ORIGINAL ARTICLE

Nucleic acid amplification testing in Indian blood banks: A review with perspectives

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ABSTRACT

Background: Nucleic acid amplification testing (NAT) is restricted to a few blood banks in India since 2008. This review was directed toward understanding NAT yield in different parts of the country and prevalence in the NAT of different types of virus. **Materials and Methods:** English literature was searched from 1990 to 2016 in PubMed, Scopus, Ind med, and Google database using properly constructed key words. Literature was collected and finally the data were synthesized. **Results:** NAT results from 11 publications and one personal communication showed that till date 389387 blood units have been NAT tested from various parts of the country. NAT yield varied from 1:476 to 1:4403 in various studies. Till date, 58/2550 (2%) blood banks of India are doing NAT testing but all of them have not published their results. Majority of the centers have used ID-NAT (Individual NAT) protocol and 21 blood banks are using minipool format of the test. One center has used in-house NAT testing system. In >70% of the time, the NAT positivity with due to hepatitis B (Hep B). For individual infection, NAT yield from the pooled data showed HIV in 1:66,000, Hep C virus 1:5484 and Hep B in 1:1761 seronegative donors. **Discussion and Conclusion:** In view of the very high NAT yield (1:1361), NAT in some from needs to be universally applied in Indian blood banks. However, the high Hep B occult infection suggests stricter donor selection and immunization of adults for Hep B may be way forward toward ensuring the viral safety of blood components in India.

KEY WORDS: Hepatitis B, hepatitis C, HIV, India, NAT testing, review, transfusion safety

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INTRODUCTION

Safety against three transfusion-transmitted viral infection (TTI), i.e., HIV-1, hepatitis B (Hep B), and Hep C became a worldwide concern in spite of the development of highly sensitive and specific serologic tests and surrogate marker tests against Hep B.[1] To close the gap between the time of acquiring infection and development of seropositivity (window period), various type of nucleic acid amplification tests (NAT) was developed in 1990s and finally applied in all developing countries by the end of 1990s.[2-4] However, NAT testing results created argument, i.e., safety at what cost? Because of the several interlocking processes, i.e., donor exclusion based on interview, serological tests, repeat donation records reduced the frequency of only NAT-positive blood donation to one in a million due to various viruses and in different countries.[5,6]

NAT testing is a costly investigation (costing at least 20–25 USD/bag of blood tested) hence developing countries including India is yet to employ even though as late as in 2000, it was believed that 21% of pediatric AIDS cases were contributed by blood and plasma infusion.[7] With mandatory HIV, Hep B, and Hep C the transmission of these viruses has been constantly reducing and at present blood donor prevalence of these viruses in India stands at 0.24% for HIV, 1.18% for Hep B, and 0.43% of hepatitis C virus (HCV). This prevalence is one order of magnitude higher than the voluntary blood donors in developing countries.[8] This finding clearly suggests that the safety of blood transfusion in India can only be improved by strict donor selection and introduction of universal NAT testing.

In India, presently, 10×10^6 units of blood collected every year. If all these units are NAT tested then country will incur an additional cost of 30×10^7, i.e., 3×10^8 or 300 million US dollar which it can ill afford at present as health budget of Government of India in 2016 is mere 1.62% of gross domestic product.[9] However, with

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burgeoning middle-class health tourism and continuous pressure by experts ultimately lead some of the corporate hospital and high-end government hospital to start NAT testing from 2008. The present review is on the collected data of NAT testing results from different parts of the country.

MATERIALS AND METHODS

All the literature was searched in PubMed from March 1995 to 2016 with following keywords NAT testing, blood bank, HIV-NAT, Hep B, NAT test, and Hep C, NAT test combined with blood bank and India.

Review articles on NAT testing were scanned and cited references for India was looked into. National blood transfusion conference abstracts and telephonic interview of blood bank directors who are known to be employing NAT tests in some manner were also recorded. NAT testing blood banks and India as terminology was keyed into Google and Scopus database.

After reading these articles, the articles which were relevant were sorted, referred, and discussed in this submission.

RESULTS

A total of 57 papers and abstracts were found and of those 14 papers were found to be relevant to our discussion.

There are 2550 public and private blood banks in the country and only 58 blood banks are doing some type of NAT testing (at 2% blood banks). Taken together, they are testing 700,000 units (7% of all collected blood units in India) of blood collected in the Country. The blood banks which are doing NAT testing in different areas of the country are presented in Table 1 and Figure 1. It can be seen that 26 of 58 blood banks are situated in or around New Delhi or in North India (50%), 13 (25%) blood banks in South India, 16 blood bank (20%) in Western India and 1 blood bank in Central and 2 in Eastern India is doing NAT testing. Except 22 blood centers all other blood centers are doing ID-NAT, 20 blood banks are using minipool NAT [marked by* in Table 1 and Figure 1] while 1 each are doing their semiautomated NAT testing.

Results from 12 (one unpublished) studies with their NAT yield for various viruses and total units tested along with journal where it was published are given in Table 2.[10-20]

NAT yield for various viruses varied from 1:476 to 1:4403. 70%–80% of the NAT yield were related to Hep B virus. HIV and HCV accounts for 10%–20% of the NAT yields. On the average, 1:1365 units of blood are seronegative. A total of 389367 units tested in various studies lead to 286 NAT yield (1:1361). As there are 10 million blood collected across the Country with 1:1361 NAT yield, it will lead to at least 7000 infected units which are seronegative and when we consider 1–3 components produced from each units at least 7000–21,000 new at risk TTI infections are

Table 1: List of the blood centers in India having nucleic acid amplification testing facility

Blood Center
North India
Indraprastha Apollo Hospital, New Delhi
Artemis Hospital, Gurgaon, New Delhi
All India Institute of Medical Sciences, New Delhi
Dr. Ram Manohar Lohia Hospital, New Delhi
Medanta - The Medicity, Gurgaon, New Delhi
Fortis Escorts Hospital, New Delhi
Armed Forces Transfusion Centre, New Delhi
Lions Blood Bank, New Delhi
Sir Ganga Ram Hospital, New Delhi
Rajiv Gandhi Cancer Hospital and Research Institute, New Delhi
Postgraduate Institute of Medical Education and Research, Chandigarh
Rotary Noida Blood Bank, Noida, Uttar Pradesh
Dayanand Medical College and Hospital, Ludhiana, Punjab
Rotary Blood Bank, New Delhi
B L Kapur Hospital*, New Delhi
Jaypee Hospital, Noida, Uttar Pradesh
SGPGI*, Lucknow, Uttar Pradesh
Fortis Memorial Research Institute, Gurgaon, New Delhi
Ambica Blood Bank, Jodhpur, Rajasthan
RNT Medical College, Udaipur, Rajasthan
Santokhaba Durlabhji Hospital*, Jaipur, Rajasthan
CMC, Ludhiana, Punjab
Max health care Hospital*, New Delhi
King George Medical College*, Lucknow, Uttar Pradesh
Bapu Maghar Singh Blood Bank*, Sirsa, Haryana
Fortis Hospital, Chandigarh*
West India
P. D. Hinduja National Hospital and Medical Research Centre, Mumbai
Rotary Blood Bank, Thane, Maharashtra
Arpan Blood Bank, Nasik, Maharashtra
Kokilaben Dhirubhai Ambani Hospital and Medical Research Centre, Mumbai
Datajivale Blood Bank*, Aurangabad, Maharashtra
Dr. Hedgewar Blood Bank*, Nagpur, Maharashtra
Sarvodaya Hospital Samarpan Blood Bank*, Mumbai
Surat Raktadan Kendra and Research Centre, Surat, Gujarat
Life Line Blood Bank, Nagpur, Maharashtra
Sir H. N. Hospital Trust, Mumbai
Rajkot Peoples' Blood Bank, Rajkot, Gujarat
Jankalyan Rakthadan Pedhi*, Pune, Maharashtra
South India
CMC, Vellore, Tamil Nadu
Apollo Hospital*, Chennai, Tamil Nadu
TTK Blood Bank, Chennai, Tamil Nadu
Amrita Institute of Medical Science, Kochi, Kerala
SRM Hospital, Chennai, Tamil Nadu
Manipal Hospital, Karnataka
Dhanvantri Blood Bank*, Rajahmundry, Andhra Pradesh
Global Hospital*, Hyderabad Andhra Pradesh
NTR hospital*, Hyderabad, Andhra Pradesh
Rashtrotthana Blood Bank*, Bengaluru
IMA Blood Bank*, Cochin, Kerala
Apollo Hospital*, Kerala

Contd...

Table 1: Contd

Blood Center
Apollo Hospital*, Hyderabad, Andhra Pradesh
Central India
Greater Kailash Hospital, Indore, MP
Eastern India
Apollo Hospital*, Kolkata, WB
Shija Hospital*, Imphal, Manipur

This list is not all embracing, few more centers might have been added after the list was compiled. *Centers running on automated minipool NAT system, two centers use semiautomated technique and rest on various automated ID NAT systems. NAT: Nucleic acid amplification testing

likely and even if 50% of these is converted into actual infection then up to 10,000 new infection are occurring every year and this number is confirmed by NACO statistics discussed recently.[21]

DISCUSSION

The present paper collates all the data on NAT testing available in public space since 2008 when first NAT testing was started in a few of the corporate hospitals in India. HIV data on NAT from India when compared to some of the developed countries, i.e., 6/389,387 donations tested, i.e., 1:66,000 donations, show better control of TTI spread with HIV infection than with HCV (71/389,387), i.e., 1:5484 donors or whose with Hep B infection (221/389387) at 1:1761 with seronegative donors.

In some of the blood banks, the NAT yield is solely contributed by Hep B infection. All India Institute of Medical Sciences, a premier government hospital in India has presently high NAT yield. In general, the average NAT yield combining all three viruses in 1:1361 in seronegative donors is a cause for great concern and 196/276 total seronegative NAT yields (71%) were contributed by Hep B infection. It is true that seroprevalence of Hep B at in Indian population is at 1.4%–1.8% while Hep C is at 0.3%–0.4% and HIV is at 0.2%–0.3% and reactive preponderance of these viruses in NAT testing closely mimic this prevalence, but HCV-positive NAT yield is proportionately much higher ($P < 0.01$) compared to HIV infection probably due to long window period of HCV.

The present NAT yield data have been obtained from the majority of the centers using ID-NAT system and some centers using minipool system and one center using its own homemade NAT testing system.

ID-NAT system has several advantages, the main being improved sensitivity. Logistically, it is easier to use hence less error-prone. On the flipside, ID-NAT tests single sample not 10-16 samples in a pool, and it is that much costlier. Moreover as theoretically, very small number of virus particles of Hep B virus is capable of transmitting hepatitis in an immunocompromised patient an improved sensitivity with less cumbersome process, like no need for mixing sixteen samples, ID-NAT obviously has been considered attractive by transfusion medicine experts. However, in the semiautomated open format of the system, tests for newer viruses can easily be accommodated without significant change in instrument profile. The pros and cons of both the process are

Figure 1: Nucleic acid amplification testing centers in India

summarized in Table 3. We should not forget that all minipool NAT machines are also capable of doing ID-NAT test. It may be noted that both minipool as well as ID-NAT format is recognized by Food and Drug Administration as valid instruments of NAT testing.

Hence, the present data can be considered as mainly individual donor NAT testing data. One of the centers which has used in-house NAT system with a NAT yield of 1:17,753 should be considered as an outlier as most of the Indian NAT yield aggregate around 1:476–1:4403 way above the results from in-house NAT testing system data. Moreover, in-house NAT testing system was cumbersome because it involved polymerase chain reaction (PCR) amplification of the virus and then gel electrophoresis to detect the virus.[10]

NAT data from India are a cause for great concern particularly in the way we are now managing our donors as well as depending only on the 3rd generation serology for the majority of the blood banks in India.

Our data are several orders of magnitude higher than NAT yield data from developed countries at 1:7.8 million for HIV, 1:2.3 million for HCV, and 1:153,000 for Hep B[22] as has been described for Canada in 2007. In developed countries and across the globe Hep B is the cause of the most common NAT yield like ours.

What insight or perspective we can get from the present data? First, our voluntary donor programme needs to be tightened

Table 2: Summary of nucleic acid amplification testing results published from India

Center	*Number of donor tested*	*NAT yield*	*Prevalence*	*HIV*	*Hepatitis C*	*Hepatitis B*	*References*
AIIMS, New Delhi	10,015	21	1:476	0	3	18	[11]
DMC, Ludhiana, Punjab	32,978	43	1:767	1	13	27 (2 hepatitis C + hepatitis B)	[12]
Apollo Hospital, New Delhi	10,302	15	1:686	-	-	15	[13]
Dhanwantari Blood Bank, Rajahmundry, Andhra Pradesh, India	8000	4	1:2000	-	-	4	[14]
Manipal Hospital Blood Bank	53,260	3	1:17753	1	-	2	[10]
AIMS, New Delhi	73,898	121	1:610	1	37	73 (10 hepatitis C + hepatitis B)	[15]
AIIMs, New Delhi	18,356	7	1:2622	-	3	4	[16]
Santokba Durlabhji Hospital, Jaipur	23,779	8	1:2972	-	-	8	[17]
Multicentric Study Apollo, Delh, Prathma, Ahmedabad, PGI, Chandigarh, Rotary Blood Bank, New Delhi, Lifecare, Kolkata	12,224	8	1:1528	1	1	6	[18]
Ram Manohar Lohia, New Delhi	28,134	25	1:1125	-	-	25	[19]
Medanta Hospital, Delhi	48,441	11	1:4403	2	2	7	[20]
Hinduja Hospital, Mumbai	70,000	20	1:3500	-	-	20	Personal communication
Total	389,387	286	1:1361	1:66,000 (6)	1:5484 (71)	1:1761 (221)	

NAT: Nucleic acid amplification testing

Table 3: Pros and cons of minipool nucleic acid amplification testing

MP-NAT (n=16)	*ID-NAT (n=1)*
FDA approved for blood donor screening only	FDA approved for blood, organ, and tissue donor
NAT testing compromised limit of detection Due to dilution (countered by improved extraction and increasing the number of PCR cycles to 40)	Best achievable lower limit of detection
Presence of DNA or RNA polymerase inhibitors (will compromise all the samples in the pool. In semiautomated system each sample also gets an internal control to see whether such inhibition is occurring)	Affects only one sample
More labor intensive (pooling step) (not a problem where labor is cheap)	No need for pooling specimens
Higher chance of clerical errors related to pooling	Low chance of clerical errors
Longer "window" of HIV and HCV undetectability	Short achievable "window" of undetectability
Lower cost	Higher cost
Detects and identifies virus in one step in our format	Needs two step detection system (now systems are available which identifies virus in one step)
Being an open system can be easily utilized for other viruses if required	No possible
Open system can also be used in ID NAT format	Not applicable

PCR: Polymerase chain reaction, NAT: Nucleic acid amplification testing, FDA: Food and Drug Administration, HCV: Hepatitis C virus

and as directed/replacement donors are still the major mode of donation in many corporate hospitals and even in apex government hospitals,[23] where patients come from thousands of kilometers and their replacement donors are always have a doubtful credential.

It has been argued that NAT testing for India may not be the solution for blood safety[24] and as the 4th generation advanced antigen antibody combined serological tests are becoming available they will significantly shorten the window period. On the other hand, it has been argued that high-quality ID-NAT testing is the only way forward.[25] The study also showed that minipool NAT with up to 16–24 pool was equally acceptable.[26,27]

As >70% of our NAT yield has been for Hep B virus, argument has been marshalled that whether all NAT-positive sample transmit infections? Some study[28] have shown that up to 19-83% of NAT positive (for Hep B) but seronegative donors may transmit infection with higher risk associated with window period donation rather than occult Hep B infection. Hence, all NAT-positive Hep B virus yield may not transmit infection and in these donor Hep B core IgM/IgG and follow-up can resolve the problem.

Finally, we may conclude from the review that there are a significant residual risk of viral transmission from seronegative donor blood in India and at 1:1400 seronegative NAT-positive blood units however if we only componenetize 70% of the collected products then it can transmit from 7000 to 20,000 new infections every year.

This can only be reduced by (i) strict voluntary blood donation and (ii) installation of some form of NAT testing keeping in mind ID-NAT is 10 time more costly than NAT testing with a minipool of 10, but ID-NAT marginally reduced the window period of the three infections compared to minipool NAT[28] by 2 days only, moreover several developed countries continue to use minipool NAT even today.

Hence if finance is the problem then minpool NAT also could be an acceptable beginning in the road to transfusion safety against the three viral infections. Although in India already 58 blood banks are doing NAT testing using different technologies, results from all of them are not in public domain and this needs to be urgently corrected.

CONCLUSION

Finally and not in the least, the blood donor interview and evaluation for the positive risk of transmission of viruses should be done in more user-friendly circumstances. Blood camps in India are often very cramped and crowded hence is not suitable for proper donor counseling. Extensive adult vaccination with Hep B virus vaccine has also been suggested[29] to reduce the Hep B viral load in the community. This may be a practical solution in the long run when all vaccinated children becomes adult and enters as voluntary blood donors, but all adults who are healthy cannot be persuaded to take Hep B vaccination at present, and its financial implication could be stupendous.

For low resource countries several challenges, for example, high cost of NAT, requirement of qualified technical staffs, laboratory facilities, reagent procurement, and maintenance of delicate equipment conspires against NAT testing as is used in developed countries. In such a situation, the 4th generation serological test which combines both antigen and antibody in an ELISA format may be a good beginning[30] along with other facets of improving the blood safety.[24] However, eventually, in the present day, scenario implementation of some sort of NAT testing is inevitable[31] to improve blood safety, this can start with in-house or minipool NAT eventually ending with ID-NAT or staying with minipool format with simultaneously improving DNA/cDNA extraction and PCR amplification efficiency.

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Conflicts of interest
There are no conflicts of interest.

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