

Advancements in Blood Donor Screening: A Comparative Study of Molecular vs. Serological Testing for Pathogen Detection

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Abstract

Background: Transfusion-transmissible infections (TTIs) remain a significant concern for blood safety, particularly in resource-limited settings. Nucleic acid testing (NAT) can detect infections during the window period before seroconversion, offering advantages over conventional serology. This study compared the efficacy of serological and molecular methods for detecting TTIs among blood donors in Bangladesh.

Methods: A cross-sectional study was conducted at the Department of Transfusion Medicine, Shaheed M. Monsur Ali Medical College, Sirajganj, from January to December 2025. A total of 150 blood donors were enrolled through consecutive sampling. Blood samples were tested using both serological (ELISA for HBsAg, Anti-HCV, Anti-HIV, and malaria antigen) and molecular (real-time PCR for HBV DNA, HCV RNA, and HIV RNA) methods. Window period infections were identified as serology-negative but NAT-positive cases. Data were analyzed using SPSS version 26.

Results: The study population was predominantly male (92.0%) and young adults (mean age 31.5 years). Voluntary donors comprised 65.3% of the sample. Overall TTI prevalence was 15.3% (23/150), with HBV most common (8.0%), followed by HCV (4.0%) and HIV (2.7%). NAT detected 25% more infections than serology (20 vs. 16), with significant differences for HBV ($p=0.023$), HCV ($p=0.045$), and HIV ($p=0.032$). Seven window period infections (4.7% of donors) were detected by NAT but missed by serology, including 3 HBV, 2 HCV, and 2 HIV cases. Serology demonstrated poor sensitivity (65.0%) but excellent specificity (99.3%). McNemar's test confirmed significant overall difference between methods ($p=0.023$), with substantial agreement ($\kappa=0.78$).

Conclusion: NAT detects significantly more TTIs than serology alone among Bangladeshi blood donors, with 7 window period infections identified that would have been missed by routine screening. Serology misses 35% of active infections, leaving unacceptable residual risk. These findings support phased implementation of NAT to improve blood safety in Bangladesh.

Introduction:

Blood transfusion is a critical life-saving therapeutic intervention performed worldwide in various clinical settings such as surgery, trauma, hematological disorders, and obstetric emergencies. Each year, millions of blood transfusions are conducted globally, saving countless lives and improving patient

outcomes. However, despite its undeniable benefits, blood transfusion carries inherent risks, with transfusion-transmissible infections (TTIs) posing a significant concern for blood safety globally.¹ TTIs are caused by pathogens that are transmitted from donor to recipient through contaminated blood or blood products. The most clinically significant TTIs

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include hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), syphilis, and malaria.² These infections present serious public health challenges, especially in developing countries where the prevalence of these pathogens is often higher, and resources for screening are limited.³ The consequences of TTI transmission can be devastating, leading to chronic liver disease, cirrhosis, hepatocellular carcinoma, acquired immunodeficiency syndrome (AIDS), and even death. To mitigate these risks, the World Health Organization (WHO) recommends comprehensive screening of all donated blood for at least HBV, HCV, HIV, and syphilis as a minimum requirement for blood safety⁴. Despite these recommendations, the risk of TTI transmission through blood transfusion has not been completely eliminated.

A significant factor contributing to this residual risk is the “window period” the time between infection and the appearance of detectable antibodies or antigens. During this window, an infected donor may test negative using conventional serological methods but still harbor infectious pathogens⁵. Serological testing, including enzyme-linked immunosorbent assay (ELISA) and rapid diagnostic tests, has been the cornerstone of blood donor screening for decades. These methods detect specific antibodies produced by the immune system in response to infection or, in some cases, viral antigens. While serological assays are cost-effective, easy to perform, and suitable for high-throughput screening, they have significant limitations⁶. The most critical limitation is the diagnostic window period, which can range from several days to weeks depending on the pathogen. During this time, serological tests may remain negative despite the presence of active infection, potentially allowing contaminated blood to enter the blood supply⁷. Additionally, serological tests may yield false-negative results in immunocompromised individuals who fail to mount an adequate antibody response, and false-positive results due to cross-reactivity⁸. In recent years, nucleic acid testing (NAT) has emerged as a powerful alternative for blood donor screening.

NAT detects the genetic material (DNA or RNA) of pathogens directly, significantly reducing the window period compared to serological methods⁹. For instance, the window period for HIV can be reduced from approximately 22 days with serology

to just 5-10 days with NAT. Similarly, for HCV, the window period can be shortened from 70 days to 3-5 days, and for HBV from 50 days to 20-25 days¹⁰. This dramatic reduction in the window period has the potential to prevent thousands of TTI transmissions annually. Several developed countries, including the United States, Germany, and Japan, have already implemented NAT screening for blood donations, either partially or universally. Studies from these countries demonstrate that NAT implementation improves blood safety by detecting window-period infections that would otherwise go unnoticed^{11,12}. However, in resource-limited settings like Bangladesh, the implementation of NAT remains restricted due to high costs, technical complexity, and infrastructure requirements¹³. Most blood banks in Bangladesh continue to rely on serological screening, which could leave gaps in blood safety.

The country bears a high burden of viral hepatitis and other TTIs. Studies have reported HBV prevalence among blood donors ranging from 5.4% to 7.5%, while HCV prevalence ranges from 0.6% to 2.2%^{14,15}. Although HIV prevalence is low in the general population, it is rising among high-risk groups. Despite the substantial TTI burden, there is limited data on the comparative performance of serological and molecular (NAT) methods in Bangladesh. The primary aim of this study is to assess the prevalence of transfusion-transmissible infections (TTIs) such as HBV, HCV, HIV, and malaria among blood donors at Shaheed M. Monsur Ali Medical College, using both serological and nucleic acid testing (NAT) methods. Additionally, the study seeks to identify infections that are detectable exclusively by NAT during the window period. A comparative analysis will be conducted to evaluate the effectiveness of serological screening against NAT, with NAT serving as the benchmark. Finally, the research aims to explore the potential benefits of implementing NAT in improving overall blood safety and reducing the risk of TTI transmission in resource-limited settings like Bangladesh.

Methods:

This was a cross-sectional study conducted to compare the efficacy of serological and molecular (Nucleic Acid Testing, NAT) methods for detecting transfusion-transmissible infections (TTIs) in blood

donors. The study was carried out at the Department of Transfusion Medicine, Shaheed M. Monsur Ali Medical College, Sirajganj, Bangladesh, over a one-year period from January 2025 to December 2025. A total of 150 blood donors were recruited, with informed consent obtained from all participants prior to enrollment. A structured questionnaire was used to collect data, which was divided into sections covering sociodemographic characteristics (age, gender, donor type), risk factor assessment (history of jaundice, surgery, transfusion, tattoos, dental procedures, travel to malaria-endemic areas, and high-risk behaviors), and laboratory test results from both serological and molecular methods. Blood samples were collected from each donor and tested using two methods.

Serological screening was performed using Enzyme-Linked Immunosorbent Assay (ELISA) to detect HBsAg (HBV), Anti-HCV antibodies (HCV), Anti-HIV antibodies with p24 antigen (HIV), and malaria antigen. Molecular testing was conducted using real-time polymerase chain reaction (RT-PCR) on the COBAS® TaqMan® system to detect HBV DNA, HCV RNA, and HIV RNA, with cycle threshold (Ct) values recorded for quantification. Window period infections, defined as cases where the pathogen is present in blood but has not yet triggered a detectable immune response, were identified by comparing serological negative but NAT positive samples. These infections were further analyzed for viral load using Ct values. All data were compiled and analyzed using Statistical Package for Social Sciences (SPSS) version 26. Descriptive statistics such as frequency, percentage, mean, and standard deviation were used

to summarize demographic data. For comparative analysis, Chi-square tests were applied to determine differences in infection rates between serology and NAT.

McNemar's test was used to analyze paired data and assess discordance between the two methods. Kappa statistics were calculated to measure agreement. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were computed for serology using NAT as the gold standard. A p-value <0.05 was considered statistically significant. Ethical approval was obtained from the Institutional Review Board of Shaheed M. Monsur Ali Medical College prior to data collection, and written informed consent was secured from all participants. Confidentiality and anonymity of the respondents were strictly maintained throughout the study.

Results:

Total 150 blood donors participated in this study. The demographic characteristics of the study population are summarized in Table I. The majority of participants were male (n = 138, 92.0%), while females comprised 8.0% (n = 12). The age distribution of donors was as follows: the largest group (34.7%) was in the 26-35 years age range (n = 52), followed by 30.0% (n = 45) in the 18-25 years range, 25.3% (n = 38) in the 36-45 years range, and 10.0% (n = 15) over 45 years of age. The mean age of participants was 31.47±8.92 years (range: 18-58 years). Voluntary donors represented 65.3% (n = 98) of the study population, whereas 34.7% (n = 52) were replacement donors.

Table I: Demographic Characteristics of Study Participants (N=150)

Characteristic	Category	Frequency (n)	Percentage (%)
Age Group	18-25 years	45	30.0
	26-35 years	52	34.7
	36-45 years	38	25.3
	>45 years	15	10.0
Gender	Male	138	92.0
	Female	12	8.0
Donor Type	Voluntary	98	65.3
	Replacement	52	34.7