Original Article

Automated nucleic acid amplification testing in blood banks: An additional layer of blood safety

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Abstract:

Context: A total of 30 million blood components are transfused each year in India. Blood safety thus becomes a top priority, especially with a population of around 1.23 billion and a high prevalence rate of human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) in general population. Nucleic acid amplification testing (NAT) in blood donor screening has been implemented in many developed countries to reduce the risk of transfusion-transmitted viral infections (TTIs). NAT takes care of the dynamics of window period of viruses and offers the safest blood pack for donation. **Aims:** The aim of this study is to show the value of NAT in blood screening. **Settings and Design:** Dhanavantari Blood Bank, Rajahmundry, Andhra Pradesh, India. **Subjects and Methods:** Over a period of 1 year from January 2012 to December 2012, a total number of 15,000 blood donor samples were subjected to tests for HIV, HBV, and HCV by enzyme-linked immunosorbent assay (ELISA) method and 8000 ELISA nonreactive samples were subjected for NAT using multiplex polymerase chain reaction technology. **Results:** Of the 15,000 donors tested, 525 were seroreactive. In 8000 ELISA negative blood samples subjected to NAT, 4 donor samples were reactive for HBV. The NAT yield was 1 in 2000. **Conclusions:** NAT could detect HIV, HBV, and HCV cases in blood donor samples those were undetected by serological tests. NAT could interdict 2500 infectious donations among our approximate 5 million annual blood donations.

Key words:

Hepatitis B virus, hepatitis C virus, human immunodeficiency virus, nucleic acid amplification testing, transfusion-transmitted infection

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Introduction

Annually, millions of people worldwide receive blood transfusions or blood-derived products. Although testing and policy decisions have combined to make blood supplies in many countries among the safest in the world, there still exists some risk of transfusion-transmitted infection (TTI) with blood borne diseases such as human immunodeficiency virus (HIV), hepatitis B and hepatitis C. Laboratory screening of donated blood and blood products for infectious diseases is a key safety measure in protecting patients and preventing the spread of serious diseases. The traditional method for screening blood donations, known as immunoassay (or serology) testing is the mainstay for screening blood donors. Immunoassays detect antibodies to viruses or viral antigens. With immunoassays; however, there is an interval between the donor's exposure to a virus until antibodies against the virus are produced, known as the "window period (WP)."[1] It is during this period that the risk of infection in donated blood can be missed by the immunoassay testing. These undetected WP infections are responsible for most of the transfusion transmission of these viruses. Nucleic acid amplification testing (NAT) shortens this WP, thereby offering blood centers a much higher sensitivity for detecting viral infections.

Subjects and Methods

This study was carried out in Dhanavantari Blood Bank located in the city of Rajahmundry, Andhra Pradesh, India. Over a period of 1 year from January 2012 to December 2012, a total number of 15,000 blood donor samples were subjected to screening for HIV, hepatitis B virus (HBV) and hepatitis B virus (HCV) by using fully automated enzyme-linked immunosorbent assay (ELISA) processor of Bio-Rad–Evolis. The tests performed were HBV surface antigen (HBsAg) to detect HBV, anti-HCV to detect HCV, 4th generation ELISA kits to detect p24 antigen and glycoprotein antibodies against HIV, slide examination using Field's stain to screen malarial parasites and syphilitic reagin antibodies detection employing the RPR technique.

During the same period, 8000 ELISA nonreactive samples were subjected for NAT. At the time of blood donation, about 7 ml of blood was collected directly into EDTA vacutainer under strict sterile conditions. Plasma was separated and used for testing. The system developed by Roche using the multiplex polymerase chain reaction (PCR) technology was used.

Two systems available to carry out NAT are the multiplex PCR technology of Roche and the

transcription mediated amplification (TMA) of Novartis. The system which wase used was multiplex PCR technology by Roche, which involves the use of separate tests for each viral genome to be detected. Testing involves 5 major steps: Initially, plasma is ultracentrifuged to concentrate viral particles. Reverse transcriptase of the target RNA is added to generate complementary DNA. Once that step is completed, amplification of target complementary DNA is achieved by PCR using bio specific complementary primers. The amplified products are then hybridized to oligonucleotide probes specific to the target, and the probe-bound amplified products are then detected by colorimetric measurement. The detection of amplified DNA is performed by using avidin-horseradish peroxidase. The entire test process takes approximately 6 hours. In comparison to the TMA of Novartis, the multiplex PCR technology of Roche is fully automated, user friendly, contamination free and processes pooled samples.[2]

Results

Of the 15,000 blood donors screened for HIV, HBV, and HCV, 525 were seroreactive (3.5%). Of these, 330 (2.2%) were HBsAg reactive, 75 (0.5%) were anti-HCV reactive , and 150 (0.8%) were anti-HIV reactive . The combined yield (seronegative/NAT reactive) for HIV-1, HCV, and HBV was 4 out of the 8,000 samples tested (0.05%) and included only HBV.

Discussion

Blood safety is a challenge in India because of the high prevalence of HIV, HCV, and HBV, the relatively low percentage of volunteer donors and the lack of standardization of screening procedures among the multitude of blood collection centers. The prevalence of HIV, HBV, and HCV in India is respectively about 1%, 4%, and 1.5%[3] compared to 0.0097%, 0.3%, and 0.07% in the US blood donors, respectively.[4] Blood screening in India is performed by three groups: Regional governments, private hospitals, and nongovernment organizations. In spite of all the precautionary measures used by blood-collecting agencies to avoid inoculating infectious agents into transfusion recipients, it is possible to transmit disease when blood from a recently infected donor fails to be identified by routine screening tests.[5] This is because of the so-called WP after a donor is infected, but before the condition is detectable by routine methods. This technological limitation puts blood recipients at a definite though infrequent risk for transmissible diseases. Since viremia precedes seroconversion by several days to weeks, tests that detect viral nucleic acids are considered a significant technological advancement and an additional step in our quest to achieve the goal of zero risk for blood transfusion recipients.

Nucleic acid amplification test (NAT) technologies have the potential to detect viremia earlier than current screening methods, which are based on seroconversion. NAT is a molecular technique to detect viral nucleic acids of HIV 1-2, HBV, and HCV at a very low concentration in donor blood by Nucleic acid amplification technology.[6] The primary benefit of NAT is the ability to reduce residual risk of infectious WP donations. The estimated reduction of the WP utilizing NAT for HCV is 70-12 days, HIV from 22 to 11 days, and HBV from 59 to 25-30 days.[7] The residual risk for HCV transmission prior to NAT was 0.64/million in France and 3.94/million in Spain which decreased to 0.1/million and 2.33/million, respectively after NAT was adopted. HIV NAT yield rates were estimated at 0.3/million donations in France and Spain as opposed to 0.59 and 2.48, respectively preceding NAT.[8]

The performance of the NAT assay is essentially dependent on its analytical sensitivity. The analytical sensitivity is generally determined by testing dilutions of standardized materials such as WHO International Standards and subsequent calculations of 95% limit of detection by probit analysis. The analytical sensitivity for HBV NAT is particularly important due to its long doubling rate of 2.6 days during which the viral count is generally low. In comparison, the HCV and HIV-1 doubling rate of 14.9 h and 20.5 h, respectively are shorter. The WHO international standards analytical sensitivity for HIV-1, HCV, and HBV is 44, 10.7, and 3.7 IU/ml.

The NAT yield of 4 in 8000 in our study assumes more significance when one considers the fact that single donation is used for generating 3 components that can be used by 3 recipients. Hence, in effect the NAT yield becomes 3 times that is, 12 in 8000. Saving 12 recipients from TTI out of 8000 (0.6%) is indeed very significant. Similar studies in other countries have also demonstrated high yields.[9-14] The most recent study done in India have noted a NAT yield of 1 in 650.[15]

Studies on the feasibility of NAT implementation in developing countries like India will help extend the message to blood centers that NAT can be an effective method for safeguarding the blood supply. The potential for NAT yield in India is staggering when compared to other countries that have already implemented the technology, especially when one takes notice of the high number of carriers of HIV (2.5 million), HBV (43 million), and HCV (15 million) as per the statistics provided by the NACO (national AIDS control organization) India.[16-18]

Conclusion

NAT technology has revolutionized the ability of blood banks to efficiently test for and intercept potentially infectious pathogens while maintaining on-time blood availability for patients and hospitals. As reducing the rate of TTI gains momentum in more and more countries, NAT can serve as a valuable tool to achieve the motto of national blood safety organization of “vein to vein 100% safe blood.”

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