International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Viral Genome Screening in Moroccan Blood Donors: Real-World Insights

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Abstract

The transmission of infectious agents remains a significant concern during blood transfusion. Real-world studies on blood safety assessment based on both serological and genomic techniques in low- and middle-income countries are scarce. This study aims to evaluate the virological quality of blood donated by volunteers from various regions of Morocco for major viral agents including HIV, HBV, and HCV. The study encompassed 1000 donors, with testing conducted using three serological kits and three genomic kits to detect these three viruses. All collected samples tested negative, both by ELISA and PCR, with low sensitivity but 100% specificity and a negative predictive value of 100% for all three viruses. The Chi-square test of independence revealed that sensitivity and specificity were independent of gender (p = 1), region (p = 1), age (p = 1), or the time of blood donation (p = 1). The reliability of these tests proved entirely satisfactory, warranting commercial authorization for the selected kits.

Keywords: Blood donors, virological quality, ELISA, PCR, Morocco

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1. Introduction

Blood transfusions, while instrumental in saving millions of lives worldwide annually, carry the inherent risk of transmitting blood-borne pathogens [1], including HIV, HBV, and HCV. These agents can lead to prolonged viremia, chronic conditions associated with increased morbidity and mortality, such as liver cirrhosis, hepatocellular carcinoma (HCC), and opportunistic infections [2]. Despite the implementation of rigorous donor selection processes and significant advancements in serological testing in recent years, a residual risk of virus transmission through blood transfusion persists [3]. Much of this risk stems from blood donation occurring during the infectious window period, which is the interval between infection and detection via screening tests [4]. Tracking the evolution of residual risks associated with transfusion-transmitted infections or the incidence of infectious agents in donated blood not only ensures the safety of the blood supply but also serves as a catalyst for the introduction of more sensitive screening techniques aimed at enhancing early infection detection [5].

Viral genomic screening (VGS) has become a standard procedure in blood transfusion testing, routinely conducted for all donated blood in most developed countries [6]. The effectiveness of VGS, along with its numerous variations, has been established in Europe, North America, Asia, and more recently, in Kenya [7]. Other regions are planning its implementation [8]. The duration of the window period has been estimated at 66 days for HCV, 22 days for HIV, and 56 days for HBV [9]. The introduction of VGS has significantly reduced this window period, bringing it down to approximately 7 days for HCV, 11 days for HIV, and 20 to 30 days for HBV [10]. Nucleic acid testing has further diminished this risk in select healthcare centers where it has been adopted. However, this added benefit comes at an additional cost to the healthcare system [11].

The objective of this study is to evaluate the feasibility of implementing viral genomic screening in primary-infected blood donors, aiming to enhance the sensitivity and specificity of HIV, HCV, and HBV diagnosis in transfusion-transmitted infections at the Blood Transfusion Center (TC) of the Mohammed V Military Hospital of Instruction (HMIMV) in Rabat, Morocco.

2. Materials and methods

2.1. Blood donors

This is a retrospective cross-sectional study conducted on a sample of 1000 blood donors at the TC of the HMIMV between March 15, 2018, and June 15, 2018. The donors included young male military recruits from diverse regions of Morocco, with ages ranging from 21 to 31 years. A pre-donation medical interview was conducted to identify individuals at risk. All participants provided their consents before enrollment in this study as per local ethical committee guidelines.

2.2. Sampling, preparation, and serological and viral genomic screening for HIV, HBV and HCV

The samples selected for viral genomic screening tested negative for all three viral tests (HIV, HBV, and HCV). Following blood collection, centrifugation was performed to separate the serum, which was then divided into two aliquots and stored at -80°C for subsequent processing.

2.3. Serological tests for HIV, HBV, and HCV

Biological screening was conducted using the enzyme-linked immunosorbent assay (ELISA) technique. The search for HIV markers was performed through the combined HIV Ag/Ab test (Murex, Abbott), enabling the detection of the p24 antigen of HIV and various antibodies associated with the HIV-1 and/or HIV-2 viruses [12]. This test has a serological window of 17 days [13,14]. Detection of the surface hepatitis B virus (HBsAg) antigen (Murex, Abbott) was performed, with a serological window of 38 days [13,14]. For the serodiagnosis of hepatitis C virus infection, HCV Ag/Ab was employed to detect the capsid antigen and Anti-HCV antibodies (Murex, Abbott) [12], with a window period of 27 days.

2.4. Viral genomic screening

Molecular analysis was conducted at the Virology Unit through the detection of HBV DNA, HIV RNA, and HCV RNA using quantitative RT-PCR. Indeed, HIV plasma viral load (pVL) was quantified using automated real-time polymerase chain reaction (PCR) performed on the Cobas Ampliprep / Cobas TaqMan system (Roche Diagnostics, Mannheim, Germany), with a detection limit of 20 HIV RNA copies/mL. The HBV plasma viral load (pVL) (DNA) was determined using automated real-time polymerase chain reaction (PCR) on the Cobas Ampliprep/Cobas TaqMan system (Roche Diagnostics, Mannheim, Germany), with a lower limit of detection equal to or greater than 20 IU/mL. The HCV plasma viral load (pVL) (RNA) was assessed using automated real-time polymerase chain reaction (PCR) with the Cobas Ampliprep/Cobas TaqMan system (Roche Diagnostics, Mannheim, Germany), having a lower limit of detection equal to or greater than 15 IU/mL.

2.5. Statistical analysis

The data, collected in Excel, underwent filtering and were subsequently transferred to SPSS statistical software (version 26.0, IBM, Armonk, NY). Qualitative variables were presented as frequencies, while quantitative variables were expressed as means with their standard deviation. The Chi-Square test of independence was employed to assess whether a statistically significant relationship exists between two categorical variables. The primary criterion for statistical significance was set at an alpha level of < 0.05. Biological indicators, including sensitivity, specificity, and negative predictive value, were also calculated.

3. Results

To ensure the sensitivity of both the ELISA and RT-PCR tests for screening HIV, HBV, and HCV viruses, serum samples were obtained from a cohort of 1000 male donors aged 21 to 31 years (with an average age of 24.99 ± 0.048). Notably, over 50% of the donors hailed from the FES/Meknes regions (n = 260), with another significant portion originating from the Rabat/Sale/Kenitra areas (n = 250). Among these donors, 863 (86.3%) were first-time blood donors, while 137 (13.7%) were regular blood donors. Table 1 presents the results of the ELISA serological test. The serum samples were subjected to ELISA screening to identify infections caused by HBV (HBS Ag), HIV (p24 Ag of HIV and anti-HIV1/2), and HCV (capsid Ag and anti-HCV), both individually and in various combinations, including dual and triple HBV/HCV/HIV co-infections. The results indicate a 0% infection rate for all three individual infections and co-infections. The ELISA-based virus detection exhibits low sensitivity but maintains a high level of specificity (100%) and a negative predictive value of 100% for all three viruses.

Table 2 displays the PCR test results. Additionally, all serum samples underwent RT-PCR analysis to detect biomarkers for the three viruses: HIV, HBV, and HCV. The PCR results corroborate those obtained from ELISA, indicating an infection rate of 0%. PCR-based virus detection exhibits high specificity (100%) and sensitivity, with a negative predictive value of 100% for all three viruses. Furthermore, the chi-square test of independence demonstrates that both sensitivity and specificity remain unaffected by variables such as gender ($p = 1$), origin ($p = 1$), age ($p = 1$), or the timing of blood donation ($p = 1$).

4. Discussion

Enhancing the services provided by blood banks not only requires strengthening transfusion safety but also ensuring the promptness and reliability of results delivered to blood donors [7]. The primary objective of our study was to assess the sensitivity and specificity of the serological and genomic kits selected for screening HIV, HBV, and HCV viruses. We conducted PCR testing on 1000 seronegative donors for the three viruses, all of whom had donated blood at the Transfusion Center of HMIMV. The results of these tests did not detect any infections with HIV, HBV, or HCV. In low-income countries, the prevalence of HIV infection among blood donors ranges from 1% to 12%, while that of hepatitis C and hepatitis B falls between 0.5% and 12% and 3% to 22%, respectively [16]. Although genomic testing is unlikely to supplant serological testing, the latter will remain the preferred mode. However, it is worth noting that in certain cases, HBsAg may test positive while HBV DNA is negative, and anti-HCV is detected but RNA is negative. Our study, however, exhibited exceptional sensitivity and specificity for all three viruses. This unique characteristic of genomic testing can be attributed to the presence or absence of the infectious agent. In essence, a positive genomic test indicates the

presence of the virus, while a negative genomic amplification suggests the absence of an infectious virus [17, 18, 19]. Additionally, the circumstances leading to a blood donation may involve the presence of a virus without detectable antibodies or antibodies without a trace of the virus.

Recent studies have revealed the presence of DNA in blood donors who exhibit no serological markers for the virus. This phenomenon is notably prevalent in specific populations, including intravenous drug users (54%), hemodialysis patients (31%), and individuals co-infected with HIV and/or HCV. In fact, 17% to 42% of those co-infected with HIV and 20% to 49% of those co-infected with HCV possess anti-HBc markers [20, 21]. In Tunisia, as reported by Hannachi et al. (2007), the prevalence rates among poly-transfused patients were 0.86% for HIV [22], 8.4% for HBs antigen, and 4.7% for HCV. The utility of nucleic acid tests (NAT yield) varies based on factors such as the donor population, the type of serological test employed, the kit's nature, and the sensitivity of the NAT test itself [23]. However, commercially available real-time PCR techniques for detecting virus DNA in serum (or plasma) exhibit sufficient sensitivity to identify numerous cases of occult infection with HIV, HBV, and HCV.

Table 1. ELISA serologic test results

Kit	**Murex HCV Ag/Ab combination**		**Murex HIV Ag/Ab combination**		**Murex HBS Ag version 3**	
Serology	**Presence**	**Absence**	**Presence**	**Absence**	**Presence**	**Absence**
Test results	(n=0)	(n=1000)	(n=0)	(n=1000)	(n=0)	(n=1000)
Sensitivity	0%		0%		0%	
Specificity	100%		100%		100%	
NPV	100%		100%		100%	

Ab: antibody, Ag: antigen, **HCV**: hepatitis C virus, **HIV**: human immunodeficiency virus, **HBS**: hepatitis B surface antigen, **NPV**: Negative predictive value

Table 2. PCR test results

Virus	**HCV**		**HIV**		**HBV**	
Kit	**AmpliPrep/COBAS® TaqMan® HCV RNA**		**AmpliPrep/COBAS® TaqMan ® HIV-1, v2.0 RNA**		**HBV, v2.0 (AmpliPrep /COBAS® TaqMan®) DNA**	
PCR	Presence	Absence	Presence	Absence	Presence	Absence
Test results	(n=0)	(n=1000)	(n=0)	(n=1000)	(n=0)	(n=1000)
Sensitivity	0%		0%		0%	
Specificity	100%		100%		100%	
NPV	100%		100%		100%	

DNA: deoxyribonucleic acid, **HBV**: hepatitis B virus **HCV**: hepatitis C virus, **HIV**: human immunodeficiency virus, NPV: Negative predictive value, **PCR**: polychain reaction, **RNA**: ribonucleic acid.

5. Conclusions

While 4th generation kits used in predictive ELISA offer valuable screening capabilities, they cannot fully supplant the detection of RNA or viral DNA, which remains inherently more sensitive. Therefore, it is advisable to promote the utilization of these kits to enhance transfusion safety. Implementing more sensitive testing methods and establishing a consistent and ongoing quality assurance and control system would represent an invaluable asset in safeguarding and consistently maintaining the virological quality of transfused blood, especially within this donor group.

Conflicts of Interest

The authors declare no conflicts of interest

Acknowledgments

None

Funding

No funding was received for this research.

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