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Review Article

Role of Nucleic Acid Amplification Testing in Blood Banks: Additional Measure for Blood Safety

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ABSTRACT

Introduction: Blood safety is a challenging task as millions of people worldwide receive blood transfusion or blood-derived products annually. The Risk of transfusion transmitted infection (TTI) with blood borne diseases such as human immunodeficiency virus (HIV), hepatitis B virus (HBV)and Hepatitis C virus (HCV) still exists even though testing and policy decisions have combined to make blood supplies in many countries among the safest in the world. Nucleic Acid Testing (NAT) shortens this window period, thereby offering blood centers a much higher sensitivity for detecting viral infections.
Objective: To understand the role of nucleic acid testing in blood banks as an additional measure for blood safety
Result and conclusion: There is sufficient evidence to support the effectiveness of using NAT as a screening test for detection of HIV, HBV and HCV in donated blood. Nucleic acid testing can help in preventing transfusion of infected unit and thus providing safe blood to the patient.

Key words: Transfusion transmitted infection, Nucleic Acid Testing, window period

METHOD

A systemic and methodical search of literature using databases published in respected peer reviewed journals was employed for selection and review in preparing this review article.

INTRODUCTION

Millions of people worldwide receive blood transfusions or blood-derived products. Every year there are about more than 92 million blood donations take place around the world. [1] Separated components can be transfused up to three people from a single whole-blood donation and to hundreds of patients from blood-derived products.

The strategy for attaining reduction in the incidence of TTI is to improve donor’s selection, test the donated blood with more sensitive serological tests, reduce exposure to allogeneic blood and provide guidelines for a robust system to achieve conservative and effective use of blood, [2,3] but transmission of diseases still occurs through transfusion and zero risk blood still is a far sight. There are several factors that can be attributed for this transmission viz inability of the test to detect the disease in the pre-seroconversion or ‘‘window’’ phase of their infection, immunologically variant viruses, non-sero converting chronic or immuno-silent carriers and laboratory testing errors. [4]

Nucleic acid testing technique is highly sensitive and specific for viral nucleic acids. It is based on amplification of targeted regions of viral ribonucleic acid

(RNA) or deoxyribonucleic acid (DNA) and detects them earlier than the other screening methods thus narrowing the window period of HIV, HBV and HCV infections. This test also adds the benefit of resolving false reactive donations on serological methods which is very important for donor notification and counseling.

Although it requires a significant investment in equipment, training, and infrastructure, the development of these molecular techniques as diagnostic tools has become increasingly important in the present day scenario. [5-9]

Nucleic acid testing:

It is a highly sensitive method of testing blood that is used to detect HIV, HBV and HCV. It detects very low levels of viral genomic material that is present soon after the infection before the body starts producing antibodies in response and thus reduces the window period (WP). The estimated reduction of the WP utilizing NAT for HCV is 70 to 12 days, HIV from 22 to 11 days and HBV from 59 to 25-30 days. [10]

Genomic screening for infectious agents using NAT is performed with several in-vitro nucleic acid amplification techniques, transcription mediated amplification (TMA), polymerase chain reaction (PCR) ligase chain reaction and nucleic acid sequence based amplification. Rapid progress and constant improvements in nucleic acid amplification technologies have resulted in a remarkable development in the molecular diagnosis and characterization of viral infections. The analytical sensitivity of nucleic acid testing in sequence specific detection of viral genomes (DNA or RNA) is several times greater than that of antigen detection or virus isolation methods.

Real-time PCR:

Nucleic acid quantification using quantitative real-time PCR (qRT-PCR) can be monitored in real time and is extremely accurate and reproducible. It requires an instrumentation platform that consists of a thermal cycler, a computer, optics for fluorescence excitation and emission collection, and data acquisition and analysis. [11-13]

Many studies have designed and developed a one-step NAT TaqMan (Thermo fisher Scientific USA) qRT-PCR method for the detection and quantification of HCV, HIVRNA and HBV DNA. These studies have shown varying range of lower limit detection of RNA copies, it was 310IU/ml for HCV,100IU/ml for HIV and 200IU/ml for HBV by Albertoni et al.[14] 500 IU/ml for HCV by Wendel et al.,[15] 100 copies/ml for HBV by Yalamanchili et al.[16] An in-house multiplex RT-PCR assay for the detection of HCV in plasma samples designed and developed by Paryan et al. had an analytical sensitivity of 200 copies/ml for HCV and 100IU/ml for HIV. [17]

Current approaches:

Several US Food and Drug Administration (FDA)-licensed NAT assays are currently available for the screening of blood donors for HIV, HCV, and HBV. The continued development of highly sensitive screening NAT systems, however, is challenging. [18] The FDA has licensed several triplex (HIV/HCV/HBV) automated NAT systems employing transcription-mediated amplification (TMA) for blood donor screening. These include the PCR-based cobas Taq Screen MPX assay using the cobas s 201 instrument (Roche Diagnostics GmbH, Mannheim, Germany), and the Procleix Ultrio assay, using the Procleix Tigris automated instrument (Novartis Diagnostics, Emeryville, CA, USA/ Gen-Probe, San Diego, CA, USA) [19-23]

NAT assays either be performed on individual donations (ID) or on Mini pools (MP) to detect the nucleic acid of the infectious agent. However, considering the cost of NAT and challenges involved in automation, several users have developed strategies based on pooling of multiple donor samples. [24] The drawback of this economic approach was reduced assay sensitivity and this type of transmission can

be avoided by NAT applied to single unit testing. To increase the sensitivity of the test the pool size was progressively decreased from 512 to 96, 36, 24, 16, 8 and finally 6, while many blood centers opted for individual donation screening.

Nucleic acid testing in resource-limited areas is difficult because of its high investment cost for instruments, high running cost for reagents, a lack of maintenance support, the need for cold-chain transport and storage of reagents. However in developed countries with usually low viral infection prevalence, NAT shows limited yield resulting in a clinical risk reduction benefit associated to an extremely low cost-effectiveness. [25] This finding initiated the development of several options intended to reduce the cost of NAT. Two non-mutually exclusive approaches have been mainly adopted. First, testing for viral genomes in plasma pools of various sizes (6 to 96 plasmas) rather than in individual donations, but with the disadvantage of reduced sensitivity

Several instances of infectious donations have been reported not detected by MP testing but reactive when tested with ID NAT. [26,27] The risk of false-negative result can be partially reduced in some cases by introducing additional procedures to concentrate viral particles in samples (e.g. ultracentrifugation of pooled plasmas prior to nucleic acid purification and increased sample volume). [28]

The second approach is to develop multiplex assays that will be able to simultaneously detect, and eventually directly identify, three or more nucleic acid targets in a single reaction. Multiplexing reduces the reagent costs, the volume of sample to process, and the time required to obtain results, but at the same time considerably complicate the already multi-step and delicate methods developed for single virus nucleic acid testing. [29] This increases the sensitivity of the test. Although single or multiplex assays initially developed in-house have been generally replaced by fully automated and relatively expensive commercial platforms/assays, they might still constitute a reliable and affordable alternative.

Effectiveness of NAT in detection of HIV, HBV and HCV:

Around the world, more than 53 million units of blood are screened with NAT annually, in USA hundred percent of blood supply is screened with NAT for HIV-1, HBV, HCV and West Nile virus. [30] Although the yield of NAT-only units is modest relative to the yield of serological screening, the infectivity of viremic donations detected by NAT (with or without detectable serological markers) is very high. Hence, the relative impact of NAT screening is arguably greater than that of serological screening, although the existence of seropositive but NAT-negative donations indicates that serological screening must be maintained even with the most sensitive NAT testing performed on individual donations. [31]

A study conducted in India by Jain et al. found that out of the randomly selected 23,779 donor units which were negative for HIV, HCV, and HBV by Enhanced Chemiluminescence immuno-assay (ECi), 8 turned out to be NAT yield (NAT reactive/sero negative). All the 8 reactive samples were positive for HBV DNA and the HBV viral load was ≥12 IU/mL (95% lower limit of detection, 12 IU/mL with 5.82 copies per IU conversion factor). [32] None was reactive for HIV and HCV. This was compared with the HBV NAT yield in developed countries like USA and Europe where prevalence rate is 1:600,000 to 1:350,000donations, in developing countries it was 1:52303 in South Africa, 1:4868 in Thailand, 1:24275 in Kuwait, 1:232 in Ghana, 1:2609in Egypt, 1:501 in Lebanon and 1:125 in Iran. [33]

In China, Shan et al. conducted a study on serologic-negative donor samples for HCV and HIV with NAT. They found that NAT yield three HCV cases and none for HIV-1. The yield rate for HCV NAT was 3.4 per10^5. The estimated incidence rate for HCV is 24.2 per 100,000 person-years.

Thus, if MP NAT is added to routine donor screening, the residual risk for HCV is estimated to be reduced to 1in 20.4. [34]

Forcicet al. conducted a study from a blood center in Croatia on 2,647 blood samples from blood center in Croatia which were screened using NAT for HCV. It was found that out of the 2,647 plasma tested, the NAT yielded 12 HCV RNA positive, but were antibody negative. [35]

In South Africa, a study carried out by Vermeulenet al. looked at the impact of ID NAT testing after one year implementation. They found that the HIV, HBV, and HCV ID-NAT window phase yielded rates were 1:45,765, 1:11,810, and 1:732,200, respectively. The residual transmission risk of ID-NAT HIV, HBV, and HCV window phase donations were estimated at 1:479,000, 1:61,500, and 1:21,000,000 respectively. [36]

A study conducted in Switzerland by Stozlet al. found that from the 306,000 blood donations screened, 31 were NAT test reactive and confirmed HBV infected. Of these 24 and 27 were HBsAg and anti-HBc positive, respectively. The study also identified seven HBV-NAT yields, two pre-HBsAg window period donations and five occult HBV infections. The introduction of ID-NAT reduced the risk of HBV window period transmission in repeat donors from 1in 95,000 to 1in 296,000. Thus it was concluded that NAT screening reduced the HBV window period transmission risk approximately threefold. [37]

Nüblinget al. conducted a study on a total of 873 plasma pools from 1996 batch of samples and 331 plasma pools from 2006 batch of samples. They analyzed for the detection of HCV RNA, HIV RNA, and HBV DNA with NAT and found that the plasma pools from 1996 detected 17.8% of HCV RNA, 0.8% of HIV-1 RNA and 0.5% of HBV DNA. Whereas, among the pools from 2006, one pool (0.3%) was found HCV RNA-positive at low titer (<10 IU/mL) and no HIV RNA or HBV DNA was detectable in any pools. It was concluded that the introduction of NAT led to marked reduction of NAT-positive plasma pools and there is a marked decrease in the frequency of NAT-positive plasma pools. [38]

Demerits of NAT:

It requires a significant investment in equipment, training and infrastructure. Technologist will need to be trained in molecular biology techniques, handling of complex new data management systems for sample pooling, resolution and supplemental testing of reactive pools.

False negative results with NAT- have also been observed, Delwartet al. [39] have reported transmission of HIV-1 from NAT negative donations with low viral load. Foglieni et al. [40] have commented that the increased heterogeneity of the HIV virus could have led to the false negative results thereby affecting the safety of blood supply as well as the diagnosis and patient management.

False positivity with NAT- The false positive rate (i.e. unconfirmed NAT reactive donations) in a study carried out by Stramer et al. [41] was 1 in 15,800 units. In India, a report by Makroo et al. [42] revealed that 27 samples out of 12,224 were Ultrioassay NAT reactive but negative on discrimination test. In a study published by Stramer that tested samples for HIV and HCV NAT, there were 193 donations that were reactive for NAT; of these 92.2% were false positive. Kleinman et al. reported that out of 23 HBV DNA positive, HBsAg, and antibodies to hepatitis B core antigen negative, only two were confirmed positive resulting in 91.3% false positive rate. [43]

The reason for these false positives has been best explained by Pisani et al. in their study, which states that false positive results with external proficiency samples of NAT are often attributed to the cross-contamination. [44]

CONCLUSION

To get the best results, implementation of quality management, good manufacturing practices and proficiency testing are of paramount

importance. The number of false positives could certainly go high if stringent quality measures and standards of cleanliness are not observed, thereby further contributing not only to blood shortage but also adding to the cost.

Cost benefit analysis needs to be done by the countries before implementation especially in low endemic areas and resources limited areas.

Nucleic acid testing can serve as a valuable additional tool to reduce transfusion transmitted infections in more and more countries and achieve motto of hundred percent safe bloods.

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