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Research Paper

# Comparison of Frequency three viruses HBV, HCV and HIV Based on ELISA and Western Blot Serology Methods at Blood Bank in Tehran, Iran

**Maryam Kamaii[1], Monir Doudi[1*], Ladan Rahimzadeh Torabi[1], Mohammad Hossein Pazandeh[2]**

*1. Department of Microbiology, Faculty of Biology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran.*
*2. Department of Pharmacology and Toxicology, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran.*

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## ABSTRACT

**Background:** Hepatitis B surface (HBs), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) are three important factors of blood and blood product-transmitted infections worldwide.

**Objective:** This study examines the prevalence of HBs, HIV, and HCV infections in the blood donor population of Tehran City, Iran, using serological techniques, specifically rapid diagnostic tests, enzyme-linked immunosorbent assay (ELISA), and Western blotting (WB).

**Methods:** In this descriptive and retrospective study, the documents of all blood donors who had been referred to the Tehran Blood Transfusion Organization from 2016 to 2017 were reviewed. The results of their tests were evaluated. The present investigation assessed the prevalence of HBs, HCV, and HIV infections among donors using various methods, such as rapid diagnostic tests, ELISA, and WB. The SPSS software, version 19 and the statistical tests (chi-square, one-way analysis of variance, Duncan test) were used for data analysis.

**Findings:** According to the results, of 159000 blood donors, 1034 positive cases were detected using rapid diagnostic tests. After implementing the ELISA and WB techniques, positive cases decreased to 743 individuals. Approximately 65.28% of the blood donors were male and 34.72% were female. A total of 511 cases (42.49%) were married while 523(58.50%) were single. The infection rate under 60 years was higher than that at >60 years. Approximately 451 cases (43.62% of the total) were labeled as unknown, indicating an unknown infection method. Meanwhile, 253(24.46%) were low-risk and 330(31.92%) were considered high-risk. However, ELISA and WB assays revealed that the prevalence rates followed a specific order. HBs exhibited the highest prevalence, followed by HCV, and then HIV. The area under curve values for HBs, HCV, and HIV were 0.945, 0.920, and 0.998, respectively.

**Conclusion:** Rapid diagnostic tests are more specific but less sensitive and are not usually used as the first test. WB and ELISA tests have a confidence percentage of over 98%. The consistency between virus prevalence among blood donors in the Tehran Blood Transfusion Center was observed using the ELISA and WB methods.

*** Corresponding Author:**
*Monir Doudi, Associate Professor.*
***Address:*** *Department of Microbiology, Faculty of Biology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran.*
***Phone:*** *+98 (13) 7420134*
***E-mail:*** *Doudi@iaufala.ac.ir*

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## Introduction

Viral infections originating from the hepatitis C virus (HCV), human immunodeficiency virus (HIV), and hepatitis B virus (HBV) are universally recognized as a significant social concern and pivotal problem [1-6]. Various studies have demonstrated a heightened prevalence and severity of viral infections, including HCV and hepatitis B surface (HBs), among individuals affected by HIV [7-14].

Simultaneous contraction of the aforementioned viral infections in humans has been observed to initiate the progression of a more severe pathological condition. As a result, the complexity of treatment approaches increases. Meanwhile, there is a noticeable rise in mortality rates related to comorbidities, such as liver disorders [15-17]. In patients infected with HBs and HIV at the same time, HIV infection leads to an increase in HBV reproduction and a faster progression of liver lesions to cancer and cirrhosis [18, 19]. HBV is a significant and prevalent etiological agent of viral infections that results in profound hepatic impairment, ultimately culminating in liver dysfunction. The progression of hepatitis B infection in humans encompasses a spectrum, ranging from acute infection to the development of chronic disease, which may ultimately advance toward the manifestation of cirrhosis [20-22].

Hepatitis B viral protein with clinical importance is a combination of envelope protein, hepatitis B surface antigen (HBs Ag), core nucleocapsid structural protein, and e antigen, which is a soluble nucleocapsid protein and is a sign of active viral replication [23, 24]. Epidemiological characteristics of hepatitis B can be classified according to the prevalence of HBs Ag in different regions worldwide. Regions with low prevalence have an HBV carrier prevalence of 1% to 2%. These regions include North America, Western Europe, Australia, and parts of South and Central America. Regions with a medium prevalence have an HBV carrier prevalence of 2% to 8%. These regions include countries around the Mediterranean, Japan, Central Asia, the Middle East, parts of South America, and Eastern Europe. Areas with a high prevalence of HBV have an HBV carrier prevalence of 8% to 20%. These areas include Southeast Asia, Alaska, parts of the Middle East, and Eastern Europe [25-29].

According to different studies, of the 2.29 million people living with HIV worldwide, approximately 5% to 10% are living with HBV co-infection. HIV infection is a serious public health and social problem worldwide [30]. Faster progression of HIV infection, even in the form of AIDS, has been observed in people infected with HBs and HCV [31, 32].

HCV infection is a prevalent and pervasive chronic infectious disease on a global scale, with an estimated worldwide burden affecting a substantial population of approximately 150 to 170 million individuals [33, 34]. This infection has the potential to give rise to liver failure, liver cancer, or chronic liver disease (cirrhosis), making it the primary precipitating factor for liver transplants in the United States of America [35, 36]. According to the epidemiology of HCV, the risk factors for co-infection with HCV in people with chronic hepatitis B include injecting drug use, history of blood transfusion, and other injection contacts. There is evidence that HCV co-infection increases the risk of progression to cirrhosis and liver cancer in people living with HBs [37, 38]. Most patients with HCV have no symptoms. However, even without symptoms, they may have physical problems decades later and can still transmit the disease to others [39].

Viral infections transmitted through blood and blood products, such as HIV, HBs, and HCV, are highly prevalent in various regions worldwide, especially in underdeveloped or developing countries. However, through the implementation of serological screening tests, such as enzyme-linked immunosorbent assay (ELISA), alongside confirmatory tests, such as the Western blot (WB), it is possible to diminish the prevalence of viral markers among blood donors. Numerous endeavors have been dedicated by blood transfusion establishments to effectively mitigate the incidence of positive findings in both serological and molecular tests, aiming for their complete elimination. The present study examines the prevalence of HCV, HIV, and HBs infections and compares the incidence rates of these viral infections among individuals seeking services from the Tehran Blood Transfusion Organization using rapid diagnostic tests (RDTs), ELISA, and WB assay methods.

## Materials and Methods

### Study population and rapid diagnostic tests

The research project was executed with the voluntary cooperation of approximately 159000 participants who provided their blood samples for analysis voluntarily. The specimens derived from the Tehran Blood Transfusion Organization were assessed using the rapid test method, specifically employing the ABON Biopharm Co. Ltd., Hangzhou, PR China. In the present study, we

offered a representative sample of individuals willing to donate blood. The specimens were combined with a unique substance to incite chemical reactions. The outcomes will be discernible using of a dipstick and compared to the control.

### Enzyme-linked immunosorbent assay test for the detection of hepatitis B surface antigen

In the first step, a 100 µL volume of the donor serum was added to the designated wells. Following this, the wells were augmented with 100 µL of positive and negative controls. These controls comprised three wells, which contained negative controls, and two wells, which contained positive controls. A volume of 25 µL of the solution containing conjugates was used for the samples and controls, followed by an incubation period of 60 min at 37˚C. Next, the washer was cleaned using an ELISA device. Subsequently, 100 µL of conjugate solution [2] was added and incubated for 30 min at 37˚C. Subsequently, the sample underwent an additional washing procedure, followed by the addition of 75 µL of chromogen solution. The resulting mixture was incubated for 30 min at ambient temperatures ranging from 20˚C to 24˚C. In the subsequent phase, 75 µL of sulfuric acid (0.25 mol/L) was introduced into the solution to terminate the reaction. The light absorption was measured using an ELISA reader at the specific wavelengths of 450 and 620 nm. The experiment was conducted following the protocol provided by SIEMENS (Germany), as prescribed in the respective kit.

### Enzyme-linked immunosorbent assay test for the detection of hepatitis B surface Ab

Initially, 25 µL of serum, accompanied by a negative control consisting of human serum lacking specific HBs antibodies and fortified with amphotericin B at a concentration of 5 mg/L and gentamicin at a concentration of 100 mg/L as preservatives, and a positive control composed of human serum containing specific HBs antibodies along with the same concentrations of amphotericin B and gentamicin as employed in the negative control, were introduced into the wells of a 96-well plate. The light absorption measurements were recorded at a wavelength of 450 nm, while reference wavelengths ranging from 615 to 690 nm were employed. The experiment was conducted following the prescribed procedure outlined in the SIEMENS (Germany) manufactured kit.

### Enzyme-linked immunosorbent assay test for the detection of human immunodeficiency virus antigen

A volume of 100 µL of negative control solution was introduced into each of the initial three wells and 100 µL of positive control solution was dispensed into the remaining two wells. In the next step, 100 µL of the donor serum was added to the wells. The plate was incubated at 37˚C for 30 min. After that, it was washed three times, and 100 µL of double conjugate was added to the wells and incubated at 37˚C for 30 min. After that, the plate was washed four times, and 75 µL of chromogen solution was added to the wells. It was placed in the dark for 30 min at a temperature of 18˚C to 25˚C (the reaction was performed in the dark at the laboratory temperature in the last step to prevent the occurrence of an additional and false absorption reaction). Then, 75 µL of stopping solution was added to each well, and the ELISA reader was read at wavelengths of 450 and 650 nm. This experiment was conducted by the protocol provided by the manufacturer of the HIV Ag kit (ADALTIS S.R.L. Italy).

### Enzyme-linked immunosorbent assay test for detection of hepatitis C virus antigen

In this experiment, three wells were designated as negative controls, two wells were assigned as calibrators, and one well was designated as a positive control. Next, 200 µL of a solution diluted with the intent of reducing its concentration was introduced into the wells designated for the sample. A 20-µL volume of the specimen was introduced into the designated sample wells, with the subsequent addition of 50 µL of assay diluent to each respective well. The plate was exposed to a constant temperature of 37˚C for 60 min. The plate underwent multiple washing cycles using an ELISA washer, specifically for 4-5 cycles. Subsequently, 100 µL of the enzyme conjugate solution was introduced into the wells, excluding the blank well, where no substance was inserted. The blank well served as a control for light absorption. Subsequently, the plates were then incubated at 37˚C for 30 min. The subsequent washing procedures were carried out. In all wells, 100 µL of 3,3’5,5’-tetramethylbenzidine solution was introduced. The plates were exposed to a temperature range of 18˚C to 24˚C for 30 min. A volume of 100 µL of stop solution, consisting of 1 mol/$m^3$ sulfuric acid was incorporated into each well to halt the ongoing reaction. Finally, light absorption within each well was quantified using an ELISA reader. The reading filter used in the experiment had a wavelength of 450 nm, while the standard filter employed for ELISA tests was in the range of 620-630 nm. The experiment was carried out following the guidelines provided by the

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manufacturer, ADALTIS S. R. L in Italy for the HCV Ag kit. In the initial phase, a 200 µL volume consisting of specimens of both positive and negative controls was introduced into the well.

### Enzyme-linked immunosorbent assay test for the detection of hepatitis C virus Ab and human immunodeficiency virus Ab

At first, 200 µL of positive and negative controls (reference serum) were added to the wells. Three wells were considered as negative controls, two wells for calibration, and one well for positive control. Then, 200 µL of dilution solution was added to the sample wells. Meanwhile, 20 µL of the patient sample was added to the sample wells and then 50 µL of the assay diluent was added to all wells. The plate was incubated at 37˚C for 60 min. The plate was washed using an ELISA washer 4-5 times. Then 100 µL of the enzyme conjugate solution was added to each well (except the blank well) (no substance was added to the blank well, and it was used as a light absorption control), and the plates were placed at 37˚C for 30 min. Then, washing steps were done. A volume of 100 µL of 3,3'5,5'-tetramethylbenzidine solution was added to all the wells. The plates were placed at a temperature of 18˚C to 24˚C for 30 min. Then, 100 µL of stop solution (1 mol/$m^3$ sulfuric acid) was added to all wells to stop the reaction. In the end, the amount of light absorption in each well was measured using an ELISA reader. The reading filter was 450 nm and the standard filter for ELISA tests was 620-630 nm. The experiment was conducted by protocols established by ADALTIS S. RL Co., Italy.

### Hepatitis B surface Western blot confirmatory test

The samples with optical absorbance exceeding 1.500 in the initial HBs Ag test were diluted with physiological serum at ratios of 1:10 and 1:100, after which the diluted samples were utilized for subsequent testing. Initially, 100 µL of each sample was combined with 25 µL of reagent 1, known as HBs antigen neutralizing solution, and an additional 25 µL of reagent 2, referred to as the dilution solution, using a plastic tube. The cap of the cylindrical container was secured by agitation, and subsequently placed in a temperature-controlled incubation chamber at 37˚C for 60 min. The present study adhered to the SIEMENS, a German manufacturer's established protocol for conducting the test, which was executed in the following manner.

### Human immunodeficiency virus and hepatitis C virus Western blot confirmatory test

Initially, nitro-cellulose strips were diligently arranged on a plate, followed by the addition of 2 mL of a washing solution, specifically a Tris solution. The aforementioned strips were subsequently positioned onto a rocker at ambient temperature for 1 to 2 min, with a rotational velocity ranging from 12 to 16 rotations per min. The washing solution was removed from the wells via aspiration using a vacuum pump, followed by the addition of 2 mL of blotting solution consisting of fat-free dry milk, distilled water, and conjugate was added to each well. Subsequently, a volume of 20 µL of the sample and control was introduced into distinct wells. Following three sequential washing steps, each lasting 5 min, 2 mL of conjugate solution was subsequently introduced into the respective wells. The wells were maintained at a constant room temperature of 25˚C for 30 min. Subsequently, the wells underwent a secondary washing process, followed by the addition of a 2 mL substrate solution. After 15 min, 2 mL of distilled water was introduced to halt the reaction. The cellulose strips were desiccated under ambient conditions for 1 h. The methodology employed bore similarity to the HIV blot technique; however, a distinguishing factor was observed in the duration of incubation. Unlike the standard 24-h incubation period, an alternative protocol was implemented with a reduced incubation time of 1 h. The experimental procedure was carried out according to the MP Diagnostics HCV and HIV Blot kit protocol issued by MP Biomedicals Co., China.

### Statistical analysis

In this study, the chi-square test was used to check the frequency distribution of antiviral antibodies positivity and confirmatory test and to check for significant differences between different groups. Receiver operating characteristic curves were used to check the diagnostic value of optical absorption for the presence of antiviral antibodies. The one-way analysis of variance, SPSS software, version 19, and the Duncan test were used to compare the frequency of positive antibody responses across various viral strains.

## Results

### Demographic characteristics of samples

Demographic characteristics, including age, marital status, exposure types, and sex of donors referred to the Tehran Blood Transfusion Organization, whose serological test results for HBs, HCV, and HIV viruses were

**Table 1.** Frequency distribution of demographic characteristics and potential risk factors in positive blood donors

| Demographic Characteristics | | No. (%) |
|---|---|---|
| Sex | Male | 675(65.28) |
| | Female | 359(34.72) |
| Age (y) | 0-10 | 23(2.22) |
| | 11-20 | 18(1.74) |
| | 21-30 | 309(29.88) |
| | 31-40 | 350(33.85) |
| | 41-50 | 223(21.56) |
| | 51-60 | 95(9.18) |
| | >60 | 16(1.55) |
| Marital status | Single | 511(49.42) |
| | Married | 523(50.58) |
| Exposure types | High risk | 330(31.92) |
| | Low risk | 253(24.46) |
| | Other (unknown) | 451(43.62) |

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Notes: High risk=the highest risk factors include a history of unsafe dental procedures, risky sexual contact, performing cupping in non-standard centers, hepatitis disease in the family, hospitalization, tattooing, surgery, drug injection, scoping (including endoscopy and colonoscopy), injection of blood and products, and contact with a contaminated needle. Low risk=Sexual activities that do not involve contact with body fluids (semen, vaginal fluid, or blood), from mother to child (chi-square test; P≤0.001).

positive. Among the demographics of blood donors, approximately 65.28% were male and 34.72% were female, approximately 511(42.49%) were recorded as being married, whereas the remaining 523(58.50%) were classified as single. The patients who were in the age range of 31 and 40 years exhibited the highest prevalence of viral infection, with a rate of 33.85%. Subsequently, the age cohort spanning 21 to 30 years exhibited the highest prevalence, amounting to 29.8%. Individuals aged below 60 years exhibited a notably higher prevalence compared to those aged 60 years or above. Approximately 451 cases (43.62% of the total) were classified as unknown, indicating an inability to determine the means of infection. Furthermore, 253 cases (24.46%) were deemed to pose low risk, while 330 cases (31.92%) were identified as high risk. The observed difference was statistically significant (P<0.001) as presented in Table 1. Also, 1034 different serum samples were used for all experiments.

## Rapid test results

Among the 159000 samples assessed, 1034 displayed positive outcomes in the rapid test analysis. Accordingly, the utilization of ELISA and WB techniques was employed to authenticate and further explore the existence of HCV, HBs, and HIV infections. Furthermore, the evaluation rigorously considered the demographic variables, including age, gender, and marital status, alongside the risk elements linked to the contributors.

## The ELISA results

The initial rapid test was performed once, and subsequently, the WB and ELISA techniques were employed to validate the findings. A total of 1034 samples were subjected to analysis using both ELISA and WB, leading to a subsequent comparison of the obtained results. According to the ELISA test, out of a sample size of 1034 blood donors, 332(32.10%) individuals tested positive for HBs Ag, while 700(67.69%) tested negative for HBs

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**Table 2.** Frequency of HBV, HCV, and HIV antigens using the ELISA test

| Western Blotting (WB) | No. (%) | | | P |
|---|---|---|---|---|
| | HIV Ag | HCV Ag | HBs Ag | |
| Positive | 162(15.67) | 249(24.08) | 332(32.10) | |
| Negative | 871(84.23) | 780(75.42) | 700(67.69) | |
| Unknown | 1(0.10) | 5(0.5) | 2(0.21) | |
| Total | 1034(100) | 1034(100) | 1034(100) | P≤0.001 |

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Abbreviations: HIV Ag: Human immunodeficiency virus antigen; HCV Ag: Hepatitis C virus antigen; HBs Ag: Hepatitis B surface antigen; ELISA: Enzyme-linked immunosorbent assay.

**Table 3.** Frequency of HBs, HCV, and HIV in donors referred to Tehran Blood Transfusion Organization by the confirmatory test (Western blotting)

| Virus Type and Number | No. (%) | | | P |
|---|---|---|---|---|
| | HIV | HCV | HBs | |
| Positive | 163(15.76) | 252(24.37) | 338(32.68) | |
| Negative | 867(83.85) | 779(75.33) | 696(67.32) | |
| Unknown | 4(0.39) | 3(0.30) | 0(0) | |
| Total | 1034(100) | 1034(100) | 1034(100) | P≤0.001 |

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Abbreviations: HIV: Human immunodeficiency virus; HCV: Hepatitis C virus; HBs: Hepatitis B surface.

Ag. In addition, 249(24.08%) tested positive for HCV Ag, whereas 780(75.42%) tested negative for HCV. Finally, 162(11.70%) individuals were found to have tested positive for HIV, while the remaining 871(88.20%) participants had a negative test result. The findings derived from the ELISA revealed that the HBs virus exhibited the highest degree of positivity.

As shown in Table 2, the chi-square test showed that the distribution of the frequency of positive antibody responses against HIV, HBs, and HCV was significantly different between different viruses (P≤0.001); accordingly, in the ELISA test, the percentage of responses with HBs Ag positivity was higher than that of HCV virus, and HCV Ag positive responses were higher than HIV.

## The Western blotting results

The WB confirmatory test was employed to validate the presence of HBs Ag in a cohort of 1034 blood donors. Our findings revealed that of the total participants, 338(32.68%) tested positive for HBs Ag, while the remaining 696(67.32%) tested negative. Regarding HCV, 252(24.37%) tested positive for the presence of HCV, while the remaining 779(75.33%) yielded negative results. A total of 163(15.76%) individuals tested positive for HIV, while 867(83.85%) tested negative for the virus.

WB results differed slightly from those of ELISA results. In the ELISA technique, the prevalence of individuals testing positive for HBs Ag was 332, while the WB method yielded a slightly higher rate of 338. In this research, the ELISA method may yield false-negative outcomes compared to the WB assay.

The findings of the chi-square analysis presented in Table 3 demonstrated that the distribution of the frequency of confirmatory tests (WB assay) in blood donors was significantly different between different viruses (P<0.001). However, in the WB assay, the frequency of positive antigen responses against HBs virus was higher than the HCV virus, and positive antibody responses against HCV were higher than HIV (Table 3).

**Table 4.** Mean value of light absorption according to the positive response in the Western blotting assay

| Viruses | Blood Donor's Results by WB | Mean±SD | P |
|---|---|---|---|
| | | OD600 (nm) | |
| HBs Ag | Positive response | 0.377±0.190 | P≤0.001 |
| | Negative response | 0.066±0.043 | |
| HCV Ag | Positive response | 0.098±0.076 | P≤0.001 |
| | Negative response | 0.162±0.032 | |
| HIV Ag | Positive response | 0.279±0.070 | P≤0.001 |
| | Negative response | 0.323±0.310 | |

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Abbreviations: HIV Ag: Human immunodeficiency virus antigen; HCV Ag: Hepatitis C virus antigen; HBs Ag: Hepatitis B surface antigen; WB: Western blotting; OD: Optical density.

The results from the independent t test are shown in Table 4. Accordingly, the mean value of light absorption in people who were positive in the WB assay. The optical density of HCV, HIV, and HBs viruses was significantly higher than that of the negative ones (P≤0.001) (Table 4). In the rapid test, 1034 positive cases were recorded out of 159000. However, after conducting the ELISA and WB tests, the overall count of positive cases was reduced to 743.

## The result of anti-viruses Abs

Based on the sensitivity and specificity values provided in Table 5, the optical absorption value can correctly identify 88.9% of cases where the anti-HBs antibody is truly positive and 95.1% of cases where the anti-HBs antibody has indeed become negative. Also, based on the values of positive and negative predictive values, 92.5% of the c–ases in which the presence of anti-HBs virus antibody was diagnosed as positive based on the cut-off point were positive for anti-HBs antibody, and 92.6% of the cases in which the presence of anti-HBs virus antibody was negative were negative for anti-HBs antibody.

According to the values of sensitivity and specificity mentioned in Table 4, 85.1% of the cases in which the antibody against HCV was positive can be correctly recognized by the optical absorption value, and 87.4% of the cases in which the antibody against HCV was negative can be correctly recognized as negative by the amount of optical absorption. Also, based on the positive and negative predictive values, 69.8% of the cases in which the presence of anti-HCV antibody was diagnosed

**Table 5.** Level diagnostic value of the presence of anti-HCV, anti-HIV, and anti-HBs antibodies based on the value of optical absorption

| Indicator | Level | | |
|---|---|---|---|
| | HBs | HIV | HCV |
| The area under the curve | 0.945 | 0.998 | 0.820 |
| Cut-off point | 0.139 | 0.204 | 0.124 |
| Sensitivity (%) | 88.9 | 100 | 85.1 |
| Specificity (%) | 95.1 | 98.8 | 87.4 |
| Positive predictive value (%) | 92.5 | 88.2 | 69.8 |
| Negative predictive value (%) | 92.6 | 100 | 94.5 |

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Abbreviations: HIV: Human immunodeficiency virus; HCV: Hepatitis C virus; HBs: Hepatitis B surface.

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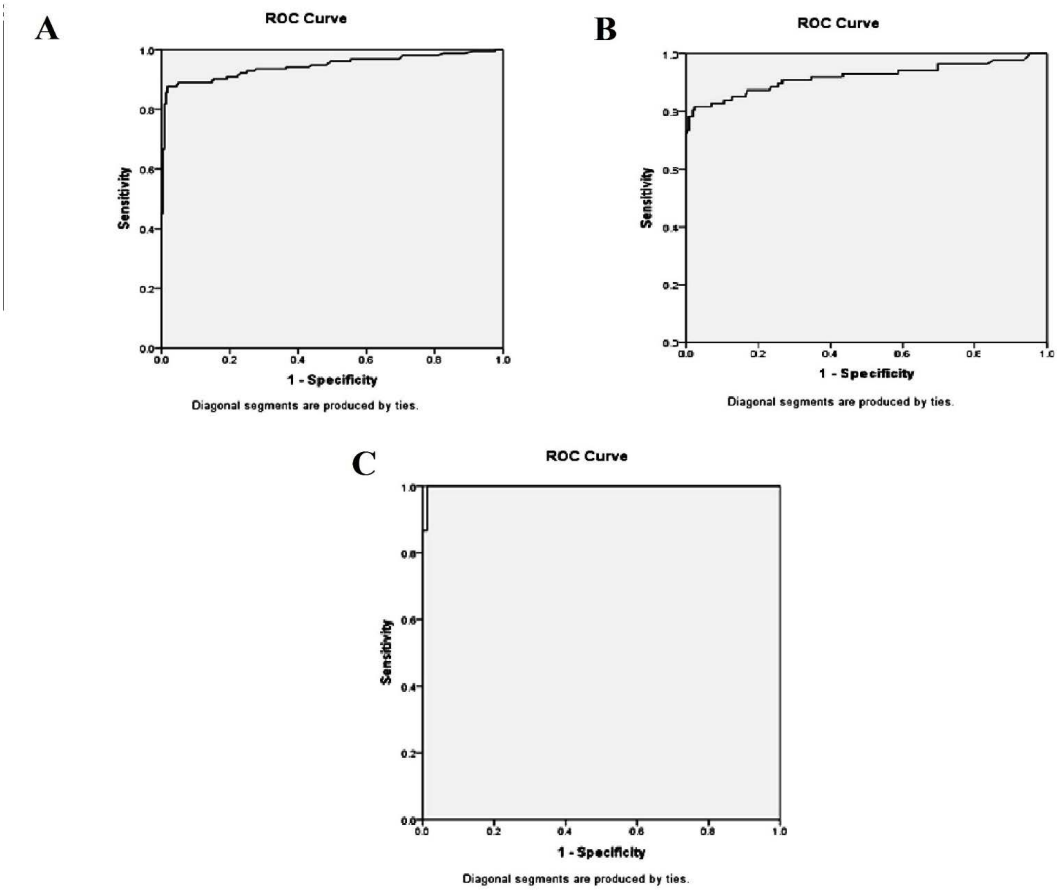


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**Figure 1.** The ROC curve of HBs (A), HCV (B), and HIV (C) antibodies based on light absorption value

Abbreviations: ROC: Receiver operating characteristic; HIV: Human immunodeficiency virus; HCV: Hepatitis C virus; HBs: Hepatitis B surface.

as positive based on the cut-off point were positive for anti-HCV antibody, and 94.5%of the cases in which the presence of anti-HCV antibody was negative were negative for anti-HCV antibody.

According to the values of sensitivity and specificity mentioned in Table 4, 100% of the cases in which the anti-HIV antibody was positive can be correctly recognized by the optical absorption value, and 98.8% of the cases in which the anti-HIV antibody has become negative can be correctly recognized as negative by the amount of light absorption. Also, based on the positive and negative predictive values, 88.2% of the cases in which the presence of anti-HIV antibody was diagnosed as positive based on the cut-off point, were positive for anti-HIV antibody, and 100% of the cases in which the presence of anti-HIV antibody was negative. Their anti-HIV antibody was negative (Table 4).

## Curve results

The calculated value for the area under the curve was 0.945, indicating that the optical density value may serve as a valuable diagnostic measure to confirm the existence of anti-HBs antibodies. The utilization of the receiver operating characteristics curve permitted the determination of the cut-off point value (0.139) to detect the presence of anti-HBs antibodies. This determination exhibited a sensitivity of 88.9% and a specificity of 95.1% (Figure 1A).

The attained value for the area under the receiver operating characteristic curve was 0.820; therefore, the optical absorption value can be a good diagnostic value to prove the presence of anti-HCV antibodies. Using the receiver operating characteristic curve, the cutoff point value of optical absorption to detect the presence of anti-HCV antibodies was 0.124 with a sensitivity of 85.1% and a specificity of 87.4%. (Figure 1B)

The area under the receiver operating characteristic curve was 0.998. Hence, the value of optical density can have a good diagnostic value for detecting the presence of anti-HIV antibodies. Using the receiver operating characteristic curve, the cutoff point value of optical density to detect the presence of HIV antibodies was 0.204, with a sensitivity of 100 and a specificity of 98.8% (Figure 1C).

## Discussion

In the present investigation, a total of 159000 blood donors were surveyed, of which 1034 samples exhibited positive results using rapid diagnostic tests at the Tehran Blood Transfusion Organization during 2016-2017. The occurrence rates of HBV, HIV, and HCV were identified using the ELISA and WB assays.

In analyzing blood donor demographics, males comprised 65.28% and females accounted for 34.72%. Of the total cases, 511(42.49%) were married, while the remaining 523(58.50%) were single. Individuals in the age range of 31-40 years had the highest viral infection rate at 33.85%. Individuals aged 21 to 30 years had the highest incidence rate at 29.8%. The population was more prevalent among those under 60 than those over 60 years. A total of 451 cases, accounting for 43.62%, were unknown, indicating infection source uncertainty. A total of 253 instances (24.46%) were low risk and 330 cases (31.92%) were higher risk.

In rapid diagnostic tests, 1034 positive cases out of 159000 were recorded. After the ELISA and WB tests, the positive case count was reduced to 743. The ELISA test found that 332(32.10%) of the 1034 blood donors were HBs Ag positive, while 700(67.69%) were negative. Moreover, 249(24.08%) were HCV-positive and 780(75.42%) were negative. Meanwhile, 162(11.70%) were HIV positive and 871(88.20%) were negative. ELISA found HBs virus to be highly positive. WB found HBs Ag in 338(32.68%) out of 1034 participants. Then, 696(67.32%) tested negative for HBs Ag and 1034 samples were analyzed in the HCV study. Out of these, 252(24.37%) were HCV-positive, while 779(75.33%) were negative.

The WB results differed slightly from the ELISA results. ELISA recorded 332 positive individuals for HBs Ag, while WB recorded a slightly higher rate of 338. The rapid diagnostic tests exhibited a significant prevalence of false-positive outcomes, thereby implying that the ELISA may yield relatively lower rates of false results in comparison to WB analysis. There is a notable similarity in the outcomes obtained from the WB and ELISA methods, as confirmed by this study. However, rapid diagnostic tests have many false positives; therefore, their specificity is not higher than that of other methods.

WB confirmation test and ELISA showed that the prevalence of these three viruses was HBs, HCV, and HIV, respectively. The area under the receiver operating characteristic curve was 0.945 for the HBs virus, 0.920 for the HCV virus, and 0.998 for HIV.

The results of this study on the donating blood population in the Tehran Blood Transfusion Organization showed that one of the ways to minimize the risk of HBs, HCV, and HIV is a suitable screening of blood donors according to the standard operating procedure guidelines and conducting confirmatory tests.

According to the standards of the Tehran Blood Transfusion Organization, donor products whose ELISA test is positive, will be eliminated, and the donors will be permanently exempt from blood donation, but a confirmation test will be done for them. The WB assay is one of the blotting methods that will be used in this organization to detect and analyze proteins. In addition to being a confirmation method, this test will also have the ability to check a type of antibody against several types of viral proteins, such as HIV, HBs, and HCV proteins, compared to the ELISA test. The rapid diagnostic tests exhibit a reduced level of sensitivity in comparison to laboratory tests, thus exhibiting inferior efficacy compared to laboratory tests in the detection of diseases during the nascent phases of infection. Therefore, rapid diagnostic tests are more specific, but it is less sensitive, and it is often not performed as the first test. It is mostly used to confirm the positive results of the ELISA. The WB and ELISA tests will have a confidence percentage of more than 98%.

The present investigation observed concordance in the prevalence of the three aforementioned viruses among the blood donor populations at the Tehran Blood Transfusion Center, using both ELISA and WB techniques. Based on research conducted in Nigeria, they concluded that hepatitis B infection was more common than hepatitis C and HIV infections. HBs was the most frequent infection, and HIV was the least frequent infection in that study [40]. In a conducted research in Turkey on 72695 blood donors, HBV showed a higher frequency than HIV and HCV, although its highest frequency was because of the simple ways of transmission of this disease [41]. Aghamohamad et al. analyzed the data from the questionnaires and the lab analysis results of the blood donors who were referred to the Semnan Province (Iran) Blood

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Transfusion Organization. The demographic characteristics and the prevalence of hepatitis B and C in blood donors were considered. A total of 124 704 blood donors were surveyed. Of 329 people with HBV and HCV, 297 were HBV positive and 32 were HCV positive. The prevalence of HBV and HCV contamination among donors was 0.24% and 0.026%, respectively [42].

Hepatitis caused by blood transfusion is still a major problem in Iraq. Therefore, HBs Ag and HBc Ab tests are essential to detect the infection in blood donors. Al-Rubaye et al. studied a total of 1625(2.3%) donors with serological evidence for hepatitis B virus infection. Of these donors, 125(0.2%) showed a positive test result for both anti-HBc and HBs-Ag, while 1475(2.1%) had positive anti-HBc results as the only positive test for HBV infection. Similar to the results from other countries, the frequency of HBS Ag was higher than the frequency of HCV Ab [42]. In a study in India, the researchers concluded that, among these three viruses, after the ELISA diagnostic test and without the use of the WB confirmation test, HBV was the most prevalent virus. The reason for its high frequency was attributed to the ease of disease transmission between prisoners and drug addicts. In another study, the prevalence of HBV in a population of 39598 blood donors in the Qazvin Blood Transfusion Organization was 1.08%, which was mostly in women and people with low education, married, and over 35 years of age; meanwhile, horizontal transmission was also the most important way [43].

## Conclusions

The rapid test demonstrated a noteworthy incidence of false-positive outcomes, suggesting that ELISA could potentially offer lower rates of false-negative results compared to WB analysis. The findings of this study demonstrate a correlation between the outcomes of WB and ELISA. The current study elucidated the agreement between the occurrence rates of the three investigated viruses among donors at the Tehran Blood Transfusion Center. This was accomplished by employing both ELISA and WB in comparison to the rapid test assay. The ELISA and WB assays found HBs had the highest prevalence, followed by HCV and HIV.

We concluded that rapid diagnostic tests offer a convenient solution for obtaining quick results. However, errors sometimes can occur, which can compromise their reliability. Therefore, it is vital to promote further improvements in the creation and application of more precise diagnostic tests and methodologies.

## Ethical Considerations

### Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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### Authors' contributions

Study design and writing: Monir Doudi; Research administration: Maryam Kamaii; Statistical analysis: Laden Rahimzadeh Torabi; Review and editing: Mohammad Hossein Pazandeh.

### Conflict of interest

The authors declared no conflict of interest.

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