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Transfusion-Transmissible Infection Screening Reactivity, Temporal Trends, and Post-donation Counselling and Referral at a Tertiary Care Centre in North India: A Retrospective Record-Based Study

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Abstract

Background and aim: Transfusion-transmissible infections (TTIs) remain an important blood-safety concern, and longitudinal donor-screening data can identify changing local patterns. This study primarily assessed overall TTI screening reactivity and, secondarily, evaluated infection-specific reactivity, temporal trends, demographic characteristics of reactive donors, and documented post-donation counselling and referral at a tertiary-care centre in North India.

Methods: This retrospective record-based study included 5,163 blood donations screened during 2021-2026, with 2026 treated as a partial-year/year-to-date (YTD) period. Reactivity for HIV, hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV), syphilis, and malaria was analysed. Temporal trends were evaluated across the complete calendar years from 2021 to 2025.

Results: Overall TTI screening reactivity was 129/5,163 (2.50%; 95% CI, 2.11-2.96%). HBsAg was the predominant reactive marker, at 66/5,163 (1.28%), followed by HCV, 36/5,163 (0.70%), syphilis, 18/5,163 (0.35%), malaria, 6/5,163 (0.12%), and HIV, 3/5,163 (0.06%). Overall reactivity differed significantly across 2021-2025 (Pearson $\chi^2 = 17.209$, df = 4, Cramer's V = 0.061, p = 0.0018), indicating a small year-wise association, and showed a significant increasing trend (Cochran-Armitage Z = 3.163, p = 0.0016). Syphilis screening reactivity also showed an increasing trend (Z = 2.996, p = 0.0027). The mean age of reactive donors was 32.5 ± 9.2 years, and 125/129 (96.9%) were male. Same-day counselling was recorded for 129/129 (100.0%) of donors; 124/129 (96.1%) were referred within one day, and 129/129 (100.0%) within seven days.

Conclusion: TTI screening reactivity showed temporal variation, with HBsAg remaining the predominant marker and syphilis showing an increasing trend. Continued centre-level surveillance and structured post-donation counselling and referral are important for blood safety.

Categories: Public Health, Epidemiology/Public Health, Infectious Disease
Keywords: blood donors, blood safety, hepatitis b virus, hepatitis c virus, syphilis, transfusion-transmissible infections

Introduction

Transfusion safety remains a fundamental component of modern health-care systems because access to safe blood and blood components is essential for emergency care, surgery, obstetric practice, oncology, haematology, and the management of severe anaemia. Despite substantial advances in donor selection, laboratory screening, quality systems, and haemovigilance, transfusion-transmissible infections (TTIs) continue to represent an important threat to recipient safety, particularly where epidemiological patterns, donor characteristics, and testing resources vary [1]. Screening of donated blood for major TTIs is therefore a central component of the blood-safety pathway and is intended to identify potentially infectious donations before transfusion [2]. Nevertheless, residual infectious risk may persist because of window-period donations and limitations inherent in screening strategies [3]. Established and emerging infectious agents also continue to require surveillance as epidemiological patterns and threats to the blood supply evolve [4].

In India, blood centres routinely screen donations for human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis, and malaria in accordance with national blood-transfusion standards [5]. Despite improvements in screening coverage and blood-safety systems, TTI screening reactivity among Indian blood donors remains heterogeneous. A recent systematic review and meta-analysis of 41 Indian studies involving more than 1.8 million blood donors demonstrated substantial geographic variation in routinely screened TTIs, with HBV contributing the largest pooled burden among the principal

viral markers [6]. Contemporary multicentre data from 87,878 donors in the Mumbai region similarly demonstrated measurable TTI reactivity and regional variation, with HBV remaining the predominant reactive infection [7]. These findings indicate that pooled national estimates cannot substitute for current centre-specific surveillance.

Longitudinal and contemporary studies from India further demonstrate that TTI patterns may change over time and that individual markers do not necessarily follow parallel trajectories [8]. A 15-year blood-bank study from New Delhi documented persistent viral TTI reactivity and demonstrated additional detection of HBV, HCV, and HIV through nucleic acid testing beyond conventional serological screening [9]. Other contemporary Delhi data have confirmed continued reactivity for all five routinely screened TTIs and variation according to donor setting [10]. Regional data from Punjab have documented continuing HCV and syphilis reactivity, emphasizing the influence of local epidemiology [11]. A recent 12.5-year North Indian analysis also demonstrated changing donor and residual-risk patterns for HBV, HCV, and HIV despite increasingly sensitive screening approaches [12]. Large contemporary donor datasets from eastern India have shown substantial differences in the distribution of HBV, HCV, HIV, and syphilis compared with other regions [13], while recent institutional data from central India have similarly demonstrated the continuing value of monitoring reactive screening markers at the blood-centre level [14]. International evidence from resource-constrained settings also demonstrates marked variation in TTI burden according to geography, donor composition, and local blood-service characteristics [15]. Collectively, these studies support longitudinal, institution-level surveillance rather than assuming that TTI patterns remain static over time.

Beyond their immediate role in transfusion safety, systematically collected blood-donor screening data may provide complementary epidemiological information on established and emerging infections [16]. However, blood donors constitute a selected population shaped by eligibility criteria, health status, age structure, donation behaviour, and the relative contribution of first-time and repeat donors. Donor screening reactivity should therefore not be interpreted as a direct estimate of community prevalence or incidence [17]. Earlier studies from Lucknow documented TTI patterns among blood donors during 2001-2007 and subsequent periods, providing important historical regional context for donor-screening epidemiology in the city [18,19]. These earlier observations represent geographic historical comparators rather than an institutional baseline for the present study. Blood safety also extends beyond the identification and disposal of reactive donations. Notification, post-donation counselling, and referral of reactive donors are important components of donor care and infection-control pathways, and recent Indian evidence continues to identify variability and operational challenges in recall and referral practices [20].

Accordingly, the primary objective of the present study was to estimate overall TTI screening reactivity among blood donations screened at the Department of Transfusion Medicine, King George's Medical University, and Hind Institute of Medical Sciences, Lucknow. The secondary objectives were to assess infection-specific screening reactivity and temporal trends across the complete calendar years 2021-2025; to describe the available partial-year/YTD 2026 observations; to characterize the age and sex distribution of TTI-reactive donors; and to evaluate documented post-donation counselling and referral intervals among reactive donors. Subsequent attendance at referral facilities, confirmatory diagnosis, treatment initiation, and long-term clinical outcomes were not assessed.

Materials And Methods

Study design and setting

A retrospective record-based observational study was conducted in the Department of Transfusion Medicine at King George's Medical University and Hind Institute of Medical Sciences, Lucknow, Uttar Pradesh, India. Routinely maintained blood-donation and TTI screening records from 2021 through the available partial-year/year-to-date (YTD) period in 2026 were reviewed. Calendar years 2021-2025 were treated as complete study years. The latest TTI-reactive record available in the analytical dataset was dated July 26, 2026; therefore, the 2026 data were treated as partial-year/YTD observations and were excluded from the primary complete-calendar-year temporal trend analyses.

Study population, record selection, and sampling

Donor eligibility and acceptance for blood donation were determined according to routine blood-centre standard operating procedures and applicable national blood transfusion standards [5]. A total-enumeration approach was used, and all blood donations included in the annual TTI screening records during the study period were included. The study comprised 5,163 screened donations: 510 in 2021, 907 in 2022, 1,147 in 2023, 1,177 in 2024, 957 in 2025, and 465 during the available partial-year/YTD period in 2026. Individual-level analytical records were available for all 129 donations recorded as reactive for at least one routinely screened TTI marker. Available variables included donor age, sex, reactive screening marker, date of donation, date of post-donation counselling, and date of referral. No reactive record was excluded because of missing age, sex, TTI-marker, counselling-date, or referral-date information. The unit of analysis was the blood donation. First-time versus repeat-donor status was not available in the analytical dataset; therefore, repeat donations from the same individual could not be identified.

Sample size and blinding

No a priori sample-size calculation was performed. All eligible blood donations available in the screening records during the study period were included by total enumeration, resulting in a final study population of 5,163 donations. Blinding was not applicable because the study was retrospective and observational, with no intervention, treatment allocation, prospective exposure assignment, or subjective outcome assessment. Screening results and dates had been recorded during routine blood-centre practice before the present analysis was undertaken.

TTI screening and classification

Blood donations were routinely screened for HIV, HBV using hepatitis B surface antigen (HBsAg), HCV, syphilis, and malaria in accordance with the blood centre's standard operating procedures. HIV screening was performed using the fourth-generation Microlisa HIV Ag & Ab ELISA kit (J. Mitra & Co. Pvt. Ltd., New Delhi, India), which detects HIV-1 p24 antigen together with antibodies to HIV-1 and HIV-2. HBV screening was performed using the Hepalisa HBsAg ELISA kit (J. Mitra & Co. Pvt. Ltd., New Delhi, India). HCV screening was performed using the fourth-generation HCV Gen4 Ag & Ab Microlisa ELISA kit (J. Mitra & Co. Pvt. Ltd., New Delhi, India), which detects HCV core antigen together with anti-HCV antibodies. Malaria screening was performed using the Malaria Ag Microlisa ELISA kit (J. Mitra & Co. Pvt. Ltd., New Delhi, India) for the detection of *Plasmodium* antigen. Syphilis screening was performed using an Aspen syphilis screening kit (Aspen Laboratories Pvt. Ltd., New Delhi, India). ELISA-based assays were read using a BeneSphera E-21 ELISA Microplate Reader (BeneSphera™, Avantor). All routine screening procedures were performed according to the respective manufacturers' instructions and blood-centre quality-control procedures. The analytical dataset contained the final recorded reactive/non-reactive screening classification. A donation was classified as TTI-reactive when a reactive screening result was recorded for at least one of the five routinely screened markers. Confirmatory diagnostic-test results were not available; therefore, outcomes were reported as screening reactivity rather than confirmed infection or seroprevalence. Raw optical-density values, assay-specific cutoff calculations, complete repeat-testing sequences, and year-specific assay-lot metadata were not available. The exact Aspen syphilis product code, assay principle, lot-specific threshold, and repeat-testing algorithm were also not available.

Study variables and outcome measures

The primary outcome was overall TTI screening reactivity, calculated as the number of TTI-reactive donations divided by the total number of donations screened during the corresponding study period. Secondary outcomes included infection-specific screening reactivity for HBsAg, HCV, syphilis, HIV, and malaria; year-wise and marker-specific temporal patterns; the distribution of reactive-marker categories over time; demographic characteristics of TTI-reactive donors; and documented post-donation counselling and referral intervals. Temporal trend analyses were conducted across the five complete calendar years from 2021 through 2025. The partial-year/YTD 2026 observations were included descriptively and were not incorporated into the primary complete-calendar-year trend tests. Age was analysed as a continuous variable and in four categories: 20-30, 31-40, 41-50, and 51-60 years. Sex was described within each reactive-marker category. Demographic analyses were restricted to TTI-reactive donors because individual-level demographic information for non-reactive donations was unavailable.

Post-donation counselling and referral

Post-donation counselling and referral were evaluated using the dates documented in departmental records. Same-day counselling was defined as a recorded counselling date identical to the donation date. The donation-to-counselling interval was calculated as the number of calendar days between the recorded donation and counselling dates. Referral was defined as documentation of referral in the departmental record. Same-day referral was defined as a counselling-to-referral interval of zero calendar days. Referral within one, two, and seven days was defined as a recorded referral date occurring within ⩽1, ⩽2, and ⩽7 calendar days, respectively, after the counselling date. Subsequent attendance at the referral facility, confirmatory testing, treatment initiation, treatment response, and long-term clinical outcomes were not recorded in the analytical dataset.

Data cleaning and quality control

Before statistical analysis, records were checked for completeness, coding consistency, chronological validity, and agreement between individual dates and study-period classifications. Five reactive records required correction using predefined internal consistency rules. Three records contained year/date inconsistencies: two records assigned to the 2021 study period contained donation, counselling, and referral dates recorded in 2020 and were corrected to the corresponding 2021 dates, while one record assigned to the 2023 study period contained a 2022 donation year that conflicted with the 2023 study-period designation and corresponding counselling and referral dates and was corrected to 2023. One record contained the non-standard sex code “MN,” which was standardized to “M.” One record contained a referral date preceding the donation and counselling dates and was corrected according to the chronologically concordant date sequence. These corrections were based on internal consistency within the analytical dataset. After completion of quality-control procedures, the analytical dataset was frozen before final statistical analysis.

Data availability and reproducibility

The fixed-margin Monte Carlo analyses were performed using 100,000 simulated tables. The random-number seed was not retained in the analytical documentation. The donor-level dataset was not deposited publicly because it was derived from confidential blood-centre records. Sharing of appropriately de-identified analytical data would be subject to institutional and ethical permissions.

Statistical analysis

Categorical variables were summarized as frequencies and percentages (n/N (%)). Continuous variables were summarized using the mean and standard deviation (SD) and median with interquartile range (IQR), as appropriate. Overall and infection-specific screening-reactivity estimates were reported with 95% confidence intervals (95% CIs) calculated using the Wilson score method. Differences in overall TTI screening reactivity across the complete calendar years 2021-2025 were evaluated using the Pearson chi-square test. The χ^2 statistic, degrees of freedom (df), p value, and Cramer's V effect size were reported. Linear temporal trends were evaluated using the Cochran-Armitage test for trend and were reported using the standardized Z statistic and corresponding two-sided p value. Separate Cochran-Armitage tests were performed for HBsAg, HCV, syphilis, HIV, and malaria screening reactivity across 2021-2025. Marker-specific analyses were exploratory, and no multiplicity adjustment was applied. The distribution of the five TTI-reactive marker categories across study periods was assessed using a fixed-margin Monte Carlo exact procedure based on the Pearson χ^2 statistic with 100,000 simulated tables. The Pearson χ^2 statistic, df, Cramer's V, and Monte Carlo exact p value were reported. Age distributions across the five reactive-marker categories were compared using the Kruskal-Wallis test, with the H statistic, df, epsilon-squared (ε^2) effect size, and p value reported. Sex distribution across reactive-marker categories was assessed using a fixed-margin Monte Carlo exact procedure based on the Pearson χ^2 statistic with 100,000 simulations, with the χ^2 statistic, df, Cramer's V, and Monte Carlo exact p value reported. Counselling and referral intervals were summarized using the median, IQR, range, and predefined time thresholds. Differences across study periods in the proportion of reactive donors referred within one day were assessed using a fixed-margin Monte Carlo exact procedure based on the Pearson χ^2 statistic with 100,000 simulations; the χ^2 statistic, df, Cramer's V, and Monte Carlo exact p value were reported. Donation-level observations were analysed as recorded. Potential within-donor correlation arising from unidentified repeat donations could not be modelled because repeat-donation status was unavailable. All statistical calculations and numerical verification were performed using Python version 3.13.5 (Python Software Foundation, Beaverton, Oregon), with SciPy version 1.17.0, NumPy version 2.3.5, and pandas version 2.2.3. All statistical tests were two-sided, and $p < 0.05$ was considered statistically significant.

Ethical considerations

The parent blood-donor research protocol was reviewed and approved by the Institutional Ethics Committee of King George's Medical University, Lucknow, Uttar Pradesh, India (Reference No. 421/Ethics/2020; dated June 8, 2020). The present study involved a retrospective analysis of routinely maintained blood-centre records, and direct personal identifiers were excluded from the analytical dataset. Donor confidentiality was maintained throughout data handling and analysis.

Results

Overall TTI screening reactivity

A total of 5,163 blood donations were screened during the study period, of which 129/5,163 (2.50%; 95% CI, 2.11-2.96%) were reactive for at least one recorded TTI screening marker (Table *1*). HBsAg was the most frequently identified reactive marker and was detected in 66/5,163 donations (1.28%; 95% CI, 1.01-1.62%), accounting for 66/129 (51.2%) of all TTI-reactive records. HCV screening reactivity was identified in 36/5,163 donations (0.70%; 95% CI, 0.50-0.96%), representing 36/129 (27.9%) of reactive records. Syphilis screening reactivity was observed in 18/5,163 donations (0.35%; 95% CI, 0.22-0.55%), representing 18/129 (14.0%) of reactive records. Malaria screening reactivity was identified in 6/5,163 donations (0.12%; 95% CI, 0.05-0.25%), representing 6/129 (4.7%), whereas HIV screening reactivity was identified in 3/5,163 donations (0.06%; 95% CI, 0.02-0.17%), representing 3/129 (2.3%).

TTI screening marker	Reactive donations, n/N (%)	95% CI (%)	Share of all TTI-reactive records, n/N (%)
Any TTI	129/5,163 (2.50)	2.11-2.96	129/129 (100.0)
HBsAg	66/5,163 (1.28)	1.01-1.62	66/129 (51.2)
HCV	36/5,163 (0.70)	0.50-0.96	36/129 (27.9)
Syphilis	18/5,163 (0.35)	0.22-0.55	18/129 (14.0)
HIV	3/5,163 (0.06)	0.02-0.17	3/129 (2.3)
Malaria/MP	6/5,163 (0.12)	0.05-0.25	6/129 (4.7)

TABLE 1: Overall and infection-specific transfusion-transmissible infection (TTI) screening reactivity among blood donations

TTI screening reactivity is presented as the number of reactive donations divided by the total number of donations screened (n/N (%)). The 95% confidence intervals (CIs) were calculated using the Wilson score method. “Share of all TTI-reactive records” represents the proportion of the 129 reactive records attributable to each screening marker.

TTI: transfusion-transmissible infection; HBsAg: hepatitis B surface antigen; HCV: hepatitis C virus; HIV: human immunodeficiency virus.

Year-wise pattern and temporal trend of overall TTI reactivity

Year-wise overall TTI screening reactivity is summarized in Table 2. Among the five complete calendar years, 10/510 donations (1.96%; 95% CI, 1.07-3.57%) were reactive in 2021, 17/907 (1.87%; 95% CI, 1.17-2.98%) in 2022, and 17/1,147 (1.48%; 95% CI, 0.93-2.36%) in 2023. Reactivity subsequently increased to 29/1,177 (2.46%; 95% CI, 1.72-3.52%) in 2024 and 39/957 (4.08%; 95% CI, 3.00-5.52%) in 2025. During the available partial-year/YTD period in 2026, 17/465 donations (3.66%; 95% CI, 2.29-5.78%) were reactive. Overall TTI screening reactivity differed significantly across the complete calendar years 2021-2025 (Pearson χ^2 = 17.209, df = 4, Cramer's V = 0.061, p = 0.0018). Although statistically significant, the magnitude of the year-wise association was small, as indicated by Cramer's V=0.061. A significant increasing linear temporal trend was also observed across 2021-2025 (Cochran-Armitage Z = 3.163, p = 0.0016). The partial-year/YTD 2026 observations were excluded from the primary complete-calendar-year temporal trend analysis.

Study year/period	TTI-reactive donations, n/N (%)	95% CI (%)
2021	10/510 (1.96)	1.07-3.57
2022	17/907 (1.87)	1.17-2.98
2023	17/1,147 (1.48)	0.93-2.36
2024	29/1,177 (2.46)	1.72-3.52
2025	39/957 (4.08)	3.00-5.52
2026, partial-year/YTD	17/465 (3.66)	2.29-5.78
Overall study period	129/5,163 (2.50)	2.11-2.96

TABLE 2: Year-wise overall TTI screening reactivity among blood donations

TTI screening reactivity is presented as the number of reactive donations divided by the total number of donations screened during the corresponding year or study period (n/N (%)). The 95% CIs were calculated using the Wilson score method. Calendar years 2021-2025 were complete study years. The 2026 data represent a partial-year/YTD observation period and were excluded from the primary complete-calendar-year temporal trend analysis.

TTI: transfusion-transmissible infection; YTD: year-to-date.

Infection-specific temporal patterns

The distribution of individual TTI screening markers across study periods is presented in Table 3. HBsAg screening reactivity was 3/510 (0.59%) in 2021, 15/907 (1.65%) in 2022, 7/1,147 (0.61%) in 2023, 18/1,177 (1.53%) in 2024, 18/957 (1.88%) in 2025, and 5/465 (1.08%) during the partial-year/YTD 2026 period. No

statistically significant linear trend was observed for HBsAg across the complete calendar years 2021-2025 (Cochran-Armitage Z = 1.805, p = 0.0711). HCV screening reactivity was 7/510 (1.37%) in 2021, 1/907 (0.11%) in 2022, 6/1,147 (0.52%) in 2023, 9/1,177 (0.76%) in 2024, 8/957 (0.84%) in 2025, and 5/465 (1.08%) during the partial-year/YTD 2026. No significant linear trend was observed for HCV across 2021-2025 (Cochran-Armitage Z = 0.327, p = 0.7435). Syphilis screening reactivity was not recorded in 2021 (0/510 (0.00%)) but was observed in 1/907 donations (0.11%) in 2022, 3/1,147 (0.26%) in 2023, 0/1,177 (0.00%) in 2024, 9/957 (0.94%) in 2025, and 5/465 (1.08%) during the partial-year/YTD 2026. A significant increasing linear trend was observed for syphilis screening reactivity across 2021-2025 (Cochran-Armitage Z = 2.996, p = 0.0027). HIV screening reactivity was not recorded during 2021-2023, was identified in 2/1,177 donations (0.17%) in 2024 and 1/957 (0.10%) in 2025, and was not recorded during the partial-year/YTD 2026 (0/465 (0.00%)). No significant temporal trend was observed for HIV across 2021-2025 (Cochran-Armitage Z = 1.473, p = 0.1409). Malaria screening reactivity was identified in 1/1,147 donations (0.09%) in 2023, 3/957 (0.31%) in 2025, and 2/465 (0.43%) during the partial-year/YTD 2026; no malaria-reactive donations were recorded in 2021, 2022, or 2024. The malaria trend across 2021-2025 yielded a Cochran-Armitage Z statistic of 1.962 (p = 0.0498). Because only six malaria-reactive records were observed overall and the marker-specific analyses were exploratory without multiplicity adjustment, this finding was interpreted cautiously. Across all six study periods, the distribution of the five TTI-reactive marker categories differed significantly (Pearson χ^2 = 39.685, df = 20, Cramer's V = 0.277; fixed-margin Monte Carlo exact p = 0.0061; 100,000 simulations), indicating that the relative composition of reactive markers varied over time.

TTI screening marker or analysis	2021 (N = 510), n/N (%)	2022 (N = 907), n/N (%)	2023 (N = 1,147), n/N (%)	2024 (N = 1,177), n/N (%)	2025 (N = 957), n/N (%)	2026 partial-year/YTD (N = 465), n/N (%)	Statistical test	Test statistic	Statistical inference
HBsAg	3/510 (0.59)	15/907 (1.65)	7/1,147 (0.61)	18/1,177 (1.53)	18/957 (1.88)	5/465 (1.08)	Cochran-Armitage trend test	Z = 1.805	p = 0.0711
HCV	7/510 (1.37)	1/907 (0.11)	6/1,147 (0.52)	9/1,177 (0.76)	8/957 (0.84)	5/465 (1.08)	Cochran-Armitage trend test	Z = 0.327	p = 0.7435
Syphilis	0/510 (0.00)	1/907 (0.11)	3/1,147 (0.26)	0/1,177 (0.00)	9/957 (0.94)	5/465 (1.08)	Cochran-Armitage trend test	Z = 2.996	p = 0.0027
HIV	0/510 (0.00)	0/907 (0.00)	0/1,147 (0.00)	2/1,177 (0.17)	1/957 (0.10)	0/465 (0.00)	Cochran-Armitage trend test	Z = 1.473	p = 0.1409
Malaria/MP	0/510 (0.00)	0/907 (0.00)	1/1,147 (0.09)	0/1,177 (0.00)	3/957 (0.31)	2/465 (0.43)	Cochran-Armitage trend test	Z = 1.962	p = 0.0498
Any TTI	10/510 (1.96)	17/907 (1.87)	17/1,147 (1.48)	29/1,177 (2.46)	39/957 (4.08)	17/465 (3.66)	Cochran-Armitage trend test	Z = 3.163	p = 0.0016
Distribution of the five reactive-marker categories across all six study periods	Five TTI-reactive marker categories evaluated across all six study periods						Fixed-margin Monte Carlo exact test based on Pearson χ^2	χ^2 = 39.685	df = 20; Cramer's V = 0.277; p = 0.0061

TABLE 3: Infection-specific TTI screening reactivity by study year/period

Infection-specific screening reactivity is presented as the number of reactive donations divided by the total number of donations screened in the corresponding year/period (n/N (%)). Marker-specific linear temporal trends were assessed using the Cochran-Armitage trend test restricted to the five complete calendar years 2021-2025 and are reported using the standardized Z statistic with the corresponding two-sided p value. The 2026 data represent a partial-year/YTD observation period and were therefore excluded from the marker-specific linear trend analyses. Because HIV and malaria had very small numbers of reactive donations, these trend findings should be interpreted cautiously. The distribution of the five reactive-marker categories across all six study periods was additionally assessed using a fixed-margin Monte Carlo exact test based on the Pearson chi-square statistic with 100,000 simulations; Cramer's V was used as the effect-size measure.

TTI: transfusion-transmissible infection; YTD: year-to-date; HIV: human immunodeficiency virus; HBsAg: hepatitis B surface antigen; HCV: hepatitis C virus.

Age and sex characteristics of TTI-reactive donors

The demographic characteristics of the 129 TTI-reactive donors are summarized in Table *4*. The mean age was 32.5 ± 9.2 years, and the median age was 30 years (IQR, 26-37; range, 20-60 years). Overall, 66/129 reactive donors (51.2%) were aged 20-30 years, 41/129 (31.8%) were aged 31-40 years, 14/129 (10.9%) were aged 41-50 years, and 8/129 (6.2%) were aged 51-60 years. Thus, 107/129 reactive donors (82.9%) were aged ≤40 years. Age distributions differed significantly across the five reactive-marker categories (Kruskal-Wallis H = 10.269, df = 4, ε^2 = 0.051, p = 0.0361). Reactive donors were predominantly male, with 125/129 (96.9%)

being men and 4/129 (3.1%) women. Among HBsAg-reactive donors, 64/66 (97.0%) were male and 2/66 (3.0%) were female; among HCV-reactive donors, 34/36 (94.4%) were male and 2/36 (5.6%) were female. All donors with syphilis screening reactivity (18/18 (100.0%)), HIV reactivity (3/3 (100.0%)), and malaria reactivity (6/6 (100.0%)) were male. Sex distribution did not differ significantly across the five reactive-marker categories (Pearson $\chi^2 = 1.587$, df = 4, Cramer's V = 0.111; fixed-margin Monte Carlo exact p = 0.7262; 100,000 simulations). These demographic comparisons were restricted to TTI-reactive donors and were therefore descriptive of the reactive cohort rather than estimates of age- or sex-specific risk.

Characteristic	Overall (n = 129)	HBsAg (n = 66)	HCV (n = 36)	Syphilis (n = 18)	HIV (n = 3)	Malaria/MP (n = 6)
Mean age, years	32.5	30.8	34.1	37.6	28.0	27.7
Age SD, years	9.2	7.9	10.9	9.2	4.0	4.6
Median age, years	30	30	30.5	37	28	27
Age IQR, years	26-37	26-35	26.8-40.5	30-44.5	26-30	24-30.8
Age range, years	20-60	20-58	20-57	22-60	24-32	23-34
Age 20-30 years, n/N (%)	66/129 (51.2)	36/66 (54.5)	18/36 (50.0)	6/18 (33.3)	2/3 (66.7)	4/6 (66.7)
Age 31-40 years, n/N (%)	41/129 (31.8)	23/66 (34.8)	9/36 (25.0)	6/18 (33.3)	1/3 (33.3)	2/6 (33.3)
Age 41-50 years, n/N (%)	14/129 (10.9)	5/66 (7.6)	4/36 (11.1)	5/18 (27.8)	0/3 (0.0)	0/6 (0.0)
Age 51-60 years, n/N (%)	8/129 (6.2)	2/66 (3.0)	5/36 (13.9)	1/18 (5.6)	0/3 (0.0)	0/6 (0.0)
Male, n/N (%)	125/129 (96.9)	64/66 (97.0)	34/36 (94.4)	18/18 (100.0)	3/3 (100.0)	6/6 (100.0)
Female, n/N (%)	4/129 (3.1)	2/66 (3.0)	2/36 (5.6)	0/18 (0.0)	0/3 (0.0)	0/6 (0.0)

TABLE 4: Age and sex characteristics of TTI-reactive blood donors according to reactive screening marker

Continuous age variables are presented separately as mean, standard deviation (SD), median, interquartile range (IQR), and range. Categorical variables are presented as n/N (%). These data describe TTI-reactive donors only and should not be interpreted as age- or sex-specific TTI risk estimates.

TTI: transfusion-transmissible infection; HIV: human immunodeficiency virus; HCV: hepatitis C virus; HBsAg: hepatitis B surface antigen.

Post-donation counselling and referral

Post-donation counselling and referral characteristics are summarized in Table *5*. Same-day counselling was recorded for all 129/129 TTI-reactive donors (100.0%). The median donation-to-counselling interval was 0 days (IQR, 0-0; range, 0-0 days). Same-day referral was recorded for 8/129 donors (6.2%), while 124/129 (96.1%) were referred within one day, 125/129 (96.9%) within two days, and 129/129 (100.0%) within seven days. The median counselling-to-referral interval was one day (IQR, 1-1; range, 0-7 days). Referral within one day was recorded for 10/10 reactive donors (100.0%) in 2021, 16/17 (94.1%) in 2022, 14/17 (82.4%) in 2023, 29/29 (100.0%) in 2024, 39/39 (100.0%) in 2025, and 16/17 (94.1%) during the partial-year/YTD 2026 period. The proportion of reactive donors referred within one day differed significantly across study periods (Pearson $\chi^2 = 12.166$, df = 5, Cramer's V = 0.307; fixed-margin Monte Carlo exact p = 0.0297; 100,000 simulations). These findings reflected documented referral timing and did not establish subsequent attendance or successful linkage to care.

Characteristic	Result
Same-day counselling, n/N (%)	129/129 (100.0)
Donation-to-counselling interval, median (days)	0
Donation-to-counselling interval, IQR (days)	0-0
Donation-to-counselling interval, range (days)	0-0
Same-day referral, n/N (%)	8/129 (6.2)
Referral within ≤1 day, n/N (%)	124/129 (96.1)
Referral within ≤2 days, n/N (%)	125/129 (96.9)
Referral within ≤7 days, n/N (%)	129/129 (100.0)
Counselling-to-referral interval, median (days)	1
Counselling-to-referral interval, IQR (days)	1-1
Counselling-to-referral interval, range (days)	0-7
Referral within ≤1 day in 2021, n/N (%)	10/10 (100.0)
Referral within ≤1 day in 2022, n/N (%)	16/17 (94.1)
Referral within ≤1 day in 2023, n/N (%)	14/17 (82.4)
Referral within ≤1 day in 2024, n/N (%)	29/29 (100.0)
Referral within ≤1 day in 2025, n/N (%)	39/39 (100.0)
Referral within ≤1 day in 2026 partial-year/YTD, n/N (%)	16/17 (94.1)

TABLE 5: Post-donation counselling and referral characteristics among TTI-reactive blood donors

Counselling and referral proportions are presented as n/N (%). Interval summaries are presented separately as median, IQR, and range. The 2026 data represent a partial-year/YTD observation period.

TTI: transfusion-transmissible infection; YTD: year-to-date; IQR: interquartile range.

Discussion

The present study demonstrated a measurable and changing pattern of TTI screening reactivity among blood donations at a tertiary-care transfusion medicine centre in North India. Overall TTI screening reactivity was 129/5,163 (2.50%), with HBsAg accounting for 66/129 (51.2%) of all reactive records. Overall reactivity showed a significant increasing linear trend across the complete calendar years 2021-2025 (Cochran-Armitage Z = 3.163, p = 0.0016). Although overall reactivity also differed significantly across these years, the magnitude of the year-wise association was small (Pearson χ^2 = 17.209, df = 4, Cramer's V = 0.061, p = 0.0018). The relative distribution of individual reactive markers also varied significantly across study periods (Pearson χ^2 = 39.685, df = 20, Cramer's V = 0.277; Monte Carlo exact p = 0.0061). Syphilis screening reactivity showed the clearest infection-specific increasing trend, whereas HIV and malaria remained infrequent. Documented counselling and referral of reactive donors were generally prompt.

Overall TTI burden and viral markers

The overall TTI screening reactivity of 129/5,163 (2.50%) should be interpreted in the context of substantial heterogeneity among Indian blood-donor populations. A longitudinal study from Northern India reported a lower overall TTI burden, whereas contemporary Delhi data continued to demonstrate measurable reactivity across routinely screened TTIs, with HBV remaining an important contributor [21,22]. Recent studies from Assam and other tertiary-care settings have also reported different overall frequencies and marker distributions, illustrating considerable centre-to-centre variation [23,24]. Changes in donation activity and donor composition, including those associated with the COVID-19 pandemic, may additionally influence observed blood-centre TTI patterns [25]. Contemporary data from Uttar Pradesh provide a further regional comparison and similarly demonstrate that infection distributions vary across blood-centre populations [26]. These observations, together with the recent Indian systematic review and meta-analysis [6], indicate that pooled national estimates cannot be assumed to represent individual institutions.

HBsAg was the predominant reactive marker in the present study, occurring in 66/5,163 donations (1.28%) and accounting for 66/129 (51.2%) of all reactive records. This finding is consistent with the continuing importance of HBV-related screening reactivity among Indian blood donors. A recent systematic review and meta-analysis specifically evaluating HBV among asymptomatic Indian blood donors similarly identified a substantial ongoing HBV burden [27]. HCV screening reactivity was identified in 36/5,163 donations (0.70%). Contemporary Indian blood-bank data continue to demonstrate measurable HCV reactivity and also show that the observed yield may differ according to donor characteristics and the screening strategy used [28]. Therefore, variation between the present estimate and other institutional reports may reflect differences in donor composition, geographic epidemiology, study period, and assay methodology.

HIV screening reactivity was uncommon, occurring in 3/5,163 donations (0.06%). This finding was broadly consistent with the relatively low HIV seroreactivity reported among screened donors in recent South Asian evidence, although important regional variation persists [29]. Low serological reactivity does not eliminate residual transfusion risk. Recent Indian data using nucleic acid testing have demonstrated the detection of NAT-only reactive donations missed by conventional serological screening [30], while contemporary international evidence similarly supports nucleic acid amplification testing as an additional risk-reduction strategy where feasible [31]. These comparisons provide blood-safety context and do not imply that NAT was performed in the present study.

Syphilis and malaria screening reactivity

Syphilis screening reactivity was identified in 18/5,163 donations (0.35%) and showed a significant increasing trend across the complete calendar years 2021-2025 (Cochran-Armitage $Z = 2.996$, $p = 0.0027$). Recent longitudinal Indian studies have likewise reported changing or increasing syphilis seroreactivity among blood donors [32,33]. Contemporary evidence also demonstrates that syphilis estimates may vary according to the screening and confirmatory strategies used. Therefore, the present finding should be interpreted as a blood-centre screening signal rather than evidence of increasing community syphilis prevalence. Because confirmatory diagnostic results were unavailable, screening reactivity could not be equated with confirmed syphilis infection.

Malaria screening reactivity remained uncommon, occurring in only 6/5,163 donations (0.12%). Recent national evidence has shown that malaria positivity among Indian blood donations remains uncommon in absolute terms but varies across blood services and screening practices [34]. Contemporary North Indian evidence similarly indicates a low yield from routine malaria screening [35]. Although the malaria trend across 2021-2025 reached the conventional statistical threshold (Cochran-Armitage $Z = 1.962$, $p = 0.0498$), only six malaria-reactive records were observed during the entire study period. In addition, marker-specific analyses were exploratory and were not adjusted for multiple testing. The malaria finding should therefore be interpreted cautiously rather than as evidence of an established increasing trend.

Temporal patterns and their interpretation

The year-wise pattern showed an initial decline in overall TTI screening reactivity to 17/1,147 (1.48%) in 2023, followed by an increase to 29/1,177 (2.46%) in 2024 and 39/957 (4.08%) in 2025. Although the difference across the complete calendar years was statistically significant, the corresponding Cramer's V of 0.061 indicated that the magnitude of the year-wise association was small. Therefore, statistical significance should not be interpreted as evidence of a large temporal effect. The relative composition of reactive markers also varied across study periods, indicating that the observed increase was not uniform across all routinely screened infections. Previous longitudinal Indian blood-centre studies have similarly demonstrated that TTI patterns may change over time and that individual viral or non-viral markers may follow different trajectories within the same donor population [8,9,21,23]. Changes in underlying infection epidemiology may contribute to such patterns; however, changes in donor recruitment, the proportions of first-time and repeat donors, donor eligibility, screening practices, or other blood-centre processes may also influence annual reactivity. The present retrospective dataset could not distinguish among these mechanisms. The partial-year/YTD 2026 observations were therefore retained as descriptive findings and were not incorporated into the primary complete-calendar-year temporal trend analyses. The observed temporal pattern should not be interpreted as direct evidence of increasing infection transmission in the general community.

Demographic characteristics of reactive donors

The TTI-reactive cohort was predominantly composed of younger adults and men. Overall, 107/129 reactive donors (82.9%) were aged ≤40 years, and 125/129 (96.9%) were male. Similar predominance of younger and male donors has been reported in contemporary Indian donor cohorts [7,21]. Age distributions differed significantly across the five reactive-marker categories (Kruskal-Wallis $H = 10.269$, $df = 4$, $\varepsilon^2 = 0.051$, $p = 0.0361$), whereas sex distribution did not differ significantly (Pearson $\chi^2 = 1.587$, $df = 4$, Cramer's $V = 0.111$; Monte Carlo exact $p = 0.7262$). These findings should remain descriptive. Individual-level demographic information for non-reactive donations was unavailable, and the present analysis therefore could not estimate age- or sex-specific TTI prevalence, relative risks, or independent demographic predictors. The marked male predominance may primarily reflect the demographic composition of the donor population rather than a biological association with TTI reactivity.

Post-donation counseling and referral

Post-donation counselling and referral represented an important operational component of the study. Same-day counselling was recorded for all 129/129 reactive donors (100.0%), 124/129 (96.1%) were referred within one day, and 129/129 (100.0%) had a referral recorded within seven days. The proportion referred within one day varied across study periods (Pearson $\chi^2 = 12.166$, df = 5, Cramer's V = 0.307; Monte Carlo exact p = 0.0297). These findings compare favourably with the Indian literature, in which considerable attrition has been reported between donor notification, response, counselling, referral, and subsequent clinical attendance. A recent Indian review documented substantial heterogeneity in notification and follow-up practices across blood centres [36]. A North Indian audit identified incomplete return for counselling among notified reactive donors, while more recent post-donation counselling data from Lucknow similarly demonstrated substantial losses between notification, counselling, and subsequent referral attendance [37,38]. Linkage to structured hepatitis-care pathways is particularly relevant for HBsAg- or HCV-reactive donors because blood-centre detection may provide an opportunity for confirmatory diagnosis and treatment [39]. More recent evidence from lower-middle-income settings has also identified notification, counselling, and linkage to care as persistent weaknesses in the post-donation pathway [40]. The present findings document the timing of counselling and referral but do not demonstrate successful linkage to care. Attendance at the referral facility, confirmatory diagnosis, treatment initiation, treatment response, and long-term clinical outcomes were not available.

Implications for blood-centre surveillance

Routinely collected donor-screening data may contribute information beyond immediate blood-component safety. Repeated standardized testing can help identify temporal changes in established infections and generate signals that warrant further investigation [16]. Blood-donor surveillance, however, represents a selected population rather than the general community. Donor eligibility criteria, health status, age distribution, donation behaviour, first-time versus repeat donation, and screening methodology may all influence observed marker reactivity [17]. Accordingly, the increasing overall TTI reactivity and changing syphilis screening pattern observed in this study should be considered centre-specific surveillance signals. They support continued longitudinal monitoring and quality assurance but should not be interpreted as direct estimates of community infection prevalence or incidence.

Strengths and limitations

This study has several strengths, including the multi-year assessment of all five routinely screened TTIs, the use of annual donation denominators, formal temporal-trend testing, infection-specific analyses, and inclusion of documented counselling and referral indicators. Several limitations should, however, be considered. The retrospective single-centre design limits generalizability, and individual-level characteristics were available only for reactive records. First-time versus repeat-donor status was unavailable; therefore, repeat donations from the same individual could not be identified, and potential within-donor correlation could not be accounted for. Changes in donor composition or screening practices over time may also have influenced the observed temporal patterns, and complete year-specific and lot-specific assay histories were unavailable for independent verification. Five reactive records required correction using predefined internal-consistency rules, but source-register verification was unavailable, introducing additional uncertainty. Confirmatory infection results and co-reactivity information were also unavailable. The small numbers of HIV- and malaria-reactive donations limited the stability of marker-specific trend estimates, and the multiple marker-specific analyses were exploratory without adjustment for multiplicity. The 2026 observations represented only the partial-year/YTD period. Finally, documented referral indicated that a referral had been recorded but did not establish attendance at the referral facility, confirmatory diagnosis, treatment initiation, or successful linkage to care. Accordingly, the temporal findings should be interpreted as centre-specific screening signals rather than evidence of changes in community infection prevalence.

Conclusions

This retrospective single-centre study demonstrated measurable temporal variation in TTI screening reactivity among blood donations during 2021-2025, although the magnitude of the overall year-wise association was small. HBsAg remained the predominant reactive marker, while syphilis screening reactivity showed a significant increasing trend. HIV and malaria screening reactivity remained infrequent, and the malaria trend should be interpreted cautiously because of the small number of reactive records. The partial-year/YTD 2026 findings were descriptive and were not included in the primary temporal analyses. Documented counselling and referral of reactive donors were timely, although referral records did not establish subsequent attendance or successful linkage to care. These findings support continued centre-level TTI surveillance, rigorous blood-screening practices, and structured post-donation counselling and referral, while emphasizing that blood-donor screening trends should not be interpreted as direct evidence of changes in community infection prevalence.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

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Disclosures

Human subjects: Informed consent for treatment and open access publication was obtained or waived by all participants in this study. Institutional Ethics Committee, King George's Medical University, Lucknow issued approval Approval No. 421/Ethics/2020; Ref. Code: 101st ECM II B-Thesis/P87; dated June 1, 2020. The study was approved by the Institutional Ethics Committee, King George's Medical University, Lucknow, Uttar Pradesh, India (Approval No. 421/Ethics/2020; Ref. Code: 101st ECM II B-Thesis/P87; dated June 1, 2020). **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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