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Original Research Paper

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DUAL SCREENING FOR TRANSFUSION-TRANSMITTED INFECTIONS IN BLOOD DONORS: A RETROSPECTIVE THREE-YEAR ANALYSIS

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ABSTRACT Blood safety is crucial in preventing transfusion-transmitted infections (TTIs). This study analysed the effectiveness of dual testing methodology (ELISA and Nucleic Acid Testing) for TTIs in blood donors over a three-year period (2022-2024). We tested 5,377 donor samples for HIV, HBV, and HCV using both 4th generation ELISA and NAT. Discordant results (ELISA+/NAT- or ELISA-/NAT+) were analysed to evaluate the effectiveness of this dual testing strategy. The overall TTI prevalence was 1.62% (87/5,377) with HBV showing the highest prevalence at 0.67% (36/5,377). Discordant results comprised 0.76% (41/5,377) of all samples, with ELISA+/NAT- being more common than ELISA-/NAT+ (0.69% vs 0.07%). NAT identified 4 additional TTI-positive donors (0.07%) that would have been missed by ELISA alone. Our findings support the implementation of dual testing methodology to enhance blood safety, particularly in detecting window period infections. This approach fulfils ethical obligations to provide optimally tested blood products while adhering to regulatory requirements outlined in the Drugs and Cosmetics Act.

KEYWORDS : Transfusion-Transmitted Infections; Enzyme Linked Immuno-Sorbent Assay; Nucleic Acid Test; Discordant Results; Window Period

INTRODUCTION

Blood transfusion remains a critical medical intervention for various clinical scenarios, yet ensuring blood safety is paramount to prevent transfusion-transmitted infections (TTIs). The risk of TTIs, particularly HIV, hepatitis B virus (HBV), and hepatitis C virus (HCV), poses a significant challenge to blood transfusion services worldwide.[1] Testing methodologies for these pathogens have evolved significantly over decades, with enzyme-linked immunosorbent assay (ELISA) and nucleic acid testing (NAT) becoming cornerstones of blood screening protocols.

As professionals working in blood centres, we have a moral obligation to provide completely safe blood to patients. This requires strict adherence to a zero-tolerance approach—there can be no room for error in TTI testing. Iatrogenic TTI from infected blood could result in significant lifelong trauma as well as financial and emotional challenges for patients.

ELISA detects antibodies or antigens produced in response to infection, while NAT directly detects viral nucleic acids.

The major advantage of NAT is its ability to detect infections during the "window period"—the time between infection and the appearance of detectable antibodies or antigens—thereby reducing the residual risk of TTIs.[2] However, NAT implementation involves significant costs, leading to debates about the cost-effectiveness of dual testing strategies, particularly in resource-limited settings.

According to the Asian Association of Transfusion Medicine's 'Consensus Document on Prevention of TTI: Future Directions,' adopting Individual Donor Nucleic Acid Testing (ID-NAT) across all blood banks could potentially prevent approximately 90,000 new infections annually in India.

A 1996 Supreme Court judgment in India mandated the government to ensure a safe and accessible blood supply, banning professional blood donation and regulating blood banks. The government also developed the National Blood Policy in 2002 directing the government to provide safe blood for all and prevent any infections or diseases that might spread through transfusions. Since establishing a NAT lab may not be financially viable for smaller blood banks, outsourcing to specialized laboratories presents a practical solution.

In India, the regulatory framework mandated by the Drugs and Cosmetics Act also requires rigorous testing of blood units for TTIs. While ELISA testing is widely implemented, NAT is increasingly being adopted to enhance blood safety. We implemented dual testing at our blood centre in 2021, reflecting our commitment to rigorous testing and the ethical obligation to provide optimally tested blood products while balancing resource utilization.[3]

This study presents a three-year analysis of TTI screening results using both ELISA and NAT methodologies at our blood centre. We specifically focus on discordant results between these two methods to evaluate the value of dual testing in enhancing blood safety.

The findings contribute to the ongoing discourse on optimal TTI screening strategies in blood transfusion services.

METHODS

Study Design and Population

This retrospective observational study analysed TTI screening results from blood donors at our blood centre over three consecutive years (2022-2024). All donor samples were subjected to both ELISA and NAT testing as per Standard Operating Procedures. The study was conducted in accordance with ethical guidelines, ensuring donor confidentiality throughout the analysis.

Testing Methodology

All blood donations were screened using 4th generation ELISA for HIV (antibodies to HIV-1, HIV-2, and p24 antigen), HBsAg, and antibodies to HCV. Syphilis testing was performed using the Treponema Pallidum Hemagglutination Assay (TPHA) cards. Concurrently, samples were outsourced for NAT, which was performed in a two-tiered approach at another lab under the aegis of Hemogenomics. Their methodology involves Initial multiplex NAT to screen for HIV, HBV, and HCV simultaneously. Samples yielding positive results in the multiplex assay undergo discriminatory NAT to identify the specific viral nucleic acid (HIV RNA, HBV DNA, or HCV RNA). Samples were also tested for malaria through ICT cards, though no positive cases were detected, likely because symptomatic individuals would not present for donation or would be deferred during screening.

Data Collection And Analysis

Data were extracted from the blood centre's records, specifically ELISA and NAT results for each donation. Samples were categorized based on concordant and discordant results between ELISA and NAT. Discordant results were further classified as ELISA+/NAT- or ELISA-/NAT+. The total number of units collected and discarded due to TTIs for each year was recorded.

Data analysis included calculation of prevalence rates for individual TTIs and evaluation of the contribution of NAT in identifying additional TTI-positive donors. The percentage of discordant results and their implications for blood safety were assessed.

RESULTS

During the study period, a total of 5,377 blood units were collected across three years: 1,545 units in 2022, 1,772 units in 2023, and 2,060 units in 2024. We anticipate reaching approximately 2,500 collections by the end of 2025.

A total of 87 blood units (1.62%) were discarded due to TTI reactivity over the three-year period: 25 units (1.62%) in 2022, 34 units (1.92%) in 2023, and 31 units (1.50%) in 2024. The prevalence of individual TTIs across the study period is presented in Table I.

TTI	2022	2023	2024	Total	Prevalence (%)
HIV	6	4	4	14	0.26
HBV	11	15	10	36	0.67
HCV	6	10	14	30	0.56
Syphilis	2	5	3	10	0.19
Total	25	34	31	90*	1.67

*Note: Three donors had dual infections (Two cases HBV + Syphilis and one case HIV + HBV), resulting in 87 unique TTI-positive donors.

Analysis of the concordance and discordance between ELISA and NAT results revealed several patterns across the three viral markers (HIV, HBV, and HCV), as presented in Table II.

Year	TTI	ELISA+/NAT-	ELISA-/NAT+	ELISA+/NAT+	Total
2022	HIV	6	0	0	6
	HBV	2	0	9	11
	HCV	2	1	3	6
2023	HIV	4	0	0	4
	HBV	2	1	12	15
	HCV	7	0	3	10
2024	HIV	4	0	0	4
	HBV	0	2	8	10
	HCV	10	0	4	14
Total	HIV	14	0	0	14
	HBV	4	3	29	36
	HCV	19	1	10	30
	All TTIs	37	4	39	80

A total of 39 samples (0.73% of all donations) showed concordant positive results (ELISA+/NAT+). HBV had the highest number of concordant positive results (29), followed by HCV (10).

Overall, 41 samples (0.76% of all donations) exhibited discordant results:

1. ELISA+/NAT-: 37 samples (0.69%) showed this pattern, with HCV accounting for the highest number (19), followed by HIV (14) and HBV (4).

2. ELISA-/NAT+: Only 4 samples (0.07%) showed this pattern, including 3 for HBV and 1 for HCV. These represent window period infections that would have been missed by ELISA screening alone.

DISCUSSION

This three-year analysis of dual testing for TTIs reveals the complementary roles of ELISA and NAT in enhancing blood safety. The overall TTI prevalence of 1.62% in our donor population is comparable to figures reported in other studies from similar settings. , HBV showed the highest prevalence (0.67%), followed by HCV (0.56%) and HIV (0.26%), which aligns with the epidemiological pattern of these infections in our region.

The discordant results between ELISA and NAT merit particular attention as they provide insights into the limitations of individual testing methodologies and justify the implementation of dual testing strategies.

ELISA+/NAT- Results

The ELISA+/NAT- pattern, observed in 37 samples (0.69%), could be attributed to several factors:

1. False-positive ELISA results: ELISA may yield false-positive results due to cross-reactivity with other antibodies or technical issues. This is particularly relevant for low-reactive ELISA results, which often do not correlate with true infection status.

2. Resolved infections: This pattern can also represent resolved infections where antibodies persist but viral nucleic acid is no longer detectable. This is particularly common in HCV, where spontaneous clearance can occur in approximately 15-45% of infected individuals.

3. Low viral load: In some cases, the viral load may be below the detection threshold of NAT, particularly in chronic HBV carriers with low-level viremia.

While ELISA+/NAT- results may suggest potential false positives, the cautious approach of discarding such units is justified from a blood safety perspective, given the residual risk of infection and the ethical obligation to provide optimally tested products to recipients. It also helps identify donors who may need counselling regarding future blood donation and timely medical management.

The limitations of ELISA as a manual procedure with potential subjective variability in recording optical density must also be acknowledged.

ELISA-/NAT+ Results

The identification of 4 ELISA-/NAT+ samples (0.07%) represents a critical finding, as these cases would have been missed by ELISA screening alone. These likely represent window period infections, where viral replication precedes the development of detectable antibodies or antigens.

The detection of these cases highlights the value of NAT in enhancing blood safety by identifying donors in the pre-seroconversion phase. While the frequency is relatively low (0.07%), each prevented TTI represents a significant public health benefit, especially considering the potential for transmission to multiple recipients from a single donation.[2]

Our findings have several implications for blood safety strategies:

1. Justification for Dual Testing: The identification of 4 ELISA-/NAT+ cases supports the continued implementation of NAT alongside ELISA, despite the additional cost and resource utilization. The incremental yield of NAT, though small, represents a significant enhancement in blood safety.

2. Handling of Discordant Results: The relatively high frequency of ELISA+/NAT- results (0.69%) raises questions about the optimal approach to such discordant results. While current practice involves discarding all reactive units, further confirmatory testing might help distinguish true infections from false positives.

3. Cost-Effectiveness Considerations: The implementation of dual testing inevitably increases the cost per unit tested. However, this must be weighed against the medical, economic, and social costs of transfusion-transmitted

infections.

The implementation of dual testing aligns with the ethical obligation to minimize harm to recipients through the provision of optimally tested blood products.

The strict adherence to donor deferral criteria, as stipulated in the Drugs and Cosmetics Act, complements the mandatory rigorous testing strategies by excluding high-risk donors.[3] This multi-layered approach to blood safety reflects the comprehensive risk reduction strategy advocated by regulatory authorities.

Limitations Of Our Study

The retrospective design limits the ability to conduct follow-up testing on donors with discordant results to determine their true infection status. It is difficult to follow up donors who may be from distant locations and reluctant to pursue further testing. Testing positive also involves informing spouses and obtaining their workup, which may not be feasible in many cases.

CONCLUSION

This three-year analysis of TTI screening results using dual testing methodology underscores the complementary roles of ELISA and NAT in enhancing blood safety.

While ELISA identified the majority of TTI-positive donors, NAT detected additional cases that would have been missed by ELISA alone, particularly those in the window period. The findings support the continued implementation of dual testing strategies to fulfil the ethical obligation of providing optimally tested blood products to recipients.

Strict adherence to donor deferral criteria, as mandated by the Drugs and Cosmetics Act, alongside comprehensive testing strategies, represents a multi-layered approach to blood safety that aligns with regulatory requirements and ethical standards.

Future research should focus on cost-effectiveness analyses of dual testing strategies and exploration of newer technologies to further reduce the residual risk of TTIs.

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