COMPARATIVE EVALUATION OF HIGH-THROUGHPUT ELISA WITH RAPID DIAGNOSTIC TEST (RDT) IN BLOOD CENTRE SCREENING

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Background: Blood transfusion is a critical component of modern healthcare but carries the risk of transmitting transfusion-transmissible infections (TTIs), particularly human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV). Reliable and efficient screening methods are essential to ensure blood safety. While rapid diagnostic tests (RDTs) are widely used due to their simplicity, concerns remain regarding their sensitivity compared to high-throughput enzyme-linked immunosorbent assay (ELISA).
Objective: To compare the diagnostic accuracy and operational efficiency of high-throughput ELISA and rapid diagnostic tests for the detection of TTIs among blood donors.
Materials and Methods:A cross-sectional analytical study was conducted over 12 months at a tertiary care blood center, including 1,200 donor samples. All samples were screened for HIV, HBsAg, and HCV using both high-throughput ELISA and RDTs. Diagnostic performance was evaluated by calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with 95% confidence intervals (CI). The difference between methods was assessed using the chi-square test, with $p < 0.05$ considered statistically significant.
Results: High-throughput ELISA detected 67 (5.59%) reactive cases, whereas RDTs identified 57 (4.76%) cases, indicating a 12% higher detection rate. ELISA demonstrated superior diagnostic performance with sensitivity of 99.5% (95% CI: 94.58–100%), specificity of 99.8% (95% CI: 99.36–99.95%), PPV of 97.2% (95% CI: 90.03–99.20%), and NPV of 99.9% (95% CI: 99.66–100%). In comparison, RDTs showed sensitivity of 94.5% (95% CI: 85.63–97.65%) and specificity of 98.7% (95% CI: 97.83–99.19%). The difference in detection rates between the two methods was statistically significant ($p < 0.05$). ELISA also demonstrated improved operational efficiency, processing all samples within approximately 4 hours.
Conclusion: High-throughput ELISA is a highly sensitive, specific, and efficient method for blood donor screening and significantly outperforms rapid diagnostic tests in detecting TTIs. Its implementation in blood centers can enhance transfusion safety and reduce the risk of undetected infections.
Keywords: Blood donor screening; transfusion-transmissible infections; high-throughput ELISA; HIV; HBsAg; HCV
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INTRODUCTION

Blood transfusion remains an essential life-saving intervention in modern healthcare, playing a critical role in the management of trauma, major surgeries, obstetric emergencies, anemia, and hematological disorders. However, despite its clinical benefits, transfusion carries an inherent risk of transmitting transfusion-transmissible infections (TTIs), particularly human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV), which continue to pose significant global health challenges. Recent evidence indicates that TTIs remain a persistent concern, especially in developing countries, necessitating continuous improvement in screening strategies (1).

Ensuring the safety of donated blood depends largely on the use of sensitive and reliable screening methods. Serological assays such as rapid diagnostic tests (RDTs) and enzyme-linked immunosorbent assay (ELISA) are commonly employed in blood centers. RDTs are widely used due to their rapid turnaround time, ease of use, and cost-effectiveness, particularly in resource-limited settings. However, multiple recent studies have demonstrated that rapid tests have comparatively lower sensitivity and may fail to detect infections with low antigen or antibody

levels, leading to false-negative results and increased residual risk (2).

Conventional ELISA methods provide improved sensitivity and specificity compared to RDTs, but they are often limited by manual processing and longer turnaround time. Recent advancements in diagnostic technologies have led to the development of automated and high-throughput ELISA systems, which enable large-scale screening with enhanced accuracy and reproducibility. Studies from India and other regions have highlighted the importance of advanced immunoassay techniques, including automated ELISA and chemiluminescence-based methods, in improving blood donor screening and reducing missed infections (3).

Furthermore, newer screening approaches such as nucleic acid testing (NAT) have demonstrated the ability to detect infections missed by serological assays, particularly during the window period. However, due to high cost and technical requirements, NAT is not routinely implemented in many resource-limited settings, making optimized serological methods like high-throughput ELISA a practical alternative (4,5).

Recent comparative studies have consistently shown that ELISA-based methods outperform rapid diagnostic tests in terms of sensitivity, specificity, and overall diagnostic accuracy in detecting TTIs among blood donors (6,7).

However, limited comparative data exist regarding high-throughput ELISA versus rapid diagnostic tests in resource-limited blood centers in India**.** Most available studies either focus on conventional ELISA or do not simultaneously evaluate operational efficiency along with diagnostic accuracy.

Therefore, the present study was undertaken to compare the diagnostic accuracy and operational efficiency of high-throughput ELISA with rapid diagnostic tests for the detection of HIV, HBsAg, and HCV among blood donors. The findings of this study aim to provide evidence-based insights for improving donor screening strategies and enhancing transfusion safety in similar healthcare settings

MATERIALS AND METHODS

Study Design and Setting

This cross-sectional analytical study was conducted at the Blood Centre of Venkateshwara Institute of Medical Sciences,Uttar Pradesh, India, over a period of 12 months from [Feb2025] to [Feb 2026].

Ethical Approval

The study was approved by the Institutional Ethics Committee of Venkateshwara Institute of Medical Sciences. Written informed consent was obtained from all participants prior to sample collection, and donor confidentiality was strictly maintained in accordance with national and international guidelines (8,9).

Sample Size Calculation

The sample size was calculated based on the expected prevalence of transfusion-transmissible infections among blood donors, using a 95% confidence level and a 5% margin of error (10). Accordingly, a minimum required sample size was estimated, and a total of 1,200 donor samples were included in the study to ensure adequate statistical power.

Study Population

A total of 1,200 consecutive blood donors, including both voluntary and replacement donors, were enrolled. Donors aged between 18 and 60 years, weighing ≥50 kg, and fulfilling eligibility criteria as per national guidelines (Drugs and Cosmetics Act, India) were included (9,11).

Exclusioncriteria:

Donors with a history of high-risk behavior (e.g., intravenous drug use, multiple sexual partners), recent blood transfusion, or chronic illness were excluded. Demographic details such as age, sex, and type of donation were recorded. Repeat donations from the same individual were counted only once.

Sample Collection and Processing

Approximately 5 mL of venous blood was collected from each donor in a plain vacutainer tube. Serum was separated within 2 hours of collection and stored at 2–8°C until analysis. All samples were processed within 24 hours to maintain sample integrity.

Screening Methods

Rapid Diagnostic Tests (RDTs)

Rapid immunochromatographic assays were used for the qualitative detection of:

- HBsAg (Hepacard)

- Anti-HCV antibodies (HCV TRI-DOT)
- Anti-HIV antibodies (HIV TRI-DOT)

Tests were performed and interpreted according to the manufacturer's instructions.

High-Throughput ELISA

Fourth-generation ELISA kits were used for the detection of:

- HBsAg (Hepalisa)
- Anti-HCV antibodies (HCV Microlisa)
- HIV-1 & HIV-2 antibodies and p24 antigen (HIV Ag & Ab Microlisa)

All ELISA procedures were performed using automated high-throughput systems following standard protocols (12).

Reference Standard

In this study, high-throughput ELISA was considered the reference method for comparative evaluation. However, to improve diagnostic reliability, all initially reactive samples were retested in duplicate using ELISA, and discrepant results between ELISA and RDTs were carefully re-evaluated. Due to resource constraints, nucleic acid testing (NAT) could not be performed, which is acknowledged as a limitation.

Quality Control

Internal quality controls provided with the kits were included in each run. External quality assurance was maintained through participation in national proficiency testing programs. Assay runs were repeated if control values fell outside the acceptable range (±2 SD) (12).

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Diagnostic performance parameters including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated with 95% confidence intervals (13, 14).

The association between screening methods was evaluated using the Chi-square (χ^2) test, calculated as:

$$\chi^2 = \Sigma [(O - E)^2 / E]$$

where O represents observed frequency and E represents expected frequency.

A p-value < 0.05 was considered statistically significant.

Biosafety

All procedures were conducted in accordance with standard biosafety guidelines and biomedical waste management rules (11).

RESULTS

In our study, A total of 1200 blood donors sample collected from Venkateshwara institute of medical sciences blood Centre. Out of these donor samples 67 (5.59%) were found reactive for one or more transfusion-transmissible infections (TTIs) by high-throughput ELISA, while rapid diagnostic tests (RDTs) identified 57 (4.76%) reactive samples. Thus, high-throughput ELISA detected 10 additional seropositive cases, representing a 12% increase in detection compared with RDTs shows in table.1 (15,18).

Table.3 shows High-throughput ELISA demonstrated superior diagnostic performance, with a sensitivity of 99.5% (95% CI: 94.58–100%), specificity of 99.8% (95% CI: 99.36–99.95%), positive predictive value (PPV) of 97.2% (95% CI: 90.03–99.20%), and negative predictive value (NPV) of 99.9% (95% CI: 99.66–100%).

In comparison, RDTs showed a sensitivity of 94.5% (95% CI: 85.63–97.65%), specificity of 98.7% (95% CI: 97.83–99.19%), PPV of 92.1% (95% CI: 70.66–87.98%), and NPV of 99.1% (95% CI: 99.08–99.86%). The difference in detection rates between high-throughput ELISA and RDTs was statistically significant (Chi-square test, $p<0.05$) (8,9,20).

The narrow confidence intervals observed for high-throughput ELISA indicate high precision and reliability of the assay for routine blood donor screening.

Table .1 Comparative Evaluation of RAPIDTest Kits with ELISA for Transfusion- Transmissible Infection (TTI) among blood donors (n=1200).

2Tests	Reactive by ELIS	Percentage Reactiv	Reactive by RAPID	Percentage Reactiv	False Negative by RAPI

	A	ity	Tests	ity	D Tests
HIV	02	0.17%	02	0.17%	0
HBV	21	1.75%	20	1.67%	01
HCV	44	3.67%	35	2.92%	09
Total	67	5.59%	57	4.76%	10

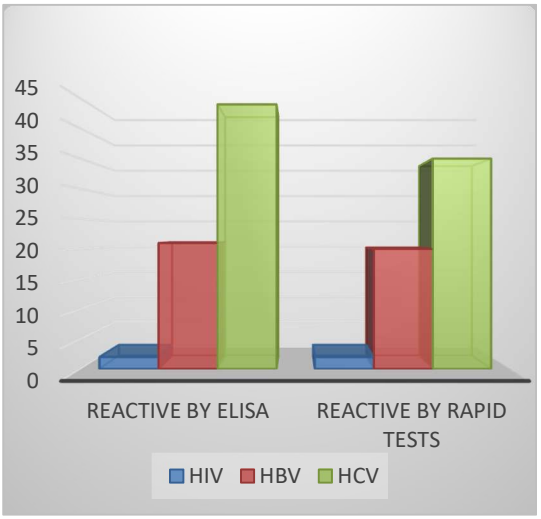

Fig.1 Comparative Evaluation of RAPIDTest Kits with ELISA for Transfusion- Transmissible Infection (TTI) among blood donors (n=1200).

Table .2 Diagnostic performance Comparison of High-Throughput ELISA and Rapid Diagnostic Tests (RDTs) in Blood Donor Screening

Rapid card Test	HCV		HBsAg		HIV	
	ELISA Reactive	ELISA non-reactive	ELISA Reactive	ELISA non-reactive	ELISA Reactive	ELISA non-reactive
Rapid Reactive	35	0	20	0	02	0
Rapid non-reactive	09	1156	01	1179	0	1198
Total Cases	44	1156	21	1179	02	1198

Table 3: Diagnostic Performance of High-Throughput ELISA and Rapid Diagnostic Tests

Parameter	ELISA	95% CI	RDT	95% CI
Sensitivity	99.5%	94.58–100%	94.5%	85.63–97.65%
Specificity	99.8%	99.36–99.95%	98.7%	97.83–99.19%
PPV	97.2%	90.03–99.20%	92.1%	70.66–87.98%
NPV	99.9%	99.66–100%	99.1%	99.08–99.86%

*Sensitivity (also called the true positive rate, the recall, power, hit rate or probability of detection)-refers to a test's ability to designate an individual with disease as positive.

*Specificity (also called the true negative rate or selectivity)-refers to a test's ability to designate an individual who does not have a disease as negative.

*Positive predictive value (PPV also called the precision)-is the ability of an assay to identify actual infected individuals among all persons.

*Negative predictive value (NPV)-is the ability of an assay to identify correctly the real non-infected individuals among persons.

Table.4 Comparison of Rapid Test and ELISA for HBsAg in other studies

Author	Place	Positive by Rapid test			Positive by ELISA		
		HBsAg	HCV	HIV	HBsAg	HCV	HIV
Ijaj H *et al.*, 2012 (6)	**Pakistan**	95	07	12	100	12	15
Adeyemi AA *et al.*, 2013 (1)	**Nigeria**	38	00	08	71	04	11
Mohammad K *et al.*, 2016 (12)	**India (Haryana)**	22	93	05	30	100	07
Bhupendra K et al., (2019)	**India(Dungarpur)**	70	06	02	78	07	02
Present Study (2026)	**India(U.P)**	20	35	02	21	44	02

DISCUSSION

The present study demonstrates that high-throughput ELISA is superior to rapid diagnostic tests (RDTs) in detecting transfusion-transmissible infections (TTIs) among blood donors. A total of 67 (5.59%) seropositive cases were detected by ELISA compared to 57 (4.76%) by RDTs, indicating a 12% higher detection rate. These findings are in agreement with previous studies that have consistently reported higher sensitivity and diagnostic accuracy of ELISA-based assays compared to rapid immunochromatographic methods (6,7,15,16). The improved performance of ELISA observed in this study can be attributed to its enhanced analytical sensitivity and ability to detect lower concentrations of viral markers.

One of the key reasons for the higher detection rate of ELISA is its ability to detect both antigen and antibody, particularly in fourth-generation assays, which improves early diagnosis of infections. In contrast, most rapid diagnostic tests rely predominantly on antibody detection, which may not be sufficient during the early phase of infection. This is particularly relevant in the context of the "window period," during which recently infected individuals may not have developed detectable antibody levels, leading to false-negative results (10,17). Advanced ELISA techniques help reduce this diagnostic gap by identifying infections at an earlier stage.

In the present study, RDTs missed a considerable number of HCV-positive cases (n=9), highlighting their relatively lower sensitivity for HCV detection. This observation is consistent with other studies that have reported reduced performance of rapid tests for HCV due to delayed seroconversion and lower antibody titers in early infection (6,18,16). Additionally, the inherent limitations of lateral flow immunoassays, including lower analytical sensitivity and variability in antigen composition, may further contribute to false-negative results (17,19). The failure to detect such infections is of particular concern in blood transfusion settings, where even a single undetected infected unit can pose a serious risk to recipients.

The findings of this study also align with reports from various regions, including India and other developing countries, where ELISA-based screening has consistently shown higher detection rates compared to rapid tests (15,18,20,21). These studies emphasize the need for more reliable and sensitive screening methods in blood centers, especially in high-prevalence settings. Furthermore, recent advancements in automated and high-throughput ELISA systems have significantly improved processing efficiency, allowing large numbers of samples to be analyzed with greater accuracy and reproducibility (8,9).

From an operational perspective, high-throughput ELISA demonstrated clear advantages in terms of automation, reduced manual error, and faster processing of large sample volumes. Although RDTs

offer the benefit of rapid results and ease of use, their lower sensitivity limits their effectiveness as a standalone screening tool. In contrast, ELISA-based systems provide a more robust approach to blood donor screening, ensuring higher reliability in detecting TTIs.

The public health implications of these findings are significant. The higher detection rate of ELISA suggests that reliance solely on rapid diagnostic tests may lead to underestimation of TTIs and increased residual risk of transfusion-transmitted infections. Strengthening screening protocols by incorporating high-throughput ELISA can substantially improve transfusion safety and reduce disease transmission. Although nucleic acid testing (NAT) remains the gold standard for early detection of infections, its high cost and technical requirements limit its widespread use in resource-constrained settings (10,22). In such scenarios, high-throughput ELISA represents a practical and effective alternative for enhancing blood safety.

CONCLUSION

High-throughput ELISA demonstrated superior diagnostic accuracy and operational efficiency compared to rapid diagnostic tests for screening transfusion-transmissible infections among blood donors. Its enhanced sensitivity allows better detection of infections that may be missed by rapid methods, thereby improving the safety of blood transfusion services. While rapid tests may still be useful for preliminary or resource-limited settings, reliance on ELISA-based screening provides a more reliable approach for routine blood donor evaluation. Strengthening screening protocols through the adoption of high-throughput ELISA can play a crucial role in reducing transfusion-related infection risks and improving overall public health outcomes.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Farheen Jahan: Visualization, Conceptualization, Methodology, writing – original draft, writing – review & editing.

Dr. Rashi srivastava: Supervision.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

LIMITATIONS

This study was conducted at a single center, which may limit the generalizability of the findings. Nucleic acid testing (NAT) was not performed, so early window-period infections could not be assessed. Additionally, the inclusion of both voluntary and replacement donors may introduce potential selection bias.

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