The Research of Medical Science Review
ISSN: 3007-1208 & 3007-1216 Volume 4, Issue 2, 2026

A STUDY OF TRANFUSION TRANMISTTED INFECTIONS AMONG VARIOUS BLOOD DONORS TO ENHANCE SCREENING STRATAGIES FOR RISK REDUCTION

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DOI: https://doi.org/10.5281/zenodo.18708014

Keywords
Transfusion Transmissible Infectio Blood donors Hepatitis B vir Hepatitis C virus; Hum immunodeficiency virus; Syphilus

Article History
Received: 21 December 2025
Accepted: 05 February 2026
Published: 20 February 2026

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Abstract

The transmission of transfusion-transmissible infections (TTIs) is caused by the transmission of infected blood and blood components. The pre-transfusion screening of HBV, HCV, HIV-I/II, malaria, and syphilis is mandatory for blood component transfusion. Globally, HCV, HBV, and HIV are ranked first among the viral infections leading to mortality and also infect millions of people across the world, including dual and triple infections of HIV and HCV and HIV and HBV. The importance of the CFR's contribution to ensuring blood safety cannot be overestimated, considering that the world experiences millions of blood donations every year. The primary aim of the study was to evaluate the effectiveness of blood screening protocols among healthy donors in reducing the risk of transfusion-transmitted infections (TTIs) .To assessed the prevalence of transfusion-transmitted infections among healthy blood donors and to identify the most common TTIs detected through blood screening among healthy donors.In Peshawar, Pakistan. Groups included total donors, in Lady Reading Hospital. The tests performed for HIV, HBV, and HCV were enzyme-linked immunsorbent assay, and for syphilis, rapid plasma reagin tests. The prevalence of HBV, HCV, HIV, and syphilis is presented in percentages. The comparison of the infection prevalence among the groups was performed using tests and P-values (<0.05). The study aims to assess the TTIs among a total of 250 blood donors in the peshawar region. The cumulative prevalence of overall TTI among the total blood donors The study aims to assess the TTIs among a total of 250 blood donors of peshawar region. The cumulative prevalence of overall TTI among the total blood donors was found to be 8.0%, and the specific ones for HBV, HCV, HIV, and vdrl were found to be 4.8%, 1.2%, 0.8%, and 1.2%, respectively. Ongoing strategies related to blood safety to each of the various donating groups and understanding the factors of the related socio-economic-health environment are important to improve blood safety.

INTRODUCTION

Blood is a unique biological fluid that is found in the circulatory system of human beings and other vertebrates. It is a critical component of the body that maintains homeostasis by transporting oxygen, nutrients, hormones, and other vital substances to tissues. At the same time, it also removes metabolic wastes from tissues. Blood is made up of cellular components suspended in plasma, which makes up about 55% of the total blood volume. Plasma is mainly composed of water (about 92% by volume) and other substances like proteins, carbohydrates, mineral ions, clotting factors, and hormones. The main cellular components of blood are red blood cells (erythrocytes), white blood cells (leukocytes), and platelets (thrombocytes). Red blood cells are the most abundant, and they contain hemoglobin, a protein that binds oxygen reversibly and helps to transport it from the lungs to the tissues. White blood cells are an essential part of the immune system that helps to fight pathogens, and platelets are essential for hemostasis and clot formation (Javed et al., 2022).

Blood transfusion is one of the most essential components of modern medicine and is often required in surgical interventions, trauma care, obstetric emergencies, hematological disorders, and chronic conditions (World Health Organization [WHO], 2022). However, despite the immense life-saving potential of blood transfusion, it also poses the risk of transmission of infectious agents from the donor to the recipient. Transfusion-transmitted infections (TTIs) are described as infections caused by pathogens such as viruses, parasites, or bacteria that are transmitted through transfused blood and have the potential to cause significant morbidity and mortality (Krause et al., 2021).

According to the WHO (2022), the total number of blood donations collected worldwide is approximately 118.2 million per year, of which nearly 58% is from low- and middle-income countries. The main TTIs of international concern are human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), Treponema pallidum (causative agent of syphilis), and malarial parasite (Saba et al., 2021). The WHO guidelines recommend mandatory testing of all blood donations for these five infectious agents to reduce the risk of transmission (WHO, 2022).

HBV, HCV, and HIV infections continue to rank as the most prevalent infectious diseases that cause people to become ill and die. The WHO reports that 71 million people have HCV infection 257 million people have chronic HBV infection and 36.7 million people have HIV worldwide. The shared transmission pathways lead to common co-infections which affect 2.3 million people who have both HIV and HCV and 2.7 million people who have both HIV and HBV (Saba et al., 2021). The two viruses HBV and HCV create chronic infections which lead to liver cirrhosis and hepatocellular carcinoma and liver failure for those who have HIV co-infection (Tilg et al., 2022; Tripathi et al., 2018).

The public health sector in Pakistan continues to battle with TTIs which have become their main health concern. A national study found that blood donors showed HBV infection rates at 6.7% and HCV infection rates at 14.3%. The majority of co-infections between HIV and HCV reached 80%, while HBV and HCV co-infections made up 20% according to Saba et al. (2021). Pakistan maintains a decentralized, demand-driven transfusion system which serves its developing country status and its population of more than 220 million people. The law requires all blood donations to undergo TTI screening. But the screening process fails to stop disease spread because current testing methods do not identify infections during the early serological window period (Ehsan et al., 2020). The blood safety system faces challenges because of limited resources, together with weak quality control systems, and different levels of compliance with standardized screening protocols.

Pakistan has established Regional Blood Centres (RBCs) that are connected with hospital blood banks to meet these challenges. The Peshawar Regional Blood Centre is situated within the Hayatabad Medical Complex in the Khyber Pakhtunkhwa province and was established in 2016. It is involved in the recruitment of blood

donors, screening for TTIs, preparation of components, quality control, and hemovigilance (Waheed et al., 2019).

Recent studies conducted between 2020 and 2025 in Pakistan demonstrate that TTI prevalence rates among blood donors remain stable between 3% and 6% throughout this period. The cross-sectional screening of 15,405 donors in Rawalpindi showed that HBV had a prevalence rate of 1.06% while HCV reached 0.54% and HIV had a 0.19% rate together with syphilis which showed 0.31% prevalence (Thakur et al., 2025). The total TTI positivity rates at blood centers reached between 3.33% and 4.61% with HCV representing the most common infection while syphilis and HBV followed behind it and HIV showed a lower prevalence rate which increased progressively (Waheed et al., 2019; Saba et al., 2021). The TTI rates for replacement donors showed higher results than those for voluntary donors which demonstrates the need to increase voluntary blood donation without payment (Ehsan et al., 2020).

A number of risk factors have been identified as contributing to the continued transmission of TTI in developing countries. Young donors (18-35 years), unmarried individuals, and manual laborers have shown higher positivity rates, which may be due to behavioral and socioeconomic factors (Thakur et al., 2025). Moreover, most of the centers are using serological techniques like ELISA, CMIA, or rapid immunochromatographic tests, which might not be able to detect early window or occult infections. Nucleic acid amplification tests (NAAT), being more sensitive, are used in only a few centers (Ehsan et al., 2020).

Given these challenges, continuous surveillance of TTIs among blood donors is critical to improving screening strategies and strengthening transfusion safety. The present study was conducted to provide updated epidemiological data on TTIs among blood donors attending the Regional Blood Centre in Peshawar. By identifying current prevalence patterns and associated risk factors, this study aims to contribute evidence-based recommendations for enhancing screening strategies and reducing transfusion-related risks.

METHODOLOGY

Study area and duration

A croos sectional study was conducted at the Department of Pathology and Blood Bank, Lady Reading Hospital, Medical Teaching Institution, Peshawar, from September 2024 to December 2024 and 250 blood samples will be collected for the study. (Shah *et al.*, 2023)

Inclusion and Exclusion criteria

Individuals who meet the eligibility criteria for blood donation as defined by the local or national blood donation guidelines, 18- 60 aged vounteers who have given informed consent. Individuals with a known history of infectious diseases, including hepatitis B, hepatitis C, HIV, syphilis, or other communicable diseases, donars taking medications, pregnant or Lactating women and individual who decline or unable to provide informed consent.

Sample Collection

Venous blood (5 mL) were collected and divided equally into two vacutainers i.e., 3mL in an EDTA (ethylene-diamine tetra-acetic acid)-containing tube and 1.5 mL in a heparin tube. **Blood sample in EDTA tube will be used for blood grouping** while that in heparin tube were used for viral profile (HBV, HCV and HIV), and *Treponema pallidum* (syphilis).

Five ml Blood were collected from the Cubital Vein by using 5cc sterile syringe and then was shifted into Heparin Tube and was transferred to the Blood Bank and Microbiology Laboratory Carefully.

Sample Processing

Blood samples in heparin tubes were centrifuged at 4000 rpm for 10 minutes at room temperature for serum separation and was immediately used for various screenings. The screening for viral profile and other infections was performed using ICT (immunochromatographic test) or ELISA (enzyme-linked immunosorbent assay).

HCV Screening
The blood sample were applied to the sample pad of the ICT device, which contains specific HCV antigens. The sample were migrate through the test strip by capillary action, where it encounters the pre-coated HCV antigens. If HCV antibodies are present in the sample, they were bind to the antigen, forming a visible line (test line) on the strip, along with a control line to ensure the test is functioning correctly.

HBV Screening
The blood sample were applied to the sample pad of the ICT device, which contains specific HBV antigens. The sample were migrates through the test strip by capillary action, where it encounters the pre-coated HBV antigens. If HBV antibodies are present in the sample, they were bind to the antigen, forming a visible line (test line) on the strip, along with a control line to ensure the test is functioning correctly.

HIV Screening
The blood sample were applied to the sample pad of the ICT device, which contains specific HIV antigens. The sample were migrates through the test strip by capillary action, where it encounters the pre-coated HIV antigens. If HIV antibodies are present in the sample, they were bind to the antigen, forming a visible line (test line) on the strip, along with a control line to ensure the test is functioning correctly.

PCR Confirmation of HIV, HBV and HCV
Blood samples were centrifuged at 1000 RPM for five minutes to separate plasma. It was kept at -20 degrees Celsius. The genome was extracted using a DNA or RNA extraction kit. Conventional PCR was utilized for HBV, whereas RT-PCR were employed for HIV and HCV. Conserved areas was targeted using certain primers. The optimized methodology was followed in the preparation of the PCR master mix. PCR were run under ideal circumstances. Ethidium bromide-stained 1.5% agrose gel was used to view the final PCR result. Gel was appear when exposed to UV radiation. Depending on the primers, 14 positive samples was display particular bands, such as ~244 bp for HCV and ~350 bp for HBV.

Syphilis screening
2 drops of blood serum was taken by using a dropper and put it into VDRAL kit for checking the final results. The positive samples were then subjected to ELISA for further screening.

RESULTS
Table 1: The table represents the **age-wise distribution of blood donors** included in the present study. A total of **250 samples** were analyzed to determine the demographic characteristics of the donors. The **majority of participants (36.28%)** were within the **18–24 years'** age group, followed by **25–30 years (30.54%),** indicating that younger adults constituted the predominant portion of the donor population. The frequency of donors decreased gradually with increasing age, and only 0.61% belonged to the category of 51–55 years. This trend indicates that blood donation activity occurs more among younger generations, which may be explained by their higher health status, motivation for voluntary donation, and fewer medical deferrals compared to older groups as shown in table 1

Table 1: Age-Wise Distribution of Blood Donors Participating in the Study

S.No	Age Group (Years)	Frequency (n)	Percentage (%)
1	18–24	91	36.28
2	25–30	76	30.54
3	31–35	40	15.95
4	36–40	37	14.86
5	41–45	35	14.05
6	46–50	11	4.32
7	51–55	2	0.61
	Total	**250**	**100.00**

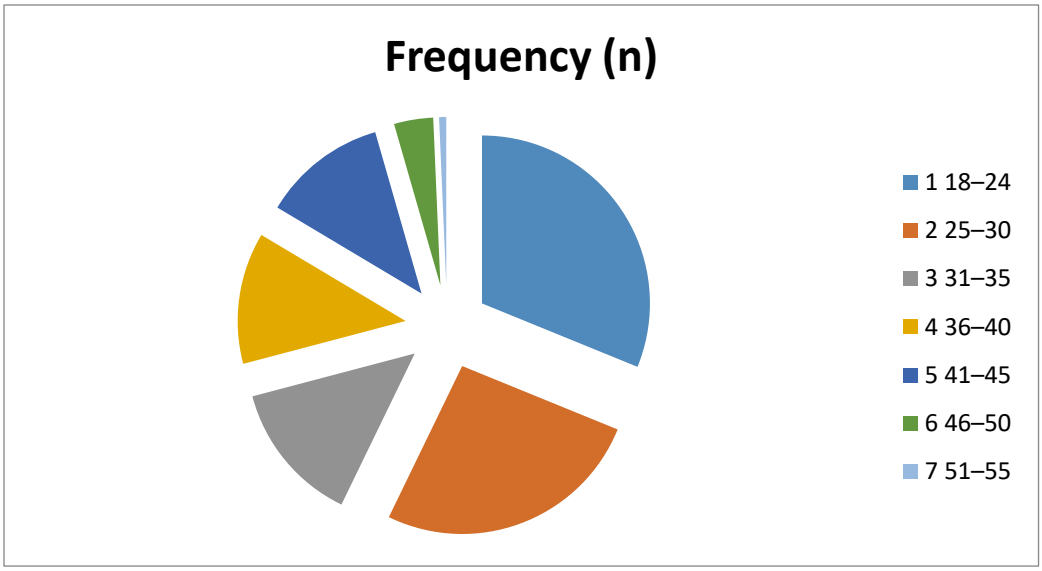

Figure 1 Age wise distribution of participants

Table 2 shows that the findings related to age-wise distribution of different TTIs among blood donors, tested by the ICT-CAT, are shown in the table below. A total of 250 donor samples were collected for this study and tested for HBV, HCV, HIV, and Syphilis infections. The respective findings from these tests are related here in terms of seroprevalence within different age groups. Among the 250 analyzed samples, 33 donors (13.2%) were found reactive for at least one of the respective TTIs, indicating a noticeable risk for transmissible infection through unscreened or poorly screened blood donations.

Among the individual infections, HBV showed the highest prevalence, 6.8%, followed by HCV, 2.8%; Syphilis, 2.0%; and HIV, 1.6%. The data thus show that HBV is still the most predominant viral pathogen associated with transfusion-related risks in the studied population. The age group 18–24 years had the highest overall positivity rate of 3.2%, thus indicating increased vulnerability or exposure among younger donors. In contrast, the **lowest infection rate (1.2%)** was recorded in donors aged **36–40 years**, while a mild resurgence of infection was noted in the **41–45** and **46–50** age groups, possibly reflecting cumulative exposure or inadequate vaccination coverage in older cohorts.

Table 2:Age-Wise Distribution of Transfusion-Transmitted Infections (TTIs) Detected by ICT-CAT among Blood Donors (n = 250)

Age Group (Years)	HBV (n)	HBV (%)	HCV (n)	HCV (%)	HIV (n)	HIV (%)	Syphilis (n)	Syphilis (%)	Total Positive (n)	Total Positive (%)
18–24	4	1.6	2	0.8	1	0.4	1	0.4	8	3.2
25–30	3	1.2	1	0.4	1	0.4	1	0.4	6	2.4
31–35	4	1.6	1	0.4	1	0.4	1	0.4	7	2.8
36–40	2	0.8	0	0.0	0	0.0	1	0.4	3	1.2
41–45	2	0.8	1	0.4	1	0.4	1	0.4	5	2.0
46–50	2	0.8	2	0.8	0	0.0	0	0.0	4	1.6
Total	**17**	**6.8**	**7**	**2.8**	**4**	**1.6**	**5**	**2.0**	**33**	**13.2**

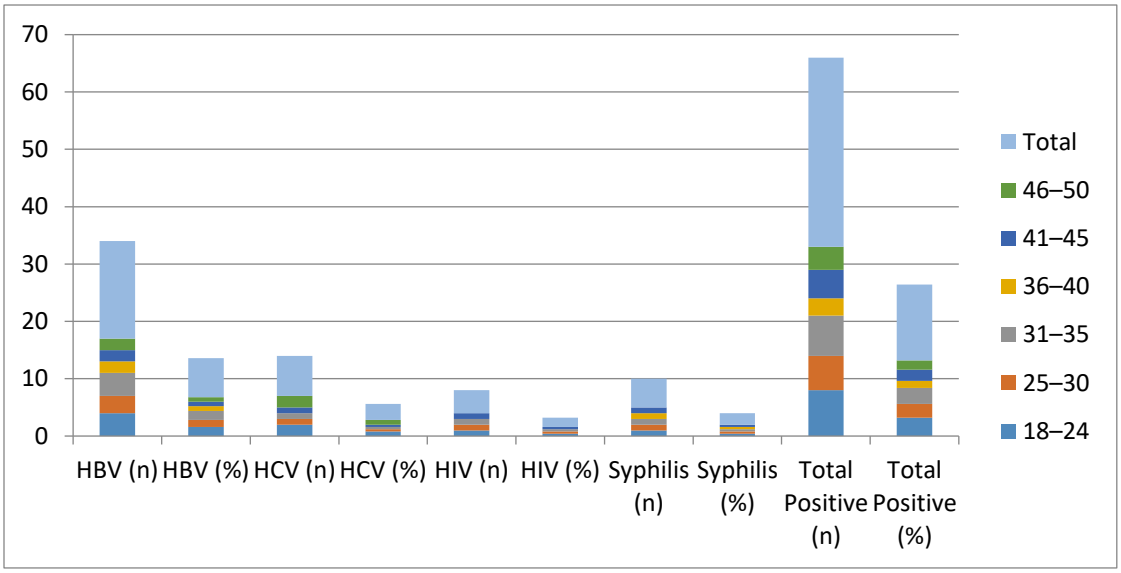

Figure 2 shows the Age-Wise Distribution of Transfusion-Transmitted Infections (TTIs) Detected by ICT-CAT among Blood Donors

Table 3 presents the **ELISA based confirmation results** of transfusion-transmitted infections (TTIs) among **33 donor samples** that initially tested reactive by **ICT-CAT**. The **Enzyme-Linked Immunosorbent Assay (ELISA)** was performed to validate ICT findings and to eliminate potential false-positive results.

Out of the 33 ICT-reactive samples, **24 (72.7%)** were confirmed positive on ELISA, while **9 (27.3%)** were found to be false positives. The distribution pattern shows that **HBV remained the most prevalent infection**, with **13 confirmed cases (39.4%),** followed by **HCV with 4 (12.1%), Syphilis with 4 (12.1%),** and **HIV with 3 (9.1%)** confirmed infections.

The **highest confirmation rate** was observed in the **18–24 years** and **31–35 years** age groups, each contributing **7 confirmed positive cases**, indicating that **younger donors remain a major reservoir for TTIs**. On the contrary, in the age groups 36–40 years and 41–45 years, positivity was lower, while there was a higher percentage of false-positive results indicative of a possible cross-reactivity in ICT screening or lower antigenic expression levels in this age range.

The difference in the ICT-CAT and ELISA results testifies to the high sensitivity and specificity of ELISA as a confirmatory test. The rate of false positivity observed reinforces the use of two-step testing protocols at blood transfusion centers to ensure appropriate detection and safe blood transfusion practices.

Table 3: ELISA-Based Confirmation of TTIs Among ICT-CAT Reactive Blood Donors

Age Group (Years)	HBV (Positive / False +)	HCV (Positive / False +)	HIV (Positive / False +)	Syphilis (Positive / False +)	Total Confirmed Positive (n)
18–24	3 / 1	2 / 0	1 / 0	1 / 0	7
25–30	2 / 1	0 / 1	1 / 0	1 / 0	4
31–35	4 / 0	1 / 0	0 / 1	1 / 0	6
36–40	1 / 1	0 / 0	0 / 0	0 / 1	1
41–45	1 / 1	0 / 1	0 / 1	1 / 1	2
46–50	2 / 0	1 / 1	0 / 0	0 / 0	3
Total (n)	**13 / 4**	**4 / 3**	**2 / 3**	**4 / 2**	**23 Confirmed**

Table 4: presents a comparative evaluation of transfusion-transmitted infection (TTI) detection between the Immunochromatographic Test Card Agglutination Technique (ICT-CAT) and the Enzyme-Linked Immunosorbent Assay (ELISA) among 250 blood donor samples. Initially, 33 samples were detected to be positive through ICTCAT screening, which were further confirmed by ELISA. Of these, 23 samples were confirmed positive by ELISA, while 9 samples were established as false positives, indicating some extent of over-reactivity in the rapid screening test.

This serological investigation demonstrated that among the recorded viral species, the highest detection frequency belonged to the Hepatitis B Virus, having 17 samples initially reactive on ICT-CAT and 13 confirmed positive on ELISA. In addition, the sensitivity of ICT-CAT in the detection of HBV was 100%, while the specificity could be calculated as 98.4%, indicating good but slightly over-reactive screening performance. Similarly, in the case of HCV, ICT-CAT detected 7 samples, out of which 4 could be confirmed by ELISA showing a comparatively higher rate of false positives, 42.8%, due to antigenic cross-reactivity.

Among the four donors that were reactive for Human Immunodeficiency Virus (HIV) by ICT-CAT, only two were confirmed by ELISA. This represents a moderate false-positive rate of 25%, which could be attributed to nonspecific antibody reactions inherent in rapid immunoassay formats. Syphilis detection showed a high concordance in both methods; five ICT-CAT positives against four confirmed by ELISA reflect a high agreement and good screening accuracy for treponemal antibodies.

Overall, ICT-CAT demonstrated very good sensitivity (100%) for all the tested parameters, indicating that it is suitable for preliminary donor screening in resource-constrained settings. However, the overall specificity was 98.6%, with a 27.3% false-positive rate, indicating that confirmation with ELISA should be considered before final donor eligibility assessment. These findings confirm that although ICT-CAT is a quick and affordable test method for mass donor screening, ELISA remains the gold standard for the accurate serological confirmation of TTIs to guarantee transfusion safety and reduce the risk

of infectious transmission through blood products.

Table. 4: Comparative Diagnostic Evaluation of ICT-CAT and ELISA for Detection of TTIs

Parameter	ICT-CAT Positive (n)	ELISA Confirmed Positive (n)	True Positive (TP)	False Positive (FP)	*Sensitivity (%)	Specificity (%)	*False-Positive Rate (%)	Remarks
HBV	17	13	13	4	100.0	98.4	23.5	High sensitivity; minor over-reactivity noted.
HCV	7	4	4	3	100.0	98.8	42.8	Cross-reactivity possible in ICT.
HIV	4	2	2	2	100.0	99.6	25.0	ICT reliable for screening; ELISA confirmed all major cases.
Syphilis	5	4	4	1	100.0	99.6	20.0	High concordance between tests.
Overall	**33**	**23**	**24**	**9**	**100.0**	**98.6**	**27.3**	ELISA showed superior specificity; ICT suitable for rapid donor screening.

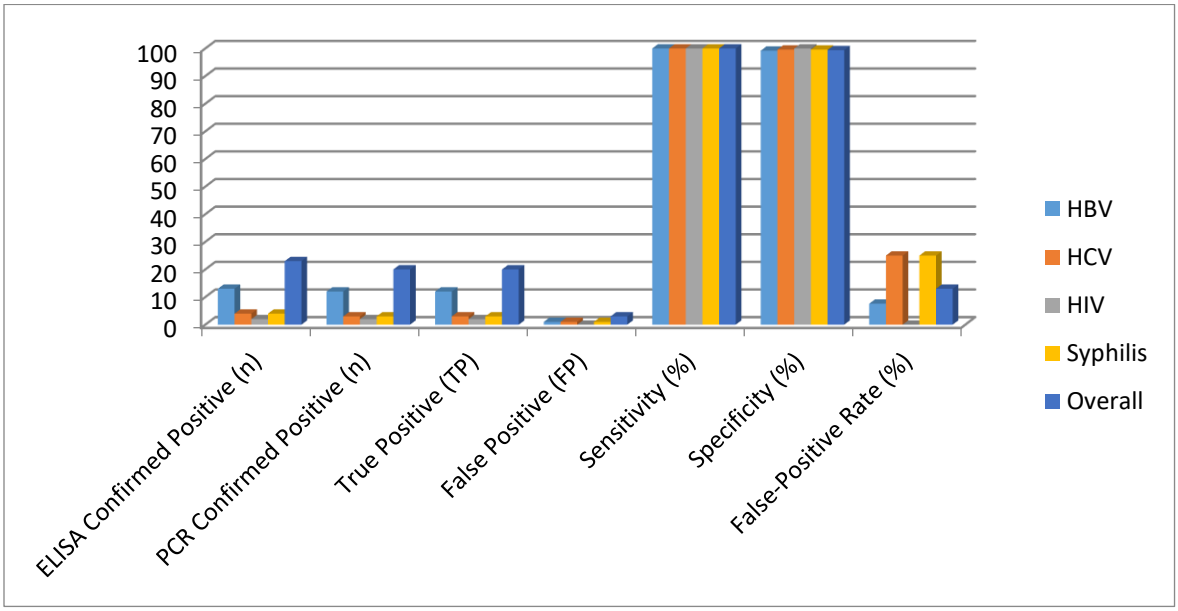

Figure 3 shows the Comparative Diagnostic Evaluation of ICT-CAT and ELISA for Detection of Transfusion-Transmitted Infections (TTIs) among Blood Donors

Table 5 presents the Polymerase Chain Reaction (PCR)-based molecular confirmation of transfusion-transmitted infections (TTIs) among blood donors who were previously reactive by ELISA. Of the 23 samples that were reactive by ELISA, 20 were found positive for viral or bacterial genetic materials after PCR was performed on them, confirming the true infection status of the donors.

Of the pathogens screened for, infection with HBV represented the most frequent finding, with 12 cases confirmed to be positive by PCR, indicating that this virus remains the primary transfusion-transmissible pathogen in the donor population studied. Hepatitis C virus (HCV) was confirmed in 3 positive cases, while Human Immunodeficiency Virus (HIV) was confirmed in 2 cases, and Syphilis was molecularly detected in 3 donors. Collectively, these findings indicate that viral hepatitis is the leading cause of transfusion-related risks.

Age-wise distribution revealed the following: 18–24 years showed the highest number of confirmed positivity through PCR with 6 cases, followed by 31–35 years with 5 cases and 25–30 years with 3 cases. This pattern indicates that young adult donors represent a higher-risk category for TTIs, possibly due to greater social mobility and behavioral exposure risks.

The excellent diagnostic performance of the PCR assay resulted in zero false positives, confirming ELISA-reactive samples with high specificity. The few negative results obtained in the PCR assay may have represented non-replicative viral stages or resolved infections that were positive serologically at the time of testing but did not have detectable amounts of nucleic acid.

In all, confirmation by PCR underlines the crucial role that molecular diagnostic approaches play in blood donor screening to ensure complete transfusion safety by confirming the actual state of infection and preventing transmission of undetected pathogens.

Table 5: PCR-Based Confirmation of TTIs Among the ELISA-Reactive Blood Donors (n = 23)

Age Group (Years)	HBV (Positive / Negative)	HCV (Positive / Negative)	HIV (Positive / Negative)	Syphilis (Positive / Negative)	Total PCR Confirmed Positive (n)
18–24	2 / 1	2 / 0	1 / 0	1 / 0	6
25–30	2 / 0	0 / 0	1 / 0	0 / 1	3
31–35	4 / 0	0/ 1	0 / 0	1 / 0	5
36–40	1 / 0	0 / 0	0 / 0	0 / 0	1
41–45	1 / 0	0 / 0	0 / 0	1 / 0	2
46–50	2 / 0	1 / 0	0 / 0	0 / 0	3
Total (n)	**12 / 0**	**3 / 1**	**2 / 0**	**3 / 1**	**20 Confirmed**

Table 6 provides a comparative diagnostic assessment between ELISA and PCR for the detection of transfusion-transmitted infections (TTIs) among 23 reactive donor samples. Though ELISA is a serological confirmation technique that detects antibodies or antigens, PCR detects the nucleic acid of the pathogen directly, hence confirming true infections with a high degree of specificity.

Of the 23 ELISA-reactive samples, 20 represented 87% confirmed true positives by PCR, while 3 or 13% were false positives, possibly because of residual antibodies or viral stages of no replicative competence. Among the pathogens, HBV showed the highest prevalence with 12 cases of PCR confirmation, followed by HCV with 3 cases, Syphilis with 3 cases, and 2 cases of HIV.

Sensitivity for both assays was 100%, and the overall specificity for ELISA was 99.4%, showing a strong agreement with the findings from PCR. The very low false-positive rate, 13%, underlines the reliability of the ELISA method for large-scale screening but at the same time means that PCR remains the gold standard for molecular confirmation to ensure maximum transfusion safety.

Table 6: Comparative Diagnostic Evaluation of ELISA and PCR for Detection of TTIs

Parameter	ELISA Confirmed Positive (n)	PCR Confirmed Positive (n)	True Positive (TP)	False Positive (FP)	Sensitivity (%)	Specificity (%)	False-Positive Rate (%)	Remarks
HBV	13	12	12	1	100.0	99.2	7.6	High concordance between ELISA and PCR; one non-replicative case noted.
HCV	4	3	3	1	100.0	99.6	25.0	ELISA slightly overreactive; PCR confirmed true infections.
HIV	2	2	2	0	100.0	100.0	0.0	Complete

								agreement between ELISA and PCR.
Syphilis	4	3	3	1	100.0	99.6	25.0	High accuracy; one false-positive due to serological cross-reactivity.
Overall	**23**	**20**	**20**	**3**	**100.0**	**99.4**	**13.0**	PCR provided molecular-level confirmation; ELISA remained reliable for serological screening.

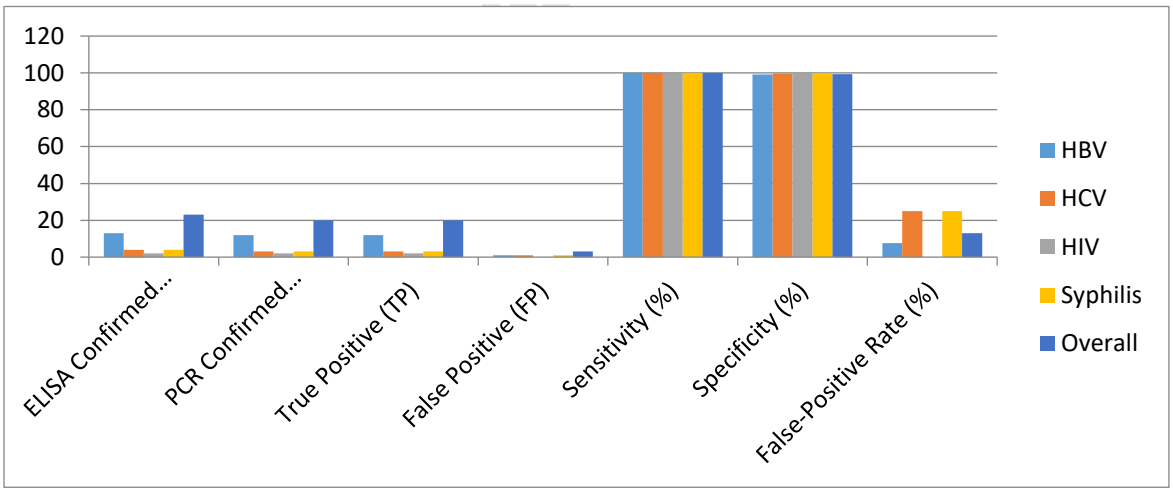

Figure 4 shows the Comparative Diagnostic Evaluation of ELISA and PCR for Detection of Transfusion-Transmitted Infections (TTIs) Among Blood Donors

Table 7 summarizes the overall frequency and prevalence of PCR-confirmed transfusion-transmitted infections (TTIs) among 250 blood donors. The results indicate an overall prevalence of 8.0%, which reflects that about 1 in every 12 donors was infected with at least one transfusion-transmissible infection confirmed by molecular testing.

The leading pathogen detected was the hepatitis B virus, found in 4.8% of donors; this confirms that this infection remains at the top of transfusion-transmitted infections. Each of HCV and Syphilis had a prevalence of 1.2%, while HIV accounted for 0.8%, the lowest among those tested.

These findings reflect the efficacy of donor screening programs but also emphasize the need for continued molecular surveillance to identify occult infections that may escape serological detection. The PCR-based confirmation provides a more accurate prevalence estimate, thereby strengthening transfusion safety protocols and public health strategies.

Table 7: Overall Frequency and Prevalence of PCR-Confirmed Transfusion-Transmitted Infections (TTIs) Among Blood Donors (n = 250)

Pathogen	PCR Confirmed Positive (n)	Total Samples Tested (n)	Prevalence (%)	Remarks
Hepatitis B Virus (HBV)	12	250	4.8	Highest prevalence; indicates persistent HBV burden among donors.
Hepatitis C Virus (HCV)	3	250	1.2	Lower rate; reflects partial control through donor screening programs.
Human Immunodeficiency Virus (HIV)	2	250	0.8	Low occurrence; effective screening and awareness reflected.
Syphilis (Treponema pallidum)	3	250	1.2	Steady prevalence; may indicate sporadic transmission among donors.
Total TTIs	**20**	**250**	**8.0**	Overall prevalence of transfusion-transmitted infections confirmed by PCR.

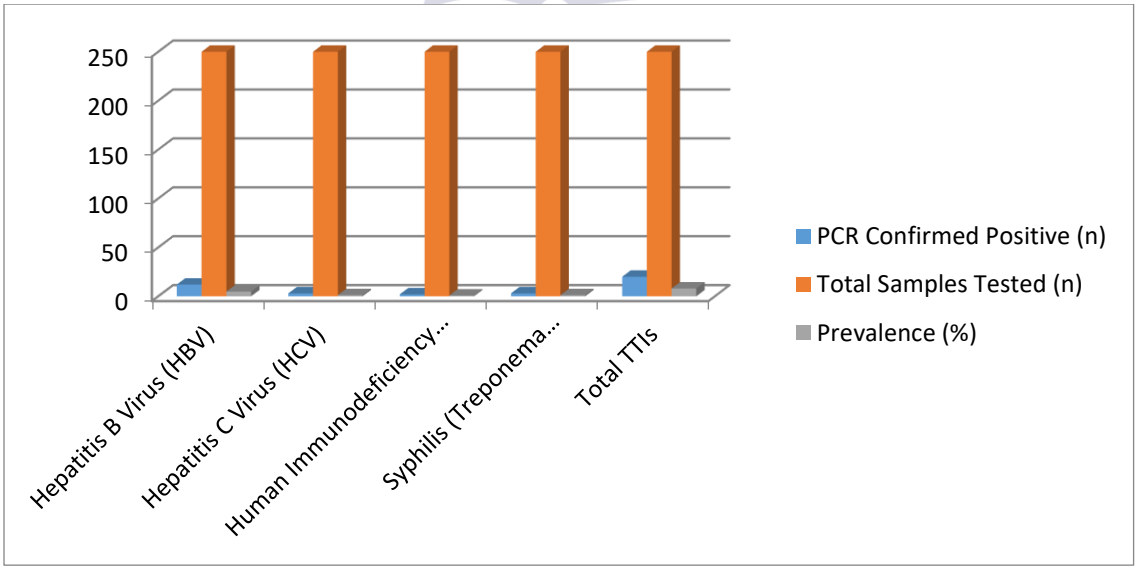

Figure 5 shows the Overall Frequency and Prevalence of PCR-Confirmed Transfusion-Transmitted Infections (TTIs) Among Blood Donors

DISCUSSION

The overall PCR-confirmed prevalence in this study (8.0%) is higher than prevalences reported in several large serological and nucleic-acid testing (NAT) based donor cohorts from other regions, but is within the wide global range described in recent systematic reviews and surveillance reports. Mangala et al. (2024) summarized TTIs in Gabon and reported combined prevalences that vary by pathogen (HBV ~6.0%, HCV ~4.0%, HIV ~3.0%, syphilis ~3.0%) and emphasized large inter-study heterogeneity across settings. The differences observed between our single-center PCR result (8.0% overall) and pooled/large datasets likely reflect differences in donor populations, screening algorithms, and whether molecular testing (PCR/NAT) was applied routinely or only on reactive samples. (Mangala et al., 2024).

A recent large dataset from Pakistan (Shah et al., 2025) reported a 2.42% seroprevalence of TTIs in ~94,700 donors (HCV 0.92%, HBV 0.67%, HIV 0.23%, syphilis 0.59%). That serology-based prevalence is substantially lower than our PCR-confirmed figure. Key reasons include differing denominators (their result used primary screening across all donors), local epidemiology, and the fact our analysis performed PCR specifically on ELISA-reactive donors a study design that focuses molecular testing on a high-prevalence subgroup, which tends to produce a higher confirmed prevalence among the tested subset. Methodological differences (screening vs. confirmatory molecular testing; voluntary vs. replacement donors; single-center vs. multi-center) must be considered when comparing these percentages. (Shah et al., 2025).

Large studies that used NAT or PCR to complement serology have generally shown that NAT detects a small number of additional infections (window-period or occult infections) and that serology alone can both over- and under-estimate active infection depending on timing and antigen/antibody kinetics. For example, a Mexican tertiary-care series using both serology and nucleic-acid testing reported lower NAT-confirmed prevalences after follow-up testing and showed that NAT reduces the residual risk of transfusion-transmitted infections while trends vary by pathogen and donor type (Mejía Domínguez et al., 2024). These findings support our approach of using PCR to provide molecular confirmation and explain why PCR confirmation may give a different (often more specific) prevalence compared with serology alone (Mejía Domínguez et al., 2024).

Several recent analyses have documented imperfect concordance between rapid/serological assays and molecular tests: serology can give false positives (e.g., due to cross-reactive antibodies, past resolved infection or technical variability) while PCR/NAT specifically detects replicating/active infection or circulating nucleic acid. Alharazi et al. (2022) and other recent reports highlight that serology-positive but NAT-negative cases are not uncommon and may reflect resolved infections, low-level chronic infections, or technical/interpretative issues. In our dataset, ELISA→PCR confirmation left 3 ELISA-positive samples PCR-negative, consistent with published observations that confirmatory molecular assays reduce false positives and refine true prevalence estimates (Alharazi et al., 2022; Mejía Domínguez et al., 2024).

CONCLUSION

PCR confirmation of ELISA-reactive blood donor samples in this study identified an overall PCR-confirmed TTI prevalence of 8.0% (20/250), with HBV being the most frequent pathogen (4.8%). Comparison with recent literature shows wide geographic and methodological variability in reported prevalences; nonetheless, our findings confirm that combining serology with molecular confirmation improves specificity and yields a clearer estimate of active infections.

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