International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 12, Issue 3, 2020, pp.-1794-1797.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

NUCLEIC ACID AMPLIFICATION TESTING IN BLOOD SCREENING: RETROSPECTIVE EVALUATION OF 5 YEARS OF EXPERIENCE

MURTHY K.S.[1] AND PRAGATI C.*[2]

[1]Managing Director, Dhanwantari Voluntary Blood Bank & Blood Components, Danavaipeta, Rajahmundry, 533103, Andhra Pradesh, India
[2]Professor, Department of Microbiology, GSL Medical College, Rajahmundry, 533296, Dr NTR University of Health Sciences, Vijayawada, 520004, Andhra Pradesh, India
*Corresponding Author: Email - ch.pragatisb@gmail.com

Received: March 02, 2020; Revised: March 26, 2020; Accepted: March 27, 2020; Published: March 30, 2020

Abstract- Introduction: The prevalence of transfusion transmitted infections (TTIs) is high in India. The aim of our study was to evaluate the sero-prevalence of TTIs in blood bank at Rajahmundry (Andhra Pradesh, India) since 2010. Materials and Methods: A total 89,639 units of blood donated at our blood bank till date. The sero-prevalence for HIV, HBV and HCV was tested by 4th generation ELISA. Only sero-negative samples were tested on mini pool nucleic acid test (MP-NAT) to detect HIV1, HIV2, HCV and HBV viral nucleic acids. Combined NAT yield was calculated and compared the NAT yield with MPX test and MPX V2.0. We also evaluated the optical density (OD) values of ELISA for MP-NAT reactive donor samples to evaluate the efficacy of NAT in detecting window period cases. Results: The overall sero-prevalence of TTIs was 2.41% and HBV was predominantly present (66%). Total 29 ELISA negative blood donor samples were found reactive on NAT. All NAT reactive donors were of HBV only. Out of 29 cases, 12 were detected by MPX test from 26,008 donations and 17 by MPX V2.0 from 25,619 donations, providing NAT yield of 1:2169 and 1:1507 respectively. OD and Signal cut off values of ELISA for NAT reactive samples were found to be much below extended grey zone value (<0.3). Conclusion: Additional testing of nucleic acids by MP-NAT in combination of mandated primary serology by ELISA provides 99.99% of safe blood to needy patients; thus significantly reduces the risk of TTIs by identifying the window period cases.

Keywords- *Transfusion transmitted infections, MP-NAT, ELISA*

Citation: Murthy K.S. and Pragati C. (2020) Nucleic Acid Amplification Testing in Blood Screening: Retrospective Evaluation of 5 years of Experience. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 12, Issue 3, pp.-1794-1797.

Introduction

The risk of transfusion transmitted infections (TTIs) is one of the major problems identified in Transfusion Medicine. The prevalence of TTIs is directly proportional to prevalence of these infections in the blood donor population. Hence, it is very critical to implement safety and quality systems in blood screening and transfusion processes to reduce the risk of TTIs in recipients. It has been noted that trends of TTIs were reported high in first time donors and replacement donors compared to voluntary donors [1-4]. NACO (National Aids Control Organization), division of Ministry of Health and Family Welfare which governs the HIV/AIDS control programme in India, has mandated blood testing for 5 TTIs, HIV (HIV1 and HIV 2 antibodies), HBV (HBsAg Antigen), HCV (HCV antibodies), Malaria and Syphilis [5]. NACO has not made Nucleic Acid Amplification testing (NAT) mandatory for blood screening but an additional test of safety in India. However, NACO in its recent guidelines on HIV testing mentioned that NAT for HIV diagnosis for early infant diagnosis and window period cases [6]. Multiple studies have shown that NAT is able to pick up the HIV HBV HCV infections in window period which may undetected leads to post transfusion TTIs in recipients with overt infection and misinterpreted as medico-legal negligence on blood banking services [7,8]. India being a second most populous country, considerably high prevalence of HIV, HBV and HCV infections and shortage of blood due to gap in demand vs supply, it possesses great risk of TTIs due to inadequate blood screening and missing cases on currently mandated serology testing. The prevalence rates in various regions in India have been published [Table-1]. The prevalence rate of HIV is decreasing over the period of time due to adoption of best practices such as thorough screening methods including donor questionnaire and avoiding blood donations from already positive cases through proper data storage facility with proper counselling of all reactive donors for treatment and follow up. HBV is a still an area of concern, due to lower viral load in early phase of infection (2.7 days of doubling time) and these infections goes undetected on routine serological testing. The objective of this retrospective assessment was to evaluate our 5 years of NAT experience and to assess NAT yield. We also compared latest NAT (cobas® TaqScreen MPX V2.0 Test) assay with the previous NAT (cobas® TaqScreen MPX Test) assay.

Table-1 *Prevalence of TTIs in India*

Study	HIV	HBV	HCV
Leena *et al*., 2012 [9]	0.27%	0.71%	0.14%
Shah *et al*., 2013 [10]	0.16%	0.98%	0.11%
Chandra *et al*.,2014 [11]	0.08%	0.24%	0.00%
Sethi *et al*., 2014 [12]	0.19%	0.63%	0.20%
Khan *et al*., 2015 [13]	0.26%	0.96%	0.02%
Patel *et al*., 2015 [14]	0.14%	0.38%	0.06%
Mohapatra *et al*., 2016 [15]	0.02%	0.40%	0.02%

Materials and Methods

The study was conducted at Dhanwantari Voluntary Blood Bank (Rajahmundry, East Godavari district Andhra Pradesh, India), established in 2007, covers twin districts of Godavari (Andhra Pradesh, India). Dhanwantari blood bank has~ 15,000 blood donations and issues ~30,000 annually. It has all the required technology mandated by NACO and DCGI. A total number of 89,639 blood donors from 2010-2016 were screened for HIV (P24antigen and GP41 antibody), HBV (HBsAg) and HCV (antibody). All positives and grey zone units are discarded. All negative samples are subjected for MP-NAT test.

We implemented mini pool NAT (MP-NAT) in 2012 with cobas®TaqScreen MPX test (Roche Molecular Systems, Branchburg, NJ) on cobas® s201 platform (Roche Instrument Center, Rotkreuz, Switzerland). We upgraded NAT to cobas® TaqScreen MPX Test, version 2.0 (MPX V2.0) in September 2014. The MPX and MPX V2.0 tests are intended as an advanced donor screening test to detect HIV-1Group M RNA, HIV-1 Group O RNA, HIV-2 RNA, HCV RNA and HBV DNA in human plasma [22, 23]. MPX V2.0 test is more advanced over MPX where the target viral infection is known in resolution test itself and no separate discrimination test is needed. Moreover, the lower level of detection of viral pathogens is better in MPX V2.0. [Fig-1] provides the screening algorithm followed at Dhanwantari Voluntary blood bank. All blood donations/units were screened by 4th generation ELISA of BIORAD employing fully automated ELISA processor-EVOLVIS. Only Seronegative samples were tested on NAT using MPX or MPX V2.0. For MPX test - NAT was performed in pools of 6 and the reactive pools were resolved by further testing of the individual blood donations/samples comprising the pools. Individual reactive specimens were further tested using the cobas Taqman monitor test (for HBV followed by HCV and then followed by HIV) for viral discrimination. All reactive samples were outsourced and discriminated at Asian Institute of Gastroenterology (AIG), Hyderabad, using the cobas TaqMan HBV, HCV and HIV viral load assay.

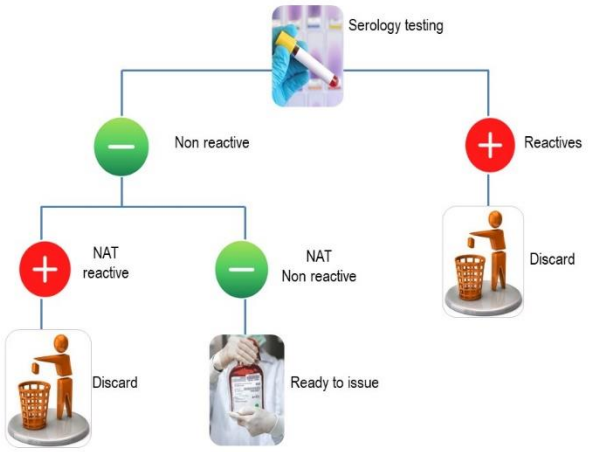

Fig-1 Blood Screening Algorithm followed at Dhanwantari Voluntary Blood Bank

For MPX V2.0-The same procedure was employed as for MPX. The reactive pool was subjected for individual testing for NAT by resolution. No viral discriminatory test was required for MPX V2.0.

The mini pool NAT is a Multiplex Polymerase chain reaction. Here 6 donor samples were pooled to one sample in Hamilton instrument. The pooled samples were transferred to Ampliprep instrument where extraction of viral nucleic acids occurs by chemical and enzymatic reactions in K tubes. Later these K tubes were automatically transferred to TAQMEN instrument through docking station. In TAQMEN Real time PCR steps occur such as Denaturation, annealing and extension and finally the target is amplified and identified by Fluorescent based 5-hydroxylase technology with advanced version of sophisticated software.

We also evaluated the optical density values of ELISA for NAT reactive donor samples, to know the efficacy of NAT in detecting window period cases.

Results

The details of number of samples tested on ELISA and sero-positivity for HIV, HBV and HCV is given in [Fig-2]. Total sero-prevalence TTIs was 2.41%. Sero-prevalence rate found to be decreased over the period years (2010 - 3.38%, 2011-3.01%, 2012-3.03%, 2013-1.91%, 2014-1.84%, 2015-1.75% and 2016-1.36%). HBV was predominantly present, ~66% of all positive cases. The sero-reactivity of HIV in blood donors is decreased over a period of years due to compulsory donor screening by qualified staff, use of donor deferral software and counselling of reactive donors with follow up for treatment in VCTC centres (sero prevalence of HIV in 2010- 0.60%, 2011- 0.61%, 2012- 0.53%, 2013- 0.33%, 2014-0.19%, 2015-0.23%, and 2016- 0.22%). Similarly, the sero-prevalence of HCV is also decreased over a period of years from 0.79% in 2010 to 0.14% in 2016.

Table-2 *NAT Reactive Samples since 2012*

Year	2012	2013	2014	2015	2016*	Total
NAT Tested Samples	4860	13008	13140	12544	8075	51627
NAT Reactive	1	7	5	11	5	29

Table-3 *NAT Yield on cobas TaqScreen MPX test and MPX test V2.0*

	NAT Tested Samples	NAT Reactive	NAT Yield
MPX	26008	12	1.54653
MPX V2.0	25619	17	1.08819

Table-4 *OD Values of ELISA in NAT reactive samples*

ELISA negative OD values in NAT reactive donor sample 2012		
NAT Reactive Donor ID	OD Value	Signal cut off Ratio
13258	0.022	0.3

Table-5 *OD Values of ELISA in NAT reactive samples (2013)*

ELISA negative OD values in NAT reactive donor sample 2013		
NAT Reactive Donor ID	OD Value	Signal cut off Ratio
3615	0.0100	0.1619
4928	0.0130	0.2167
6984	0.0110	0.1796
8538	0.0140	0.2267
8737	0.0090	0.1552
9729	0.0140	0.2286
12939	0.0120	0.1983

Table-6 *OD Values of ELISA in NAT reactive samples (2014)*

ELISA negative OD values in NAT reactive donor sample 2014		
NAT Reactive Donor ID	OD Value	Signal cut off Ratio
61	0.0250	0.0849
86	0.0130	0.2105
1866	0.0110	0.1796
2248	0.0050	0.0881
9541	0.0060	0.1081

Table-7 *OD Values of ELISA in NAT reactive samples (2015)*

ELISA negative OD values in NAT reactive donor sample 2015		
NAT Reactive Donor ID	OD Value	Signal cut off Ratio
115	0.0190	0.2686
2713	0.0120	0.1912
3851	0.0150	0.2419
3857	0.0120	0.1935
5428	0.0140	0.2295
6218	0.0170	0.2731
6219	0.0180	0.2892

Table-8 *OD Values of ELISA in NAT reactive samples (2016)*

ELISA negative OD values in NAT reactive donor sample 2016		
NAT Reactive Donor ID	OD Value	Signal cut off Ratio
3165	0.0080	0.1356
4385	0.0120	0.2056
4397	0.0090	0.1545
4961	0.0100	0.166
8789	0.0230	0.3695

Table-9 *HBV Viral loads by Real time PCR in MP-NAT MPX*

Bag No.	Viral load
13258/ 2012	17 IU/ml
3615/ 2013	20 IU/ml
4928/ 2013	<6 IU/ml
6984/ 2013	14 IU/ml
8538/ 2013	<6 IU/ml
8737/ 2013	6 IU/ml
9729/ 2013	<6 IU/ml
12939/ 2013	<6 IU/ml
61/ 2014	61 IU/ml
86/ 2014	<6 IU/ml
1866/ 2014	7 IU/ml
2248/ 2014	1002 IU/ml

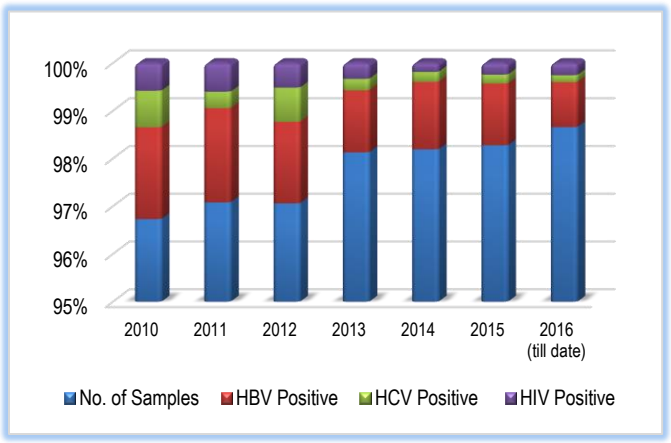

Fig-2 Seropositive cases by ELISA test

A Total of 51,627 donations were tested on Mini pool NAT of Roche Cobas. Of which 26,008 samples tested on MPX test and 25,619 donations were on MPX V2.0 test. Total 29 donors were detected on NAT screening which were negative on ELISA test. All NAT reactive donors were of HBV. No NAT reactive samples found for HIV and HCV. Out of 29 cases, 12 were detected by MPX test from 26,008 donations and 17 by MPX test V2.0 from 25,619 donations, providing NAT yield of 1:2169 and 1:1507 respectively [Table-2, 3]. Optical Density (OD) values of ELISA for NAT reactive samples were found to be much below from the signal cut off ratio [Table-4-8].

Discussion

In our retrospective analysis, we found that NAT yield of 1 in 2,167 for MPX and 1 in 1,507 for MPX V2.0 and all were of HBV. Total 29 donors found reactive on NAT who were negative on serology testing. No NAT reactive donors found for HIV and HCV. With consideration of three blood components from one blood donation, total 87 lives saved from getting infected from HBV.

As given in [Table-1], prevalence rate of HBV in blood donors is comparatively high than HIV and HCV. It is mainly due to HBV carriers, viral mutants, and occult HBV cases, which could not be detected in serological screening tests [16]. Additionally, blood donation during window period infection is another reason for high prevalence. Another alarming factor for HBV infection is it remains viable at least 7 days outside human body, on environmental surfaces at room temperature [17] and 37% cases the route of transmission in acute cases is unknown, and probably fomites play an important role of transmission [18]. With high populous country like India, these would lead to high prevalence of HBV.

No NAT reactive donor for HIV could be due to implementation of best screening practices at Dhanwantari Blood Bank, such as donor database, repeat non remunerative donors, thorough screening, health questionnaires and counseling for HIV positive donors to exclude such high-risk donors from donor list. Similar findings were observed for HBV and HCV with reduction in detected cases over the period of 5 years. The findings are in aligned with the prevalence data published by Khan *et al* on HBV (0.96%) and HCV (0.01%) in blood donors (13). Our study results are similar to results published by Jain *et al*. As compared to sero-prevalnce of 2.41%, Jain *et al* published the sero-prevalence rate of 2.62%. Combined NAT yield of their study was 0.034% (0.057% in our study) and all 8 cases NAT reactive were of HBV DNA (1:2972 donations) [19].

Other publication from India reported NAT yield of 156 cases from 35,722 donations in northern India, with high NAT reactivity (69.2%) for HBV. NAT yield for HBV was 1:627 in this study which was much higher to our results *i.e*. 1:1507 [20]. However, another study has shown much lower NAT yield for HBV (1 in 26,630) on in house MP-NAT probably due to lower sero-positive donor or low sensitivity of assay [21]. The NAT yield was improved after up gradation from MPX to MPX V2.0, detected higher number of HBV cases per number of donations. MPX V2.0 detects (LOD) as low as 2.3 IU/ml (95% limit of detection) viral load for HBV compared to MPX 3.8 IU/ml [22, 23], which is best in class available test to detect HBV at such low concentration. Improved yield was due to improved sensitivity to HBV. Our study showed that MP -NAT screening detects the low viral load for HBV, could be found in window period infections or inactive chronic hepatitis B infections or chronic carriers. OD values of ELISA tests for NAT reactive samples was much below the signal cutoff ratio, indicating the window period infection with low viral load which were undetected by the fourth generation ELISA. NAT due to high sensitivity could able to detect these donors at low viral load. With 4th generation ELISA or chemiluminiscence assay, the sensitivity would be expected to improve in detecting such cases [24,25].

In case of MP-NAT by MPX, the discrimination test was outsourced at Asian Institute of Gastroenterology, Hyderabad to know the viral pathogen and load by Real time PCR technology. Analysis of results shows all are HBV positive and viral loads are less than 200 IU/ml except one which is 1002 IU/ml. All these donors were in low viral load probably they may be in window period phase, or Occult hepatitis B infection. Transfusion of such blood or blood components would definitely produce overt hepatitis B infection in recipients due to high infective dose. In one case of viral load of 1002 IU/ml could be due to false occult hepatitis B infection. The ELISA test could not pick up the positive result of surface antigen and NAT is able to give positive reaction probably due to surface antigen mutation leading to viral mutants.

Conclusion

The challenges in India such as high prevalence rate of TTIs, blood supply - demand gap, low sensitive ELISA tests (3rd generation) warrants the use of mini pool NAT technology for effective and efficient blood screening to reduce the risk of TTIs. Implementation of centralized blood banking or mother banking equipped with MP-NAT enhances the safety standards to provide safe blood to the patients. It is happy to see that some States (Orissa, Karnataka) in India are in way to implement mother blood banking concept in NAT testing to achieve 100% Vein to Vein safe blood.

In countries with high population like India, the risk of TTIs is high due to high TTIs prevalence in blood donors and window period donations. The single ELISA test for TTI prevention is not 100% safe for transfusion. The addition of MP-NAT test is able to detect TTIs in window period, chronic carriers, chronic hepatitis cases, viral mutants and occult hepatitis B infections

Application of research: NAT should be made mandatory in blood bank screening to eliminate the burden of TTIs in India

Research Category: Medical Microbiology

Acknowledgement / Funding: Authors are thankful to Professor, Department of Microbiology, GSL Medical College & General Hospital, Rajahmundry, 533296, Dr. NTR University of Health Sciences, Vijayawada, 520004, Andhra Pradesh, India

****Principal Investigator or Chairperson of research: Dr Pragati Chigurupati**
University: Dr. NTR University of Health Sciences, Vijayawada, 520004, India
Research project name or number: Clinical study

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Dhanwantari Voluntary Blood Bank & Blood Components, Danavaipeta, Rajahmundry, 533103, Andhra Pradesh

Conflict of Interest: None declared

Ethical approval: Ethical approval taken from Department of Microbiology, GSL Medical College & General Hospital, Rajahmundry, 533296, Dr. NTR University of Health Sciences, Vijayawada, 520004, Andhra Pradesh, India.
Ethical Committee Approval Number: Nil

References

[1] Kaur P. and Basu P. (2005) *J Postgard Med*., 51,146-151.
[2] Morsi H.A. (2011) *Journal of the Egyptian Society of Hematology & Research*, 7(2), 1-5.
[3] Zou S., Stramer S.L., Notari E.P., *et al*. (2009) *Transfusion*, 49,1609-20.
[4] Zou S., Dorsey K.A., Notari E.P., *et al*. (2010) *Transfusion*, 50, 1495-504.
[5] http//www.saatm.org accessed on 27 October 2016.
[6] http//www.naco.gov.in as accessed on 27 October 2016.
[7] Comanor L., Holland P. (2006) *Vox Sang*, 91,1-12.
[8] Busch M.P., Glynn S., Stramer S., Strong D.M., Caglioti S. and Wright D. (2005) *Transfusion*, 45, 254-64.
[9] Leena M.S., Mohd. Shafee (2012) *Journal of Pathology of Nepal*, 2, 203-06.
[10] Shah N., Shah J.M., Javeeri P., Patel K., Shah C.K., Shah N.R. (2013) *Gujarat Medical Journal*, 68 (2), 35-39.
[11] Chandra T., Rizvi N.F. and Agarwal D. (2014) *Scientific World Journal*, 1-4.
[12] Sethi B., Kumar S., Butola K.S., Mishra J.P., Kumar Y. (2014) *Internet Journal of Medical Update*, 9(1),10-15.
[13] Khan M.I., Khan T., Vaghela M. (2015) *J Cont Med A Dent*, 3(2),17-21.
[14] Patel P. (2014) *Medical Journal of Dr. D.Y. Patil University*, 7(5), 620-24.
[15] Mahapatra S., Ray G. and Bhuyan P. (2016) *EC Bacteriology and Virology Research*, 2.1, 49-53.
[16] Said Z. N. A. (2011) *World Journal of Gastroenterology*, 17(15), 1927-1938.
[17] https//www.cdc.gov as accessed on 22 December 2016.
[18] Veldhuijzen I.K., Smits L.J., van de Laar M.J. (2005) *Epidemiol Infect*.,133(1),113-9.
[19] Jain R., Aggarwal P., and Gupta G. (2012) *International Scholarly Research Network ISRN Hematology*, Article ID 718671, 5 pages.
[20] Tulika Chandra S., Nishat F.R., Agarwal D. (2016) *International Journal of Contemporary Medical Research*, 3(6),1818-1821.
[21] Chandrashekar S. (2014) *Asian J Transfus Sci*., 8(1), 35-8.
[22] cobas® TaqScreen MPX Test, version 2.0-Information for Use document (Package insert)
[23] cobas® TaqScreen MPX Test-Information for Use document (Package insert)
[24] Bhattacharya P., Kaur R. (2013) *Asian Journal of Transfusion Science*, 7(2),107-108.
[25] Kim S., Kim J.H., Yoon S., Park Y.H., Kim H.S. (2008) *J Clin. Microbiol.*, 46(12), 3919-23.