Nucleic acid testing-benefits and constraints

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Nucleic acid testing (NAT) is a molecular technique for screening blood donations to reduce the risk of transfusion transmitted infections (TTIs) in the recipients, thus providing an additional layer of blood safety. It was introduced in the developed countries in the late 1990s and early 2000s and presently around 33 countries in the world have implemented NAT for human immunodeficiency virus (HIV) and around 27 countries for hepatitis B virus (HBV).[1] NAT technique is highly sensitive and specific for viral nucleic acids. It is based on amplification of targeted regions of viral ribonucleic acid or deoxyribonucleic acid (DNA) and detects them earlier than the other screening methods thus, narrowing the window period of HIV, HBV and hepatitis C virus (HCV) infections. NAT also adds the benefit of resolving false reactive donations on serological methods which is very important for donor notification and counseling. In a recent Malaysian study[2] 1388 donor samples were tested by serology as well as NAT, authors found 1.37% samples reactive on standard serology methods but non-reactive by NAT. These samples were confirmed to be "false reactive" on confirmatory serological tests.

NAT for HCV was first introduced in Germany in 1997 and it was performed on pooled samples of 96 blood donations (Minipool NAT [MP-NAT]).[3] Later on, as other countries adopted this technique there was a progressive decrease in pool size to 16, 8 or 6 donation samples. MP-NAT may have the advantage of being cost-effective, but there are some limitations. The whole size of pooled blood donations is blocked until the NAT report is available. Moreover, as viral nucleic acid concentration gets diluted in the large pool of samples, the sensitivity of NAT might decrease and if a pool is tested reactive, the whole pool requires resolution to identify the single positive unit and this process requires an additional step of handling, additional time for testing and hence delay in the release of units. NAT is also available for testing each donation individually (ID-NAT). This format of NAT seems more sensitive as shown by data from many studies where ID-NAT has been compared to MP-NAT with pools of 16 or 8 or 4 samples[4,5] and if a unit is ID-NAT reactive, donation number is identifiable for performing discriminatory test. That single unit is removed from the inventory, releasing other units on time. However, this has limitations of higher cost. In a study conducted in United States, it was seen that over a 10-year period, approximately 66 million donations were screened with 32 HIV (1:2 million) and 244 HCV (1:270,000) NAT yield donations identified. HCV prevalence among first time donors decreased by 53% for 2008 compared with 1999.[6] The introduction of HBV NAT in the United States, along with the HBV vaccination policy made a measurable contribution to blood safety and decreased residual risk of HBV infection.[7] In United Kingdom, NAT has reduced the risk of HCV by 95% and that of HIV by 10%.[8] The American Red Cross implemented automated triplex NAT for HIV, HCV and HBV in June 2009. They analyzed their results of the initial year of testing and found that the yield of MP-NAT (MP16) had little measurable impact on blood safety in detecting seronegative donations.[9] Other studies showed that sensitive, ID-HBV NAT detects significantly more DNA-positive, hepatitis B surface antigen (HBsAg)-non-reactive donations than MP-NAT, regardless of the anti-hepatitis B core status of the donor.[10,11] In a pilot study of 18 months from China, ID-NAT was compared with enzyme immunoassays. It was observed that HBV yield rate in their population is 1:1056 for blood donations.[12] In a study from Egypt 5 window period HCV donations were identified among 15,655 1st time donors (yield 1:3100).[13] NAT screening may thus prove to be more beneficial where the seroprevalence of transfusion transmissible infectious agents is high, as is the case in most developing countries.

In India, mandatory blood screening for HBV, HIV and HCV is done by serological tests for HBsAg and antibodies to HIV 1/2 and HCV. The screened seronegative donations are still at risk for TTIs and thus, need for a sensitive screening test arises to decrease this residual risk which has been reduced significantly over the last two to three decades in western countries where NAT has been implemented. NAT testing has been started in few centers in India, but it is not a mandatory screening test for TTIs as per Drug and Cosmetics Act, 1940 and the rules therein.[14] Major barriers in implementing routine NAT testing in India is its high cost and lack of technical expertise in most of the blood centers.

In the present issue of the journal three articles on NAT - one review and two original studies highlight the current dilemma for India. The review article by

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Shyamala draws attention to the high seroreactivity of HBV, HCV and HIV in multitransfused thalassemic patients which indicates window period transmission of TTIs by seronegative units. A case for ID-NAT testing has been put forward. The article by Chatterjee et al. has compared the sensitivity of ID- and MP-NAT testing as assessed by dilution of NAT yield samples. The authors observed that samples with high viral load were detected by all dilutions, but 67% of samples of low viral load are missed by MP-NAT and concluded that ID-NAT is ideal methodology for TTI screening. In a study by Shivaram seronegative samples were tested by in-house MP-NAT, using reverse transcriptase-polymerase chain reaction over a period of 5 years and found a nominal increase in cost per test. Their NAT yield was 0.0006%, low as compared to studies from other parts of India. However, the analytical sensitivity of the in house MP-NAT had not been established and the results may not represent the true yield.

In India blood centers are gradually introducing NAT to provide safe blood to their patients. First multicenteric study was done by Makroo et al.[15] where a total of 12,224 samples along with their serological results were obtained from eight blood banks in India and were tested individually manually by procleix ultrio assay for HIV 1, HCV and HBV. They observed eight NAT yield cases. According to a study from the western part of India combined NAT yield (NAT reactive/seronegative) for HIV, HCV and HBV was 0.034% (1 in 2972 donations)[16] which is high when compared to studies from developed countries. In another study conducted in north India, 18,354 donors were tested by both ID-NAT and fourth generation enzyme-linked immunosorbent assay (ELISA), 7 were found to be NAT-positive but ELISA-negative (NAT yield) for HBV and HCV. The prevalence of NAT yield cases among routine donors was 1 in 2622 donations tested (0.038%).[17] This high yield of NAT is due to the high prevalence of TTIs in India, further highlighting the need for NAT in India. In another study from a tertiary care center from north India ID NAT results were compared to serological method for 73,898 samples, 1.49% were reactive by NAT, HIV-1 (0.09%), HCV (0.25%), 1.05% were reactive for HBV only and around 0.08% were HBV-HCV co-infections with a combined yield of 1 in 610 donations (total 121 NAT yields).[18]

NAT is a highly sensitive and advanced technique which has reduced the window period of HBV to 10.34 days, HCV to 1.34 days and HIV to 2.93 days[19] but it is highly technically demanding, involving issues of high costs, dedicated infrastructure facility, equipments, consumables and technical expertise. The need for NAT depends on the prevalence and incidence rate of infections in blood donor population, available resources and the evidence of benefit added when combined with serology tests. Hence the decision of starting NAT should be considered when basic quality assured blood transfusion system is already in place such as volunteer base for blood donation, provision of donor self-deferral, donor notification and counseling along with quality assured sensitive serological methods for testing TTIs.

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