised by mobile teams.

Inclusion criteria: Those donors who tested negative for any of the mandatory transfusion transmissible diseases by routine serologic testing were included in the study.

Exclusion criteria: Those donors who tested positive for any of the mandatory transfusion transmissible diseases by routine serologic testing were excluded from the study.

Study Procedure

Sample collection: All voluntary and replacement candidate blood donors were consecutively enrolled in the study. Donor demographic data, such as age, sex, nationality, as well as lifestyle, travel history, medical and drug history, were all collected as part of the hospital's necessary predonation selection questionnaire (sex and age already tabulated, rest are the part of Donor Health Questionnaire). Also, medical examination was done and informed consent was obtained from each donor.

Blood sample collection: Two separate blood samples were collected from the donor unit at the end of the donation bleeding session for each donor maintaining complete aseptic hygiene and both sample tubes were labeled on site after comparing with the donor questionnaire data and confirming with the donor himself. The samples were as follows:

One 2 mL Ethylenediaminetetraacetic Acid (EDTA) tube plasma sample for routine serologic testing. All samples were stored at 2-4°C until testing.

One 5 mL EDTA tube sample for NAT testing for HIV, HBV and HCV.

Blood sample storage and handling: All the samples were stored at a temperature of 2-4°C in order to maintain the vital properties till performing the serologic testing.

Serologic screening: The serologic enzyme immunoassays done for donated blood in the current study at the inhouse laboratory included HBsAg, HCV antibodies, combined HIV p24 Ag/Ab, and HIV-1 and HIV-2 antibodies [16], in addition, tests for malaria antigen (not antibodies) and syphilis screening by Venereal Disease Research Laboratory (VDRL) or Rapid Plasma Reagin (RPR) testing [16]. They were performed prior to NAT testing. Test for HIV screening was done using sensitive fourth generation ELISA (Merilisa HIV Gen 4, Meril Diagnostics) and manufacturer's instructions were followed. ELISA positive samples were rechecked using Rapid test, nevertheless the reactive donations were discarded following "Strategy 1" of testing strategy for HIV at blood centres.

NAT: The other vacutainer of each sample was sent to All India Institute of Medical Sciences (AIIMS), Jodhpur for NAT run using the Procleix Ultrio Elite Assay (Panther system by GRIFOLS, USA) is a qualitative in-vitro NAT to screen for HIV RNA, HCV RNA, and/or HBV DNA. The assay was performed on the fully automated Procleix Panther system.

The Procleix Ultrio Elite Assay involves three main steps which take place in a single tube on the Procleix Panther system:

1) Sample preparation/target capture.
2) HIV RNA, HCV RNA, and HBV DNA target amplification by Transcription-Mediated Amplification (TMA) and
3) Detection of the amplification products (amplicon) by the Hybridisation Protection Assay (HPA).

The assays incorporate an internal control for monitoring assay performance in each individual reaction tube.

STATISTICAL ANALYSIS

Statistical analysis was performed using Microsoft excel 2016. All the categorical variables were expressed in terms of frequencies and percentages and continuous variables as mean/median and standard deviation.

RESULTS

A total of 2000 seronegative blood donors were included in this study. The mean±SD and participants' median age were 28.98±8.05 and 27 years, respectively. The oldest donor was 61 years old, and the youngest was 18 years old. Maximum 1061 (53.05%) donors were in the age group 18-27 years, while 8 (0.4%) donors were in the age group 58-67 years [Table/Fig-1].

Age group (years)	Frequency	Percentage (%)
18-27	1061	53.05
28-37	616	30.8
38-47	265	13.25
48-57	50	2.5
58-67	8	0.4
Mean±SD	28.98±8.05	
Median	27	
Min-Max	18-61	

[Table/Fig-1]: Age group wise distribution of blood donors.

There were more males 1978 (98.9%) than females 22 (1.1%) and there were 1367 (68.35%) voluntary and 633 (31.65%) replacement donors [Table/Fig-2].

	Replacement		Voluntary		Total	
Sex	n	%	n	%	n	%
Males	626	31.3	1352	67.6	1978	98.9
Females	7	0.35	15	0.75	22	1.1
Total	633	31.65	1367	68.35	2000	100

[Table/Fig-2]: Frequency of males, females, voluntary and replacement blood donors.

Out of these 2000 donors, the most common blood group in ABO blood group system found to be was 'B' followed by 'O', 'A', and 'AB' in frequencies of 687 (34.35%), 628 (31.4%), 522 (26.1%) and 163 (8.15%) respectively and in Rh blood group system, 1826 (91.3%) were RhD positive and 174 (8.7%) were RhD negative [Table/Fig-3].

	Positive		Negative		Total	
Blood group	n	%	n	%	n	%
O	573	28.65	55	2.75	628	31.4%
B	621	31.05	66	3.3	687	34.35%
A	477	23.85	45	2.25	522	26.1%
AB	155	7.75	8	0.4	163	8.15%
Total	1826	91.3	174	8.7	2000	100%

[Table/Fig-3]: Blood group distribution among donors.

All the samples were non reactive for mandatory transfusion transmissible diseases on serologic testing. When these samples were made to run for nucleic acid testing, all turned out to be non reactive for all the three viruses (viz. HIV, HBV, HCV) [Table/Fig-4].

Results	Frequency	Non reactive
Serologic test	2000	2000 (100%)
NAT	2000	2000 (100%)

[Table/Fig-4]: Serological test (ELISA) and nucleic acid test results of blood donors.

DISCUSSION

The effectiveness of blood screening is a crucial concern when it comes to blood transfusion safety. TTIs cause mortality and morbidity

that are linked to infectious markers, including HBV, HCV, and HIV markers. TTIs poses a burden on healthcare systems all throughout the world, including in India. As a result, TTI screens are critical for determining the risk of blood and blood product transfusion.

By putting through ELISA negative samples to NAT, this study evaluated the efficacy of a fourth generation (Merilisa HIV Gen 4, Meril Diagnostics) assay.

Out of 2000 fourth generation ELISA negative p24 samples, none was reactive when analysed with NAT. This finding was alike to that reported by Makroo RN, Jain R et al., Dong J and Wu Y, Chigurupati P and Murthy KS, Chaurasia R et al., Datta S et al., and Ayuba Z et al., in 2007, 2012, 2014, 2015, 2016, 2019 and 2021 respectively [17-23]. Other studies, however, reported a positive yield after ELISA negative samples were subjected to NAT. In a study by Ohnuma H et al., in 2001, among 6,805,010 serologically negative donation, four HIV- 1 RNA positive cases were found by pooling 50 units of serologically negative units [24].

Among 37,164,054 units screened, 12 were confirmed to be positive for HIV-1 RNA in a study conducted by Stramer et al., in the United States at various blood centres [25]. Further this study states that the presence of virus below the limit of detection of minipool testing is the source of the residual risk after the implementation of NAT; individual nucleic acid screening of each sample, rather than screening of small pools of multiple samples, would reduce the residual risk even further, but at a much higher cost. Some have questioned the value of HIV-1 NAT because of its low yield and high cost effectiveness. Costs rise even more if each donated blood unit is examined individually rather than in minipools, with additional costs rising due to the automation necessary to do vast numbers of individual screening tests. As a result, the cost of HIV-1 NAT would have to drop significantly to match the cost of most other acceptable medical practices.

There was a lot of debate in the mid 1990s over whether new NAT techniques, like as Polymerase Chain Reaction (PCR) or TMA, should be used to test every blood donation for viral nucleic acids. The general consensus was that a marginal gain could only be obtained at extremely large prices. Furthermore, there was worry that NAT from a technological standpoint to be used for routine testing of thousands of blood donors per day. As a result, in most nations, the choice to implement the technology was deferred until commercial NAT systems became available.

The residual risk of incurring a TTIs was assessed at 1 in 4,300,000 for HIV-1 based on the incidence of WP donations reported between January 1997 and December 2005 in a study conducted by Hourfar MK et al., in the Institute of Transfusion Medicine and Immunohematology, German Red Cross, Germany [26]. In this investigation, minipool NAT was used to screen a total of 31,524,571 blood donations collected between 1997 and 2005, with pool sizes averaging 96 donations. Approximately 80% of the blood collected in Germany during that time period was covered by these donations. During this study, 7 HIV-1 NAT only positive donors were confirmed. Similarly during a one year Individual Donor-Nucleic Acid Testing (ID-NAT) screening of 732,250 donors, 16 HIV infections were detected by Vermeulen M et al., in the year 2005 in South Africa [14].

Interestingly, the discovery of two donors who were antibody positive but p24 antigen and HIV RNA negative in a South African study by Cable R et al., emphasises the importance of continued HIV antibody screening. These donors fall under the group of elite controllers, who have had detectable quantities of HIV RNA in their plasma on a regular basis for a lengthy period of time. In this five year review study, a total of 649, 745 donations were tested by ID-NAT, which yielded 6 HIV RNA positive donations in the anti-HIV-negative window period [27].

A multiplex NAT was used to begin routine ID-NAT screening at AIIMS blood bank in June 2010 as stated in a study at All India Institute of Medical Sciences, New Delhi, India, by Agarwal N et al., in which approximately 73,898 samples were tested for all three viruses using both ELISA and NAT over a 27 month period from June 2010 to August 2012. The results of both assays were compared and evaluated. There was one HIV case out of 73,898 samples that was not detected by serology but was positive on NAT testing [28]. Testing of the 12,032 seronegative donor samples included over a period of almost 2.5 years from January 2009 to June 2011 in Egypt revealed two samples were positive for HIV-1, according to NAT results published in the study Selim HM et al., [29].

The majority of asymptomatic HIV carriers were detected by first generation ELISA tests for anti-HIV, but the WP preseroconversion in newly infected individuals was approximately 56 days. Anti-HIV tests of the second and third generations shortened the window to 33 and 22 days, respectively. In comparison to third generation antibody tests, the currently used EIA tests included the p24 antigen along with IgM/IgG detection (Ag/Ab combined fourth generation assays), reducing the diagnostic WP from three weeks to about two weeks [29]. The routine serologic assay for blood donors at our blood centre relied on this combined HIV p24 Ag/Ab detection, which increased sensitivity in detecting HIV infectivity compared to assays relying solely on antibody detection. NAT has been shown to shorten the WP even more and detect HIV-1 infection 11-16 days earlier than serological tests when used as a screening tool [30].

Kumar R et al., conducted the ID-NAT study in the Punjab region of India, which was the first of its kind in Punjab. Out of 32,978 blood donor samples tested, 43 were NAT reactive and serologically non reactive for any of the three viruses. One (1/32978) of the 43 NAT yields tested positive for HIV-1 [1]. They also stated in this study that blood donors with a low viral load may go unnoticed by the discriminatory assays. The differences between multiplex and discriminatory assays found in this study should be attributed to the low viral load of the sample tested rather than false positive results or decreased discriminatory assay sensitivity. The most likely cause of discrepant results in low viral load samples is stochastic sampling variation. It is recommended to discard all initial NAT reactive donations regardless of the results of the discriminatory probe assay or multiplex repeat assays in order to avoid the infusion of a very low level viremic unit [1].

In contrast to above study, Delwart EL et al., in the United States reported a case of HIV transmission via RNA screened blood donation. The transmission of HIV by blood transfusion was confirmed by phylogenetic linkage of HIV sequences in the blood donor and recipient. ID-NAT was able to reliably detect viral RNA, whereas Minipool NAT (MP-NAT) was only able to detect it inconsistently. Even after MP-NAT was introduced, a preseroconversion donation with a very less viral load went undetected, resulting in HIV transmission. These findings show that very low level HIV viraemia during the preseroconversion window is infectious by blood transfusion, and that, as predicted, there is still a small risk of transfusion-associated HIV transmission even after MP-NAT screening [31].

In similar context, one literature showed some clusters of HIV-1 recombinant forms escaping detection by commercial nucleic acid amplification assays. In a study by Foglieni B et al., in Italy, in which a repeat blood donor seroconverting to anti-HIV was observed, and HIV RNA was initially undetectable with routine NAT. HIV RNA was detected during donor follow-up, but the viral load was 2-3 logs less

than that measured with other targeting regions in NATs. The poor performance of routine NAT was attributed to a novel recombinant with mutations affecting primers and probe annealing, as revealed by genome sequencing [32]. In 19 (3.5%) cases, the routine assay significantly underestimated viremia (1-5 log), 11 (58%) of which were infected with the same strain found in the index donor samples. Two other non-B circulating recombinant HIV-1 forms had been identified as being difficult to detect. As a result, increasing HIV-1 heterogeneity has an impact on the effectiveness of NATs, as well as the safety of the blood supply, diagnosis and patient management [32].

The positive results of NAT on fourth generation ELISA negative samples may have been influenced by the greater sample sizes used in the research mentioned above as opposed to those employed in this research. The type of blood donors used may be the reason why there were no NAT yields in this study. The majority of the participants in this study were voluntary non paid blood donors, which are thought to be the safest kind of blood donors. It's possible that all cases lacking amplification had extremely low virus loads, making it difficult to identify the target. As a result, it would be assumed that a positive sample with a virus load below the instrument's detection limit was negative. A negative test may not always indicate non infectivity as a result. Although, HIV-1 and HIV-2 have a similar virion structure and overall genomic organisation, HIV-2 cannot be detected using test techniques made for HIV-1. The HIV is well known for its potential to mutate; hence, the NAT may miss the targeted sequence and result in no amplification [23].

According to reports from developed countries, NAT blood screening has limited value in improving blood safety. The Scottish study reported a NAT yield rate of 1 per 1.9 and 0.77 million donations for HIV and HCV, respectively. The NAT yield of screening 3.6 million blood donations from continental Europe for HBV, HCV, and HIV-1 was 1 per 0.6 million for HBV, HCV, and 1 per 1.9 million for HIV-1, according to reports. The low prevalence of HIV-1, HBV, and HCV in these countries was the main reason for this. In contrast, the prevalence of these viral infections is generally high in resource-limited countries [33-35]. The [Table/Fig-5,6] shows various Indian and foreign studies comparing yield for HIV [1,14,17-29,36-48].

The fourth generation ELISA's effectiveness in this study shows that it may be depended upon for blood donor screening in present context, offering a reasonably priced alternative to NAT.

S. No.	Place	Author	Sample size (n)	Period of study	Multicentre/Single centre study	Year of publication	ELISA-/NAT+ samples/NAT yield for HIV
1	Japan	Ohnuma H et al., [24]	68,05,010	15 months	Multicentre	2001	04
2	USA	Stramer SL et al., [25]	3,71,64,054	38 months	Multicentre	2004	12
3	Germany	Hourfar MK et al., [26]	3,15,24,571	108 months	Multicentre	2008	07
4	South Africa	Cable R et al., [27]	6,49,754	60 months	Single centre	2013	06
5	South Africa	Vermeulen M et al., [14]	7,32,250	12 months	Multicentre	2009	16
6	Germany	Selim HM and ElBashaar MA, [29]	12,032	30 months	Single centre	2014	02
7	Thailand	Phikulsod S et al., [36]	4,86,676	12 months	Single centre	2009	05
8	Germany	Nubling CM et al., [37]	5,79,50,409	108 months	Multicentre	2009	17
9	USA	Zou S et al., [38]	6,60,00,000	120 months	Multicentre	2010	32
10	Many Nations of Europe	Roth WK et al., [39]	3,77,00,000	12 months	Multicentre	2012	72
11	Croatia	Safic Stanic H et al., [40]	5,45,463	37 months	Multicentre	2017	10
12	China	Huang W et al., [41]	1,06,488	24 months	Single centre	2017	21
13	Saudi Arabia	Alaidarous M et al., [42]	3028	20 months	Single centre	2018	02
14	China	Dong J and Wu Y, [19]	1,78,447	17 months	Single centre	2014	0
15	Nigeria	Ayuba Z et al., [23]	1018	3 months	Single centre	2021	0
16	India (Present study)	Mathur R et al.,	2000	24 months	Single centre	2022	0

[Table/Fig-5]: Comparison of various foreign studies with present study [14,19,23-27,29,36-42].

S. No.	Place	Author	Sample size (n)	Period of study	Multicentre/Single centre study	Year of publication	ELISA-/NAT+ samples/NAT yield for HIV
1	New Delhi	Makroo RN, [17]	22,277	15 months	Single centre	2007	0
2	Rajasthan	Jain R et al., [18]	47,558	21 months	Single centre	2012	1
3	New Delhi	Chatterjee K et al., [43]	18,354	7 months	Single centre	2012	0
4	New Delhi	Agarwal N et al., [28]	73,898	27 months	Single centre	2013	1
5	Karnataka	Chandrashekhar S, [44]	53,729	60 months	Single centre	2014	1
6	Andhra Pradesh	Chigurupati P and Murthy KS, [20]	8,000	12 months	Single centre	2015	0
7	Punjab	Kumar R et al., [1]	32,978	12 months	Single centre	2015	1
8	New Delhi	Mangwana S and Bedi N, [45]	16,997	31 months	Single centre	2016	1
9	New Delhi	Chaurasia R et al., [21]	10,015	10 months	Single centre	2016	0
10	Gujrat	Mishra KK et al., [46]	79,532	30 months	Single centre	2017	5
11	New Delhi	Datta S et al., [22]	1,01,411	71 months	Single centre	2019	0
13	Karnataka	Aramani SS et al., [47]	6000	19 months	Single centre	2019	1
14	New Delhi	Jagani R et al., [48]	54,895	48 months	Single centre	2022	3
15	Rajasthan (Present study)	Mathur R et al.,	2000	24 months	Single centre	2022	0

[Table/Fig-6]: Comparison of various Indian studies with present study.

Limitation(s)

Being a single centre hospital-based study, its results cannot be generalised. The samples selected for the study were specifically intended to be tested with NAT for the use in the patients of thalassaemia only. Due to the SARS-CoV-2 pandemic, there was restricted transportation, hence sample transportation was affected.

CONCLUSION(S)

Serology remains the cornerstone for donor screening in India, but NAT testing is not a mandatory TTI screening test in India. The fourth generation ELISA is recommended as the minimum test for TTIs; additional blood safety tests can be advocated. Cost/effectiveness and affordability of implementation are the most important factors to consider when making such a decision.

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