A study on optimization of plasma pool size for viral infectious markers in Indian blood donors using nucleic acid amplification testing

Access this article online	
Website: www.ajts.org	Quick Response Code:
DOI: 10.4103/0973-6247.95056	

Sir,

To make transfusion therapy safe, nucleic acid amplification testing (NAT) may be started in India. But, implementation of NAT for every blood unit or individual donor screening may not be cost effective. Therefore, pooling of samples may be employed

for NAT. This study was planned to determine the optimum pool size to detect viral infectious markers, hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) in Indian blood donors by using NAT.

This study was conducted in a regional blood transfusion center of Western India; a total of 300 samples were collected only from voluntary repeat donors and tested for HIV, HBV, and HCV by enzyme linked immunosorbent assay (ELISA). Within 6 hours of collection, plasma was frozen.

Samples were divided in pool sizes, 16, 24, and 48. A total of 36 pools were prepared. Extraction of ribonucleic acid (RNA) for HIV and HCV as well as deoxyribonucleic acid (DNA) for HBV was carried out for and preserved at -30°C. This preserved DNA/RNA was used for NAT testing for HIV-1, HBV, and HCV. A total of 288 samples were pooled in 18 mini pools of size 16, 12 of size 24, and 6 of size 48. Following the qualitative real time NAT protocol, all specimens were tested and analyzed if amplification was present or not, using specific thermal cycle conditions. None of the mini pools showed amplification for any of the three viruses [Table 1].

For determination of appropriate plasma pool size for NAT, one positive sample with a viral load of 650 copies/ml for HIV, 500 copies/ml for HBV, and 5000 copies/ml for HCV was prepared. From one mini pool of 16 samples, 20 µl of plasma was taken out and 20 µl of prepared HCV positive sample having fixed viral load was added and tested by NAT. In the same way, sample having fixed viral load was added to a mini pool of 24 and 48 also and all the three diluted positive samples were tested by NAT [Table 2]. Similar procedure was done for HIV and HBV also. For testing real time NAT system, ABI PRISM 7000 and kits of Sacace Biotechnologies SRL, Como, Italy, for HIV, HBV, and HCV were used.

All these diluted positive samples were tested for HIV-1, HBV, and HCV by NAT. In case of HBV, only mini pools, 16 and 24 were showing amplification, but mini pool 48 showed no amplification. However, for HIV and HCV, all the three mini pools were showing amplification.

NAT screening of blood donors would be able to reduce the window period by 59, 25, and 11 days for HCV, HBV, and HIV-1, respectively. The remaining residual risk of transmitting one of these viruses can be reduced by 72, 42, and 50%, respectively, with NAT.[1] In the present study, for determination of appropriate plasma pool size for NAT, one positive samples with a viral load of 650 copies/ml for HIV, 500 copies/ml for HBV, and 5000 copies/ml for HCV were prepared. This viral load is present in preseroconversion stage; therefore, sensitivity of mini pool should be of this level.

Table 1: Cumulative results of all mini pools prepared from ELISA negative plasma samples

Pool size	Total no. of pools	No. of pools pos NAT for HBV	No. of pools pos NAT for HCV	No. of pools pos NAT for HIV
16	18	None	None	None
24	12	None	None	None
48	6	None	None	None

Table 2: Cumulative results of NAT on all diluted positive samples at different dilutions with fixed viral load

Pool size	HIV	HBV	HCV
16	Yes*	Yes	Yes
24	Yes	Yes	Yes
48	Yes	No**	Yes

*Yes: amplification seen, **No: No amplification seen

The doubling time for HBV DNA is estimated at a mean of 2.56-2.84 days. In other words, during the early stage of infection, HBV replicates rather slowly and reaches a viral load of 100-1100 or 720-11500 copies/ml before hepatitis B surface antigen (HBsAg) can be detected.[2] When NAT for HBV DNA was done with all the three diluted positive samples having a viral load of 500 copies/ml, only mini pools, 16 and 24 showed amplification. It is suggestive of that if mini pool NAT is applied for HBV DNA, pool size cannot be put higher than 24. Same mini pool size was found to be optimum by others.[3]

With the high titer viremic phase, HCV is very amenable to detection by pooled specimen NAT with pool size as large as 100 to 500 donations.[4,5] According to recommendation by Paul Ehrich Institute and US Food and Drug Administration, sensitivity of NAT for HCV RNA should be 5000 IU/ml. Following this recommendation, these numbers of copies were added to mini pools of 16, 24, and 48. All the three diluted samples were showing amplification. Therefore, it is quite safe to pool up to 48 sample size for screening of HCV RNA by NAT.

In the present study, 650 copies of HIV were added in dilutions of 16, 24, and 48 mini pools. All the three mini pools showed amplification in NAT. It gave an impression that again 48 samples could be pooled for detection of HIV-1 RNA without compromising on sensitivity. According to Busch MP and Kleinman SH,[6] estimated viral load for these periods were 10,000 copies/ ml. According to Roth et al.,[1] single donation testing would not add significant benefit to transfusion safety, but would increase the cost significantly. Present study supports the application of mini pool NAT with pool sample size 24. It would be cost effective as well as without compromising on safety.

Keeping in mind the high endemicity of transfusion transmitted diseases like HIV, HBV, and HCV in South Asian region, implementation of NAT has become necessary in developing countries of Asia. However, cost effectiveness also has to be evaluated before implementing any new test for transfusion services. Mini pool NAT with pool sample size of 24 will be optimum as well as a cost effective approach for implementation of NAT. It is also recommended that for large plasma sample screening for viral infection among blood donors in fractionation industry, 24 pool sizes may be safely used. Though this study was on a small sample size, but it gives a view towards mini pool NAT testing and the size of mini pool. Certainly, it can be commented that it will improve the safety of blood transfusion as well as will be cost effective for developing countries like India. A large multi centric study is recommended to consolidate this fact.

Ankit Mathur, Jwalant Shah[1], Ripal Shah[2], Priti Shah[3], V. Harimoorthy[1], N. Choudhury[4], Sunita Tulsiani[5]

Rotary TTK Blood Bank, Bangalore, [1]Prathama Blood Center, Vasna, Ahmedabad, [2]Gujarat State Blood transfusion Council,

Ahmedabad, [3]Tata Memorial Hospital, Parel, Mumbai,
[4]Tata Memorial Center, Kolkata, [5]Jeevan Jyothi Blood Bank,
Nagpur, Maharashtra, India

Correspondence to: Dr. Ankit Mathur,
Rotary Bangalore TTK Blood Bank,
Bangalore Medical Services Trust, HAL III stage,
Bangalore – 560 075, India.
E-mail: ankit_avni@yahoo.com

References

1. Roth WK, Buhr S, Drosten C, Seifried E. NAT and viral safety in blood transfusion. Vox Sang 2000;78:257-9.
2. Fang CT. Blood screening for HBV DNA. J Clin Virol 2006;1 suppl 36:30-2.
3. Kleinmen SH, Strong DM, Holland PV. Hepatitis B DNA screening of blood donation by minipool NAT with COBAS Ampliscreen HBV test. Transfusion 2005;45:1247-57.
4. Busch MP. HIV, HBV and HCV: New developments related to transfusion safety. Vox Sang 2000;78:253-6.
5. Lefrere JJ, Coste J, Defer C, Mercier B, Ferec C. Screening blood donations for viral genomes: Multicenter study of real time simulation using pooled samples on model of Hepatitis C virus detection. Transfusion 1998;38:915-23.
6. Busch MP, Kleinman SH. Nucleic acid amplification testing of blood donors for transfusion transmitted infectious diseases. Transfusion 2000;40:143-58.