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Original Article

Analysis of HIV infection among voluntary blood donors based on HIV ELISA and nucleic acid detection

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Abstract: Objective: To analyze the epidemiological characteristics of human immunodeficiency virus (HIV) infection among voluntary blood donors and provide a foundation for improving the donor recruitment strategies and developing a more scientific and effective HIV screening strategy. Methods: HIV testing data from voluntary blood donors in Nanchang, collected from January 2021 to February 2024, were analyzed. According to the serologic and nucleic acid sequence detection mode, two different reagents were used for ELISA detection and NAT detection. The reactive samples were subjected to western blot confirmatory test, and the confirmed positive samples were sent to the provincial CDC for viral load detection. Results: Among 264,615 voluntary blood donors, 166 cases (0.627‰) were initially reactive: 165 cases detected by ELISA and 1 by NAT. Confirmed positive samples showed 100% positivity for gp160, gp120, and p24, while the p24 detection rate in the HIV indeterminate group was highest at 78.13%. Among the 24 confirmed positive specimens, 21 (87.5%) had a viral load (VL) > 5,000 copies/mL, and 3 (12.5%) had a viral load (VL) < 5,000 copies/mL. Conclusions: Confirmed HIV-positive donors exhibited varying levels of viral replication. It is necessary to develop more scientific and effective HIV screening strategies and conduct viral load testing for indeterminate cases to retain qualified blood donors while ensuring blood safety.

Keywords: Voluntary blood donation, HIV, nucleic acid detection, ELISA detection, western blot, viral load

Introduction

Acquired Immunodeficiency Syndrome (AIDS) has become one of the most remarkable public health and social problems in the world [1], affecting millions. Transmission mainly includes unprotected sexual contact, sharing of contaminated needles among intravenous drug users, and mother-to-child transmission during pregnancy, childbirth, and breastfeeding. In high prevalence regions, certain populations are at higher risk, such as men who have sex with men, commercial sex workers, and people with multiple sexual partners [2]. The spread of AIDS is not only related to individual behaviors but also influenced by social, economic, and cultural factors. Prevention efforts, including education on safe sex, needle exchange programs, and early detection and treatment, play a crucial role in controlling the epidemic [3]. Additionally, continuous research and surveillance are essential to monitor trends and adapt prevention and control strategies effectively.

Over the past 20 years, advancements in transfusion-transmitted pathogen detection technologies, such as nucleic acid testing, have significantly enhanced blood supply safety. However, a residual risk of HIV infection caused by blood transfusion still exists, with increasingly diverse HIV testing results among voluntary blood donors. Variability in viral load during the HIV window period and the presence of elite controllers (ECs) and long-term non-progressors (LTNPs) contribute to these complexities [4, 5]. Detecting HIV infection indicators in blood donors is essential in preventing HIV transmission through blood transfusion [6, 7]. Blood collection and supply institutions bear the critical responsibility of developing robust testing strategies, implementing control measures, and effectively reducing HIV transmission through blood transfusion [8].

Therefore, this study statistically analyzed the initial screening, confirmatory testing, and viral load results of HIV among voluntary blood

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donors in the local area, in order to enhance voluntary blood donation promotion and recruitment strategies and support the development of more scientific and effective HIV screening strategies.

Materials and methods

Study population

From January 2021 to February 2024, a total of 264,615 uncompensated blood samples were donated in Nanchang. This study was approved by the Ethics Committee of Jiangxi Provincial Blood Center.

Instruments and reagents

Enzyme linked immunosorbent assay (ELISA) equipments: STAR fully automatic sampler (Hamilton, Switzerland); XANTUS fully automatic sampling and blood type analyzer (Shenzhen Aikang Company); FAME 24/20 fully automatic enzyme immunoassay analyzer (Hamilton, Switzerland); URANUS AE368 automatic enzyme immunity instrument (Shenzhen Aikang Company). Nucleic acid testing equipment: COBAS S201 nucleic acid testing system (Roche, USA). Western blot confirmation testing equipment: fully automatic immunoblotting machine (Bee Robotics, UK). Virus load detection equipment: COBAS TaqMan96 analyzer. The instruments and equipment were regularly calibrated and maintained every year.

Human Immunodeficiency Virus Antibody Diagnostic Kit (Beijing Wantai Biopharmaceutical Co., Ltd., third-generation reagent, abbreviated as WT reagent) and Human Immunodeficiency Virus Antigen Antibody Diagnostic Kit (Yingke Xinchuang (Xiamen) Technology Co., Ltd., fourth-generation reagent, abbreviated as XC reagent); Nucleic acid testing reagent: Cobas TaqScreen MPX test, version 2.0 (Roche, USA); Immunoblotting test (WB) reagent: HIV (1+2 type) antibody detection kit (MPD HIV BLOT 2.2) (Singapore MP Biomedical Asia Pacific Private Limited); Virus load reagent: COBASTaqManHIV-1Test (Roche). All reagents were batch tested and used within their validity period, strictly following the instructions of the reagent kit.

Methods

Following the Quality Management Standards for Blood Station Laboratories [9], Technical Operating Procedures for Blood Stations [10], and National Guideline for Detection of HIV/AIDS [11], samples initially screened reactive by ELISA and NAT were sent to our center's HIV confirmation laboratory for further confirmation experiments using WB. The confirmed positive samples were forwarded to the provincial CDC for virus load testing.

ELISA testing: ELISA detection of HIV Ab/Ag was carried out using a double-reagent approach with kits from Yingke Xinchuang (Xiamen) Technology Co., Ltd. and Beijing Wantai Biological Pharmacy Enterprise Co., Ltd., in combination with the FAME 24/20 fully automatic enzyme immunoassay system (Hamilton, Switzerland) and the URANUS AE368 automatic enzyme immunity instrument (Shenzhen Aikang Company). S/CO represents the ratio of the absorbance value (S) of the sample to the cut-off value (CO, critical value). An S/CO $\geq$ 1.0 is considered reactive, while an S/CO value between 0.8 and 1.0 falls within a designated "gray area". Samples with an S/CO $\geq$ 0.8 in any reagent undergo double-well retesting with the same reagent. If both reagents yield S/CO values $\geq$ 0.8, the original sample undergoes further retesting in duplicate. Any reactivity in retesting is marked as initially reactive.

NAT detection: If the ELISA test results show double-reagent non-reactivity, NAT (HBV, HCV, HIV) screening is conducted using a six-sample pooling method. If the pooled sample shows reactivity, each sample is individually tested to distinguish the specific reactivity of HIV, HBV, or HCV.

Confirmatory test: HIV - reactive blood samples from primary screening were sent to the AIDS confirmation laboratory for protein blot test (WB). Results were interpreted based on the kit's instructions. Two env bands plus any other band are judged as HIV-1 antibody positive; absence of specific bands or detection of only p17 antibody is indicative of HIV antibody negative; presence of bands but not meeting the positive evaluation criteria, it is judged as uncertain for HIV antibodies.

Viral load testing: Viral load testing followed the reagent kit's protocol. Results above the minimum detection limit (20 copies/mL) were reported as the detected value. Samples below this threshold were reported as "below the detection limit, no HIV-1 RNA detected".

Table 1. Characteristics and distribution of unpaid blood donors in recent three years

	Distribution	Total blood donors	Composition ratio (%)
Age (years)	18-24	154,707	58.46
	25-34	42,134	15.92
	35-44	35,162	13.29
	45-55	29,227	11.05
	56-60	3,385	1.28
Gender	Male	169,732	64.14
	Female	94,883	35.86
Educational background	Graduate student	7,746	2.93
	Undergraduate Course	114,903	43.42
	Junior college	81,839	30.93
	High school/Technical secondary school	28,234	10.67
	Junior high school and below	31,893	12.05
Occupation	Worker	2,499	0.94
	Peasant	1,788	0.68
	Student	137,077	51.80
	Soldier	2,328	0.88
	Civil servant	6,151	2.32
	Teacher	3,605	1.36
	Medical personnel	10,760	4.07
	Company staff	31,086	11.75
	Self-employed	2,510	0.95
	Institution staff	2,344	0.89
	Migrant worker	1,560	0.59
	Unknown	62,907	23.77
Blood donation history	The first time	154,855	58.52
	Repeat	109,760	41.48

Statistical analysis

SPSS 22.0 statistical software was used for data analysis. Counted data were expressed as percentages (%) or per thousand (/‰). The continuous data in this study did not follow normal distribution, so it was described in the form of median (lower quartile to upper quartile), and Inter-group comparisons were analyzed using Kruskal-Wallis rank sum test. Rate comparisons were performed using the chisquare test or Fisher exact probability method. A *p*-value of less than 0.05 ($P < 0.05$) was considered significant.

Results

Characteristics of blood donors

Over the past three years, this center has screened 264,615 samples from unpaid blood donors. The gender distribution showed that males accounted for 64.14% (169,732/264,615) and females accounted for 35.86% (94,883/264,615). In terms of age distribution, 18-24 age group had the highest proportion, accounting for 58.46% (154,707/264,615). In terms of educational distribution, junior college and undergraduate accounted for the majority, 74.35% (196,742/264,615). In terms of the occupation of blood donors, students were the main group, accounting for 51.80% (137,077/264,615). In terms of the distribution of blood donation history, first-time blood donors accounted for 58.52% (154,855/264,615), and repeat blood donors accounted for 41.48% (109,760/264,615) (**Table 1**).

HIV screening and confirmatory test results

HIV screening was conducted on 264,615 samples of voluntary blood donors, with a total of 166 reactive samples screened (including 165 reactive samples screened by ELISA and 1 by NAT screening). Following WB confirmation, 32 samples were classified as indeterminate, and

Table 2. Demographic statistics of HIV-positive blood donors

	Sample size of initial reactivity	*Positive coincidence rate (%)	*Positive proportion (%)	χ^2	*P*
Gender				15.709	P < 0.01
Male	98	23 (23.47)	95.83		
Female	68	1 (1.47)	4.17		
Age				10.608	P < 0.05
18-24	108	9 (8.33)	37.50		
25-34	23	6 (26.09)	25.00		
35-44	20	4 (20.00)	16.67		
45-55	15	5 (33.33)	20.83		
56-60	0	0	0.00		
Educational background					P < 0.01①
Graduate student	5	0 (0.00)	0.00		
Undergraduate course	66	4 (6.06)	16.67		
Junior college	67	8 (11.94)	33.33		
High school/Technical secondary school	13	4 (30.77)	16.67		
Junior high school and below	15	8 (53.33)	33.33		
Occupation					P < 0.01①
Worker	3	0 (0.00)	0.00		
Peasant	1	0 (0.00)	0.00		
Student	101	6 (5.94)	25.00		
Civil servant	5	0 (0.00)	0.00		
Teacher	3	0 (0.00)	0.00		
Medical personnel	18	0 (0.00)	0.00		
Company staff	13	1 (7.69)	4.17		
Self-employed	0	0 (0.00)	0.00		
Institution staff	1	0 (0.00)	0.00		
Migrant worker	1	1 (100.00)	4.17		
Unknown	20	16 (80.00)	66.67		
Blood donation history				4.756	P < 0.05
First time	122	22 (18.03)	91.67		
Repeat	44	2 (4.55)	8.33		

Note: *The positive composite rate is the ratio of the number of confirmed positive cases in this population to the number of preliminary reactivity samples in this population. The positive proportion was the proportion of confirmed positive cases to the total number of confirmed positive cases (24 cases) in the population. ①Fisher exact probability method is adopted HIV, human immunodeficiency virus.

24 were confirmed positive (including 23 from ELISA and 1 case from NAT, which confirmed positive after two follow-up visits). The initial screening positivity rate was 0.627‰ (166/264,615), and the confirmed positivity was 0.091‰ (24/264,615).

Characteristics of HIV-confirmed positive blood donors

A total of 24 blood donors were confirmed HIV-positive (0.091‰). Among these confirmed positive donors, 95.83% were male. The age distribution showed that 37.50% were between 18-24 years, followed by 25.00% in the 25-34 age group. Educationally, 33.33% had junior college, and 16.67% had undergraduate or senior high/technical secondary school education. Students represented 25%, while 66.67% had an unknown occupation. The differences were all statistically significant (all P < 0.05) (**Table 2**).

ELISA preliminary screening and confirmation

Among the 165 HIV reactive specimens initially screened by ELISA, 15.76% (26/165) were reactive with dual regents, 42.42% (70/165)

Table 3. WB confirmation results of HIV primary screening reactive specimens using ELISA reagents

	Reactivity number using ELISA initial screening	WB [n (/%)]		
		Confirmed positive	Indeterminant	Negative
WT single reagent reactivity	70	0 (0.00)	15 (21.43)	55 (78.57)
XC single reagent reactivity	69	0 (0.00)	16 (23.19)	53 (76.81)
Dual reagent reactivity	26	23 (88.46)	1 (3.85)	2 (7.69)
Total	165	23 (13.94)	32 (19.39)	110 (66.67)

Note: There was a significant difference in the WB confirmation results of single reagent reactivity and dual reagent reactivity ($x^2 = 142.98$, $P < 0.05$); There was no significant difference in the WB confirmation results between two single reagent reactivity ($x^2 = 0.062$, $P > 0.05$). HIV, human immunodeficiency virus.

Table 4. S/CO situation and WB confirmation results of ELISA initial screening of reactive samples

	Sample S/CO value	WB		
		Confirmed positive (n = 23)	Indeterminant (n = 32)	Negative (n = 110)
Single reagent Reactivity (n = 139)	0.8-1.0	0 (0.00)	3 (9.38)	41 (37.27)
	1.0-5.0	0 (0.00)	25 (78.13)	59 (53.64)
	5.0-10.0	0 (0.00)	3 (9.38)	8 (7.27)
	≥ 10.0	0 (0.00)	0 (0.00)	0 (0.00)
Total		0	31	108
Dual reagent Reactivity (n = 26)	0.8-1.0	0 (0.00)	0 (0.00)	2 (1.82%)
	1.0-5.0	0 (0.00)	1 (3.13%)	0 (0.00)
	5.0-10.0	1 (4.35)	0 (0.00)	0 (0.00)
	≥ 10.0	22 (95.65)	0 (0.00)	0 (0.00)
Total		23	1	2

Note: One specimen with no ELISA reactivity/NAT reactivity was not included in this table. S/CO: the ratio of the absorbance value (S) of the sample to the cut-off value (CO, critical value).

Table 5. Analysis of variance results of initial screening S/CO values with different confirmation results

	Positive	Negative	Indeterminant	Kruskal-Wallis test statistic H value	*P*
S/CO	20.00 (20.00, 24.19)	1.23 (0.99, 1.97)	1.48 (1.09, 3.04)	106.44	P = 0.000

Note: S/CO: the ratio of the absorbance value (S) of the sample to the cut-off value (CO, critical value).

with WT single reagent, and 41.82% (69/165) with XC single reagent. WB confirmation showed an 88.46% (23/26) positive rate among dual reagent-reactive samples. No positive confirmations were found in the single reagent-reactive groups, with a 77.70% negative rate in WB (108/139). Statistical analysis revealed a significant difference in WB confirmation results between single and dual reagent reactivity ($P < 0.05$) (**Table 3**).

Comparison and analysis of different confirmatory results and ELISA initial screening values

WB indeterminate results were predominantly associated with single reagent S/CO values between 1.0 to 5.0, accounting for 78.13% (25/32) of the total indeterminate cases. No confirmed positives were found with S/CO values below 5.0. Among the confirmed positive samples, 95.65% (22/23) had S/CO values ≥ 10 from initial ELISA screening reactivity. The initial screening S/CO values of confirmed positive specimens were significantly higher than those of confirmed negative or indeterminate results, with statistically significant differences ($P < 0.05$) among the three groups, as determined by F-test (**Tables 4**, **5**).

WB confirmation in NAT reactive specimens

One blood donor initially tested non-reactive by ELISA but reactive by NAT. After follow-up, on the 10th day, the WB result changed to indeterminate on day 10, showing a gp160 band. By day 27, WB confirmed HIV-positive with bands

Table 6. WB reaction bands of HIV-1 antibody positive and HIV antibody uncertain specimens

	Banding pattern	Positive (n = 24)		Indeterminant (n = 32)	
		Number of cases	%	Number of cases	%
Env envelope glycoprotein	gp160	24	100	11	34.38
	gp120	24	100	0	0
	gp41	22	91.67	0	0
Pol reverse transcription protein	p66	22	91.67	2	6.25
	p51	22	91.67	2	6.25
	p31	22	91.67	0	0
Gag core protein	p55	4	16.67	0	0
	p24	24	100	25	78.13
	p17	17	70.83	4	12.50
	P39	6	25	0	0

Note: HIV, human immunodeficiency virus.

for gp160, gp120 and p24, and a viral load (VL) of 9.1 × 10^5 copies/mL. The transmission pathway for this donor was identified as MSM (men who have sex with men).

WB reaction bands of confirmed HIV positive and indeterminate specimens

In the WB bands of 24 confirmed HIV-positive specimens, the presence of gp160 and gp120 encoded by anti-env genes (outer membrane protein) and p24 encoded by anti-gag genes (core protein) showed a positive detection rate of 100%. Additional bands, such as gp41 and proteins p66, p51, and p31 encoded by the anti-pol gene, were detected in 91.67% (22/24) of cases. Detection rate of p17, also encoded by the ant-gag gene (core protein) was 70.83% (17/24). The p55 and p39 bands were relatively less frequent, with rates of 16.67% (4/24) and 25% (6/24), respectively. Among the 32 indeterminate specimens, the detection rate of p24 was the highest, at 78.13% (25/32), with a single P24 band in 50% (16/32). gp160 had a detection rate of 34.38% (11/32), with a single gp160 band present in 18.75% (6/32) and a gp160+p24 combination detected in 12.50% (4/32). The detection rate of P17 was 12.50% (4/32), with a P17+p24 combination type detected in 9.38% (3/32) (**Table 6**).

Viral load comparison in confirmed HIV-positive individuals by gender, infection route, and age

Among the 24 confirmed positive donors, males accounted for 95.83% (23/24) and females accounted for 4.17% (1/24). Samples with VL values above 5,000 copies/mL accounted for 87.5% (21/24), while 3 samples (12.5%) had VL values below this threshold. The mean VL was 1.81 × 10^5 copies/mL, with a standard deviation of 1.46 × 10^5 copies/mL. Epidemiologic investigations showed that there were 14 cases (58.33%) of infections caused by heterosexual contact, 8 cases (33.33%) of male-male sexual behavioral infections (including 6 students aged 18-24), and 2 cases (8.33%) of unknown reason due to untraceable phone numbers. There was no significant difference in VL among the three route groups (P > 0.05) or among different age groups (P > 0.05) (**Table 7**).

Discussion

Blood and blood component transfusion are vital, life-saving interventions supporting countless patients globally. However, contaminated blood transfusions play a key role in the transmission of blood-borne infectious agents. Monitoring HIV prevalence trends in the donor population remains valuable to assess the effectiveness of existing intervention strategies [12]. This study analyzed the HIV screening and confirmation outcomes among voluntary blood donors in Nanchang City from January 2021 to February 2024. The confirmed positive rate of HIV was 0.091‰, lower than the rates reported in Shanghai (0.18 ‰) [13], Ningxia (0.10‰) [14], Fuzhou (0.16‰) [15], and close to Jiaxing (0.09‰) [16]. Despite high population mobility as a provincial capital, HIV infections in Nanchang are relatively well-controlled. This is related to the fact that in recent years, the center has organized personnel to compile relevant materials on blood donation knowledge and selected lecturers to strengthen the

Table 7. Comparison of viral load among confirmed HIV-positive donors of different genders, infection routes, and ages

	N	VL > 5,000 copies/mL	VL < 5,000 copies/mL	χ^2	*P*
Gender				1.34	> 0.05
Male	23	21	2		
Female	1	0	1		
Age					> 0.05①
18-24	9	9	0		
25-34	6	5	1		
35-44	4	3	1		
45-55	5	4	1		
56-60	0	0	0		
Route of infection					> 0.05①
Male-Male sexual behavior	8	8	0		
Heterosexual contact	14	11	3		
Unclear route due to wrong contact information	2	2	0		

education on health knowledge before blood donation.

Confirmed HIV-positive blood donors were analyzed by gender, age, occupation, and donation frequency. The positive rate among females (4.17%) was much lower than among males (95.83%). The highest proportion of confirmed cases was among young donors aged 18-24 (37.5%), with those of unknown occupation showing the highest positivity rate (66.67%), followed by students (25%). Repeat donors (8.33%) had a lower positive rate than that of first-time blood donors (91.67%). In response to the demographic characteristics of HIV-positive donors, further targeted health education is essential, and recruitment and pre-donation information should be optimized. Strengthening consultation and screening skills among staff to identify high-risk donors, retaining regular donors, and expanding the pool of repeat donors will help reduce the residual risk of HIV transmission through blood [17-19].

In this study, one specimen was identified as ELISA non-reactive but NAT reactive. Follow-up revealed that the donor had engaged in male-male sexual activity 12 days prior to this blood donation, confirming that the donor was in the HIV window period. This cases underscores the critical role of NAT testing at blood stations [20-24]. The sensitivity and specificity of first to fourth-generation HIV test reagents have increased, with sensitivity progressing from 99.0%, > 99.5%, > 99.5% to > 99.9% and the specificity increasing from 95.0-98.0%, > 99.0%, > 99.5% to 99.5%, respectively [22]. However, there is still a risk during serological testing window period. Studies have shown that in the first 3-4 days following HIV infection, viral concentrations are very low, with HIV RNA typically undetectable until 6-8 days post-infection. During this period (before HIV RNA is detected), infected individuals are still capable of transmitting the virus [23]. Therefore, blood collection staff should prioritize comprehensive health consultations during donor recruitment and exclude individuals who may be donating blood primarily as a means of obtaining health screening.

As a new diagnostic method for HIV, viral load testing is a valuable addition to HIV antibody confirmation testing methods [25, 26]. In this study, 87.5% of confirmed positive specimens had VL values exceeding 5,000 copies/mL, indicating that most newly confirmed HIV-infected individuals exhibit varying degrees of HIV virus replication in their bodies, with active virus replication necessitating immediate antiviral treatment. Epidemiological survey showed that 6 of the 8 blood donors with male-male sexual infection were students aged 18-24 years old. At present, HIV transmission in China is mainly through sexual transmission, with a significant rise in MSM-related infections, particularly among young people [27]. This trend underscores the need for disease prevention

and control departments to focus on this demographic, actively engaging in AIDS prevention within college environments and enhancing the scientific rigor and effectiveness of educational outreach [28]. In addition, two positive blood donors of unknown reasons due to untraceable phone numbers suggests the potential for high-risk individuals to exploit blood donation as a means of health screening, warranting vigilance against malicious donations.

However, this study has some limitations. Firstly, the sample size may be relatively small, potentially limiting the generalizability to the overall voluntary blood donor population. Secondly, the study focused on a specific region and timeframe, lacking a more comprehensive and long-term perspective. In addition, potential biases could exist in the data collection or methodology. Further research should aim to expand the sample size and cover more diverse regions and populations to obtain more accurate and generalized results.

Conclusions

To further ensure clinical blood safety, blood collection and supply institutions should rigorously evaluate HIV testing reagents under the guidance of *Technical Operating Procedures for Blood Stations*, scientifically refine HIV screening strategies, and establish more precise "gray area" intervals. Introducing chemiluminescence immunoassay (CLIA) technology for joint testing, conducting viral load testing for uncertain donors, and retaining more qualified blood donors are essential to maintaining safety. At the same time, institutions should develop more effective recruitment strategies, strengthen HIV education efforts, enforce thorough pre-donation consultations, establish regional blood donor monitoring systems, and optimize the exclusion of high-risk individuals from the donor pool to ensure the healthy development of voluntary blood donation.

Disclosure of conflict of interest

None.

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