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ORIGINAL ARTICLE

Correlation Analysis of HIV Infection Epidemiology and Detection Characteristics Among Voluntary Blood Donators in Hefei City

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SUMMARY

Background: **This study aimed to explore the characteristics of HIV infection prevalence and laboratory detection results among voluntary non-remunerated blood donators in Hefei.**
Methods: **Statistical analyses were performed on 609,230 blood samples from blood donation volunteers receiving HIV screening tests in Blood Center of Anhui Province from 2017 through 2021. Blood samples were screened and/or confirmed by HIV ELISA, nucleic acid testing (NAT), and western blotting (WB)-based HIV confirmation detection if appropriate. The reactive rates, correlation, and consistency of HIV ELISA screening and WB confirmation tests as well as NAT were comprehensively analyzed in a large scale. The efficacies of HIV ELISA reagents were assessed through ROC curve analyses.**
Results: **The WB confirmed HIV-positive rate averaged 0.013% (80/609,230) among the blood donation cases between 2017 and 2021. In the HIV-positive population, the gender ratio (male to female) was over 25, the age group of 18 - 30 years was predominant, with blood group types of mainly A and B, and most were office employees and students. The total number of HIV preliminary screening tests with reactive results by two ELISA reagents (reagent 1 and reagent 2) amounted to 1,948 (0.320%), with 1,828 of single-reagent reactive and 120 of double-reagent reactive. The HIV ELISA initial and retest reactive rates for reagent 1 vs. reagent 2 were 0.077% vs. 0.405% ($p < 0.001$) and 0.054% vs. 0.286% ($p < 0.001$), respectively, but their ELISA retest compliance rates were comparable. The ROC curve analyses showed that the area under curve (AUC) and specificity of reagent 1 were relatively higher than those of reagent 2, but both shared an identical sensitivity.**
Conclusions: **From 2017 through 2021, the local participation of voluntary non-remunerated blood donation showed an overall increasing trend, and the HIV infection prevalence maintained relatively stable in the blood donating population, with the infected individuals being dominated by sexually active young male office employees and students. There existed certain differences in the screening efficacies of distinct HIV ELISA reagents. The authorized laboratories should comprehensively evaluate the selection of blood screening programs and reagents to effectively conserve blood resources and reduce the risk of blood-borne HIV transmission.**
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KEYWORDS

HIV infection, voluntary non-remunerated blood donation, epidemiological characteristics, HIV screening and confirmation

INTRODUCTION

Since acquired immune deficiency syndrome (AIDS) was officially documented in the 1980s, human immunodeficiency virus (HIV) infections in China have undergone introduction and spreading periods, and currently have entered into the stage of rapid growth, to which the HIV infection prevention and control is increasingly concerned [1-3]. With the continuous development of testing approaches, the risk of HIV transmission through blood transfusion has been substantially reduced. However, due to the influence of host factors, demographic characteristics, detection window period, virus mutation, and others, there is still a certain false negative possibility in blood screening, resulting in the risk of HIV transmission [4-6]. Therefore, understanding the epidemiological characteristics of HIV infections among different blood donation populations and the effectiveness of screening strategies are particularly important for efficiently controlling the risk of HIV transmission via blood and developing reasonable blood screening programs under the premise of saving blood resources [7,8]. In the present study, we conducted a comprehensive analysis on the prevalence trend of HIV infections and the baseline characteristics of the confirmed positive population among blood-donating volunteers in Hefei city, the capital of Anhui province in East China, in a large scale, from 2017 through 2021. We specifically compared the current serological screening reagents for HIV and their consistency with HIV RNA test (or nucleic acid testing, NAT) as well as HIV confirmation test, with a view to guiding the optimization of blood donor recruitment and blood screening strategy in the region, hopefully reducing the risk of HIV transmission via blood transfusion.

MATERIALS AND METHODS

General data

A total of 609,230 samples from blood-donating volunteers were collected and screened for HIV infections in Blood Center of Anhui Province, in Hefei city of Anhui province in East China, from January 2017 to December 2021. Each blood sample was split presumably for both the HIV serological assay and NAT. The physical examination of all blood-donating volunteers met the requirements for blood donor health examination of the local government, and all blood samples were centrifuged and well preserved after collection in accordance with the Technical Operating Procedures for Blood Stations, and underwent all the tests in a timely manner [9, 10].

Instruments and reagents

The Microlab FAME automatic ELISA analyzer was used for HIV screening tests, assisted with the STAR automatic sample-adding device (Hamilton Company, Switzerland). The Novartis TIGRIS blood nucleic acid screening analyzer was applied (Novartis, Switzerland). The Roche Cobas s201 automatic nucleic acid extraction and amplification detection system, Cobas Ampli-Prep and Cobas Taqman analyzers were employed (Roche, Switzerland). Procleix Panther automated nucleic acid detection and analysis system was utilized as well (Grifols, USA). The third generation of HIV ELISA detection reagents (Beijing Wantai BioPharm, China; referred to as "Reagent 1") and the fourth generation of HIV ELISA detection kits (DiaSorin, UK; referred to as "Reagent 2") were commercially available. Nucleic acid detection reagents were purchased from Roche (PCR-fluorescence method) or Grifols (TMA-chemiluminescence method). The quality control (QC) reagents for the HIV ELISA and NAT were provided by Beijing Controls & Standards Biotechnology Co., Ltd (Beijing, China).

Detection procedures

All samples were routinely preliminarily screened for HIV twice using the two ELISA reagents (Reagent 1 and Reagent 2), respectively. The reactive samples from the preliminary screening tests were then sent to the AIDS Laboratory of the Blood Center for HIV confirmation tests by western blotting (WB). Certain ELISA single-reagent reactive (i.e. either of the two ELISA reagents presented reactive results, defined as ELISA single-reagent reactive) samples or negative (i.e. both ELISA reagents gave non-reactive results) samples were further tested for HIV NAT. ELISA double-reagent reactive (i.e. both of the two ELISA reagents presented reactive results, defined as ELISA double-reagent reactive) samples were regularly no longer subject to NAT (i.e. "Exemption"). The flow chart is shown in Figure 1. The third-party QC products were used and set in accordance with the manufacturer's instructions.

Result interpretation

Each HIV ELISA testing result was considered as reactive if its S/CO [ratio of the optical density (OD) of sample (S) to cutoff (CO)] was no less than the gray zone (GZ) with $0.5 \leq S/CO < 1$ (for Reagent 1) or $0.85 \leq S/CO < 1$ (for Reagent 2). The reactive samples from the preliminary screening tests need to experience a "double-reagent, duplicate-well" double-check detection, in which the samples would be regarded as true reactive if any one of the 4 wells could give rise to the $S/CO \geq GZ$. The samples receiving mixed or combined NAT with reactive results would either be divided to retest or undergo discrimination detection. The WB positive results from HIV confirmation tests in the AIDS

Table 1. Analysis of the epidemiological trends and test results of HIV infections among blood-donating volunteers in Hefei from 2017 to 2021.

Year	# of blood donations n (%)	HIV ELISA single-reagent reactive # n (%)	HIV ELISA double-reagent reactive # n (%)	# of HIV ELISA preliminary screening tests with reactive results			# of HIV confirmation tests (WB) with positive results			# of HIV confirmation tests (WB) with uncertain results		
				n (%)	χ^2	p	n (%)	χ^2	p	n (%)	χ^2	p
2017	110,905 (18.204)	394 (0.355)	21 (0.019)	415 (0.374)			14 (0.013)			2 (0.002)		
2018	118,807 (19.501)	309 (0.260)	40 (0.034)	349 (0.294)			29 (0.024)			2 (0.002)		
2019	128,449 (21.084)	259 (0.202)	18 (0.014)	277 (0.216)	71.126	< 0.001	7 (0.005)	17.670	0.001	6 (0.005)	2.773	0.608
2020	126,362 (20.741)	421 (0.333)	21 (0.017)	442 (0.350)			16 (0.013)			3 (0.002)		
2021	124,707 (20.470)	445 (0.357)	20 (0.016)	465 (0.373)			14 (0.011)			5 (0.004)		
Total	609,230 (100.000)	1,828 (0.300) *	120 (0.020) *	1,948 (0.320) #			80 (0.013) #			18 (0.003)		

* **Comparison between the frequencies of HIV ELISA single-reagent and double-reagent reactive cases (χ^2 = 1,499.967, p < 0.001),** # **Comparison between the frequencies of HIV ELISA preliminary screening tests with reactive results and HIV confirmation tests (WB) with positive results (χ^2 = 1,723.492, p < 0.001), Fisher's exact tests were applied if appropriate. WB western blotting.**

Laboratory were determined to be confirmed HIV positive.

Receiver operating characteristic (ROC) curve analysis

The ROC curves were prepared and the area under curve (AUC) was calculated to compare the testing efficacy of the two types of reagents (Reagent 1 and Reagent 2), with positive WB results as the gold standard for confirmation of HIV infections (as the state or dependent variables) and the corresponding S/CO values of the two reagents as the test or independent variables.

Statistical analysis

All data were statistically processed by IBM SPSS Statistics 25.0 software (SPSS Inc., USA). Qualitative variables were presented as frequency (%), and analyzed by the chi-squared test or Fisher's exact test. Screening efficiencies of Reagent 1 and Reagent 2 were evaluated by the ROC curve analysis. Statistical significance was determined using two-tailed tests. A p-value of < 0.05 was considered significant in all analyses.

RESULTS

Analysis of prevalence characteristics and test results of HIV infections among blood-donating volunteers in Hefei

Between 2017 and 2021, the number of participation in voluntary non-remunerated blood donation in Hefei was 609,230 in total, with a rising trend year by year. The number of HIV ELISA preliminary screening tests with reactive results reached 1,948 during the five years, accounting for 0.320% of the total tests, among which 1,828 cases were HIV ELISA single-reagent reactive and 120 cases were HIV ELISA double-reagent reactive. There was a statistically significant difference when compared the frequencies of HIV ELISA single-reagent and double-reagent reactive cases (χ^2 = 1,499.967, $p < 0.001$, Table 1). The number of WB confirmed HIV-positive cases amounted to 80 in total, which accounted for 0.013% in all the cases. The comparison between the frequencies of HIV ELISA preliminary screening tests with reactive results and WB confirmed HIV-positive tests showed a statistically significant difference (χ^2 = 1,723.492, $p < 0.001$, Table 1).

Baseline characterization of WB confirmed HIV-positive population

From 2017 through 2021, there were no statistically significant differences in gender, age, blood group and occupation/career among HIV-positive blood-donating volunteers ($p > 0.050$). The analyses revealed that the HIV-positive blood donators were predominantly males aged 18 - 30 years, whose blood group types were mainly A and B, followed by O, and most of whom were office employees and students (Table 2).

Consistency analysis of results from HIV ELISA preliminary screening tests using different reagents and HIV confirmation tests

There were statistically significant differences between the numbers of ELISA initial test- and retest- reactive

Table 2. Analysis of baseline characteristics of the HIV-positive voluntary blood donators in Hefei from 2017 to 2021.

Characteristics		Total # n = 80 (%)	# of HIV confirmation tests (WB) with positive results each year						
			2017 n = 14 (%)	2018 n = 29 (%)	2019 n = 7 (%)	2020 n = 16 (%)	2021 n = 14 (%)	$\chi 2$	p
Gender	male	77 (96.250)	14 (17.500)	27 (33.750)	7 (8.750)	16 (20.000)	13 (16.250)	2.295	0.762
	female	3 (3.750)	0 (0)	2 (2.500)	0 (0)	0 (0)	1 (1.250)		
Age (year)	18 - 30	48 (60.000)	7 (8.75)	22 (27.500)	3 (3.750)	9 (11.250)	7 (8.750)	17.098	0.086
	31 - 40	17 (21.250)	3 (3.750)	4 (5.000)	3 (3.750)	6 (7.500)	1 (1.250)		
	41 - 50	14 (17.500)	4 (5.000)	3 (3.750)	1 (1.250)	1 (1.250)	5 (6.250)		
	51 - 60	1 (1.250)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.250)		
Blood group	type A	27 (33.750)	5 (6.250)	9 (11.250)	2 (2.500)	6 (7.500)	5 (6.250)	6.915	0.892
	type B	25 (31.250)	3 (3.750)	10 (12.500)	3 (3.750)	3 (3.750)	6 (7.500)		
	type O	22 (27.500)	6 (7.500)	7 (8.750)	2 (2.500)	5 (6.250	2 (2.500		
	type AB	6 (7.500)	0 (0)	3 (3.750)	0 (0)	2 (2.500)	1 (1.250)		
Occupation	office employee	24 (30.000)	5 (6.250)	8 (10.000)	4 (5.000)	3 (3.750)	4 (5.000)	14.303	0.880
	students	15 (18.750)	1 (1.250)	8 (10.000)	1 (1.250)	3 (3.750)	2 (2.500)		
	peasants	5 (6.250)	2 (2.500)	1 (1.250)	0 (0)	1 (1.250)	1 (1.250)		
	factory workers	5 (6.250)	1 (1.250)	1 (1.250)	0 (0)	2 (2.500)	1 (1.250)		
	medical personne	1 (1.250)	1 (1.250)	0 (0)	0 (0)	0 (0)	0 (0)		
	others	30 (37.500)	4 (5.000)	11 (13.750)	2 (2.500)	7 (8.750)	6 (7.500)		

Fisher's exact tests were applied if appropriate. WB western blotting.

results from the two HIV ELISA reagents (Reagent 1 and Reagent 2) (χ^2 = 1,359.285, p < 0.001, and χ^2 = 965.732, p < 0.001, respectively). It didn't observe any statistical differences in the compliance rates of retests between the two HIV ELISA reagents (χ^2 = 0.183, p = 0.669). Furthermore, HIV confirmation tests via WB were carried out for the samples with HIV ELISA single- or double-reagent reactive results. The analyses indicated that there existed no any WB confirmed HIV-positive outcomes among those samples with HIV ELISA single-reagent reactive results. There were a total of 120 samples with HIV ELISA double-reagent reactive results, of which 80 cases were WB confirmed HIV-positive, accounting for 4.107% (80/1,948) of the HIV ELISA preliminary screening tests with reactive results (Table 3).

Correlation analysis between S/CO values of HIV ELISA preliminary screening tests and HIV confirmation test results

Both Reagent 1 and Reagent 2 showed statistically significant differences in their corresponding S/CO intervals with HIV confirmation test results (χ^2 = 283.398, p < 0.001, and χ^2 = 445.731, p < 0.001, respectively). In particular, during the intervals of 5 ≤ S/CO < 10 and S/CO ≥ 10, the agreement of positive results from the two ELISA reagents and HIV confirmation test was higher than the other S/CO intervals, and the WB confirmed HIV-positive rates were statistically different between the two ELISA reagents (p = 0.003 and p = 0.015, respectively; Table 4). The ROC curve analyses showed that the AUC and specificity of Reagent 1 were relatively higher than Reagent 2, but both shared an

Table 3. Consistency analysis of the results from HIV ELISA preliminary screening and HIV confirmation tests.

Parameters	Reagent 1 n (%)	Reagent 2 n (%)	χ^2	p
Total #	609,230	609,230		
ELISA initial test-reactive	472 (0.077)	2,469 (0.405)	1,359.285	< 0.001
ELISA retest-reactive	328 (0.054)	1,740 (0.286)	965.732	< 0.001
Compliance rate of ELISA retest	69.492%	70.474%	0.183	0.669
ELISA single-reagent reactive	208 (0.034)	1,620 (0.266)	1,092.308	< 0.001
WB positive	0 (0)	0 (0)		
WB uncertain	3 (1.442)	5 (0.309)	5.437	0.020
WB negative	205 (98.558)	1,615 (99.691)		
ELISA double-reagent reactive	120 (0.020)			
WB positive	80 (66.667)			
WB uncertain	10 (8.333)			
WB negative	30 (25.000)			

Fisher's exact tests were applied if appropriate. WB western blotting.

Table 4. Correlation between S/CO values of HIV ELISA preliminary screening tests and HIV confirmation results.

ELISA Reagents	S/CO intervals	# of ELISA preliminary screening tests with reactive	HIV confirmation test results (western blotting)				
			positive n (%)	uncertain n (%)	negative n (%)	χ^2	p
Reagent 1 (n = 328)	0.5 ≤ S/CO < 1	75 (22.866)	0 (0)	2 (2.667)	73 (97.333)	283.398	< 0.001
	1 ≤ S/CO < 5	149 (45.427)	0 (0)	7 (4.698)	142 (95.302)		
	5 ≤ S/CO < 10	11 (3.354)	0 (0) *	1 (9.091)	10 (90.909)		
	S/CO ≥ 10	93 (28.354)	80 (86.021) #	3 (3.226)	10 (10.753)		
Reagent 2 (n = 1,740)	0.85 ≤ S/CO < 1	353 (20.287)	0 (0)	1 (0.283)	352 (99.717)	445.731	< 0.001
	1 ≤ S/CO < 5	1,238 (71.149)	0 (0)	13 (1.050)	1,225 (98.950)		
	5 ≤ S/CO < 10	97 (5.575)	44 (45.361) *	1 (1.031)	52 (53.608)		
	S/CO ≥ 10	52 (2.989)	36 (69.231) #	0 (0)	16 (30.769)		

* **Comparison of WB confirmed HIV-positive case frequencies between the two reagents in the range of 5 ≤ S/CO < 10 (Fisher's exact test, p = 0.003).** # **Comparison of WB confirmed HIV-positive case frequencies between the two reagents in the range of S/CO ≥ 10 (χ^2 = 5.877, p = 0.015).**

Table 5. Detection efficacy analyses of two different HIV ELISA reagents via ROC curve.

ELISA Reagents	Standard error (SE)	Area under curve (AUC)	Maximal youden index	Optimal cut-off point	Sensitivity (%)	Specificity (%)	95% confidence interval (CI)
Reagent 1	0.001	0.999	0.996	11.692	100%	99.568%	0.998 - 1.000
Reagent 2	0.002	0.989	0.964	5.080	100%	96.432%	0.985 - 0.993

Table 6. Agreement of HIV ELISA preliminary screening, HIV confirmation and NAT test results.

HIV confirmation test results (WB)	Total # (n = 100)	ELISA Reagent 1	ELISA Reagent 2	NAT
+	55	+	+	/
+	18	+	+	+
+	7	+	+	-
±	4	+	+	/
±	6	+	+	-
±	5	-	+	-
±	3	+	-	-
/	2	-	-	+

+ reactive results of ELISA or NAT, or positive results of HIV confirmation tests via WB, - non-reactive or negative results of ELISA or NAT, ± uncertainty in the results of WB, / that WB and NAT were not tested or were exempted. WB western blotting. NAT nucleic acid testing.

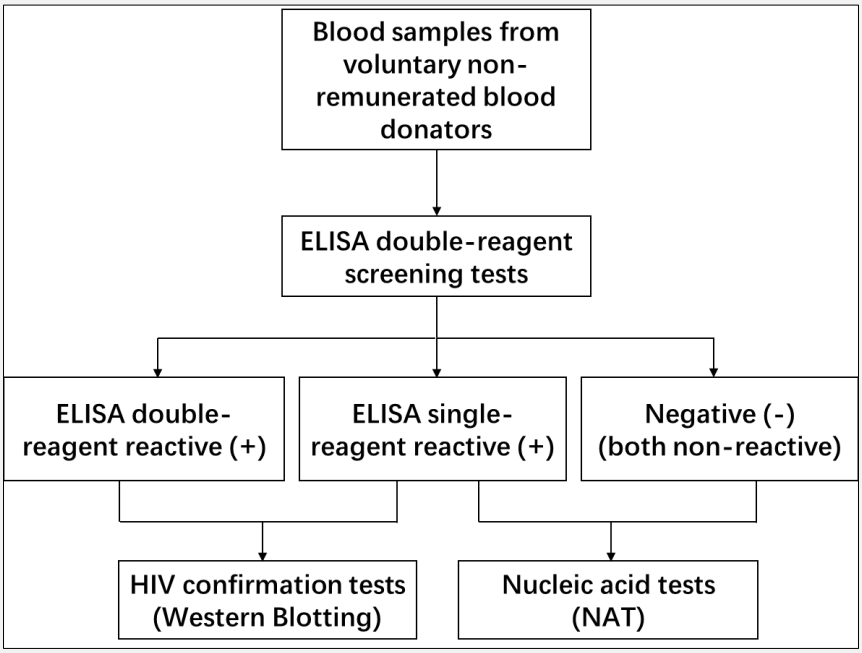

Figure 1. Flow chart of HIV screening and confirmation tests of blood samples from voluntary non-remunerated blood donators.

identical sensitivity (Figure 2 and Table 5). The HIV confirmation tests with uncertain results were excluded out of the analyses.

Agreement analyses of HIV ELISA preliminary screening, HIV confirmation and NAT test results

Out of the 80 WB confirmed HIV-positive samples, 73 showed HIV ELISA double-reagent reactive, for which the NAT results were positive or exempted; the remaining 7 cases were HIV ELISA double-reagent reactive but NAT negative. Among the 18 samples with uncer-

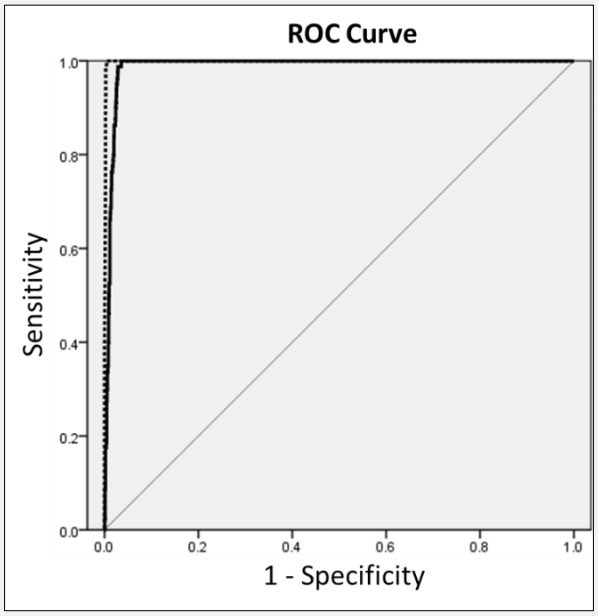

Figure 2. The ROC curve analyses of HIV ELISA preliminary screening tests using different reagents (dotted line - reagent 1, solid line - reagent 2, diagonal grey line - reference line).

tain results of HIV confirmation tests, 6 were HIV ELISA double-reagent reactive but NAT negative, and 8 were HIV ELISA single-reagent reactive and NAT negative. Interestingly, 2 samples were found non-reactive for both the ELISA reagents but NAT positive (Table 6).

DISCUSSION

From 2017 through 2021, it turned out to be an overall increase trend in the participation of voluntary blood donation in Hefei city, the capital of Anhui province. On average, the year of 2018 gave rise to a relatively better efficacy of HIV ELISA preliminary screening tests for the donated blood samples in terms of the frequency of WB confirmed HIV infections (0.024%), especially of which the HIV ELISA double-reagent test efficacy was the highest (0.034%). Unexpectedly, it observed no significant decline in the number of voluntary blood donation cases during the COVID-19 period of 2020 and 2021 (accounting for 20.741% and 20.470% of the total cases, respectively), and the WB confirmed HIV infection rates were both close to the average level, suggesting that the prevalence trend of HIV infections in this population of the region was not significantly affected by the impact of COVID-19 pandemic [11,12]. In general, the average frequency of HIV ELISA preliminary screening tests with reactive results for the voluntary blood donators in this region (0.320%) was nearly three times higher than that in Zhengzhou city, the capital of neighboring Henan province, but merely half lower than that in other parts of the country [13]. Out of the 1,948 cases of HIV ELISA preliminary screening tests with reactive results, a total of 80 samples were of WB confirmed HIV infections, accounting for 0.013% of the total number of cases, which was much higher than the reported average rate of HIV prevalence among voluntary blood donators in Anhui province between 2000 and 2009 [14]. To be noted, the average level of HIV prevalence in the voluntary blood donation population of this region was much lowered when compared with the data from the six cities (Chongqing, Liuzhou, Luoyang, Mianyang, Urumqi, and Shenzhen) with different geographic distributions in China [15,16].

Since the proposal and implementation of the “Four Frees and One Care” policy in December 2003, the rising trend of HIV infections in China has been basically controlled [17,18], and its main epidemiological pattern has changed from predominantly blood-borne transmission to sexual contact transmission, and the HIV infection rate among men who have sex with men (MSM) has been on the rise [19,20]. Therefore, enhancing comprehensive interventions for MSM to screen out and then treat individuals acquiring HIV as early and as soon as possible would be able to help reduce the risk of

HIV transmission and improve the safety of blood transfusion [7]. The present study also revealed that the gender ratio varied considerably among the local population with WB confirmed HIV infections, and males were the majority of the infected blood donators. The age of the HIV-positive blood-donating volunteers in this region mainly fell in the age group of 18 - 30 years, which was the sexually highly active period. The occupational classification showed that the student group was second only to the office employees. Such baseline and epidemiologic characteristics of the local population acquiring HIV were similar to those reported by Shi et al. and Chiou et al. [15,21], with the infection rate among young students increasing year by year. In this study, the population acquiring HIV was dominated by the blood group of types A and B, followed by type O, which was consistent with the reports by Batool et al. from Pakistan and Davison et al. from South Africa [22, 23]. However, there existed controversial viewpoints. For instance, a case report indicated that ABO blood group was associated with the risk of HIV infections, and the type O had the preferential probability of HIV infections [24]. Some investigators even suggested that ABO blood group appeared not to be significantly correlated with the HIV infection tendency [25]. It was possible that various sample sizes presented in different studies contributed to conflicting conclusions, more multicenter studies need to be conducted. It is believed that understanding the correlation between the ABO blood group system and the susceptibility to the specific disease in the population may be beneficial for healthcare departments including the Center for Disease Control and Prevention (CDC) to better optimize the prevention and control strategies of HIV infections.

The analyses of HIV ELISA preliminary screening tests demonstrated that there was a significant difference between the two ELISA reagents in terms of the agreement with the outcome of WB confirmed HIV infections, with Reagent 1 harboring a higher agreement rate of reactive results (24.390%, 80/328) than Reagent 2 (merely 4.598%, 80/1,740). The ELISA initial test-reactive rate (0.405%) and retest-reactive rate (0.286%) of Reagent 2 were remarkably higher than those of Reagent 1 (0.077% and 0.054%, respectively); however, the two reagents exhibited a similar compliance rate of ELISA retest. These findings suggest that the testing specificity of Reagent 1 would perform better than that of Reagent 2. It was reported that the testing sensitivity of the fourth-generation HIV ELISA reagents was higher than that of the third-generation ones [26], which was consistent with our observations. However, the false positive result rates of Reagent 2 ought to elevate over an increased sensitivity, resulting in a decreased specificity, which inevitably would exclude a large proportion of HIV false positive-blood donators, leading to a waste of blood resources and loss of scarce blood supplies [27]. In the HIV ELISA preliminary screening tests with the two reagents, both WB confirmed HIV-positive rates would increase significantly with the up-regulation of the corresponding ELISA cutoff GZ criteria. Reagent 1 and Reagent 2 illustrated the highest WB confirmed HIV-positive rates when the S/CO values were greater than or equal to 10, indicating a certain correlation between the ELISA cutoff GZ setting and the agreement of the outcome from WB-based HIV confirmation tests [28]. In addition, the ROC curve analyses showed that both Reagents performed well, with Reagent 1 slightly better than Reagent 2 in terms of screening efficacy. Some investigators claimed that the fourth-generation HIV ELISA reagents could be applied to screening the very early-stage individuals acquiring HIV whose HIV antigens, but not anti-HIV antibodies, might be detectable in the window period during blood screening settings [29]. According to our findings and the report by Chen et al., the fourth-generation HIV ELISA reagent (Reagent 2) did not seem to present such an advantage mentioned above [13]. Hence, when compared with the comprehensive performances of Reagent 1 and Reagent 2 in this study, it is recommended to cancel the tests by Reagent 2 to save the local blood resources, and to increase the ELISA cutoff GZ setting in combination with the HIV NAT to further reduce the false-elimination of blood donators, as well as to reduce the daily workload [27,30]. Among the WB confirmed HIV-positive population of the study, 7 cases were found to be HIV ELISA double-reagent reactive but NAT negative, which presumably were HIV elite controllers (i.e., individuals acquiring HIV with serological test reactive, WB confirmation test positive and NAT negative) or carriers of HIV gene variants, and these cases were likely subject to false negative results by the HIV NAT [2,31,32]. Interestingly, there were two cases with HIV NAT positive but neither of the ELISA reagents reactive, which may be in the window period of HIV infections; it was difficult to confirm whether they were infected or not, due to the lack of WB confirmation tests. Therefore, serological test and NAT are highly recommended to carry out in combination to effectively avoid missing HIV elite controllers and individuals in the HIV infection window period. As stated in the 2019 edition of the Technical Operating Procedures for Blood Stations of the local government, the HIV serological screening tests can be ELISA single-reagent alone, if combined with the NAT [10]. To date, however, many blood centers in China still adopt the HIV screening strategy with two ELISA reagents [33]. Through the comprehensive comparisons of the HIV screening test results from different HIV reagents and methodological strategies in the present study, the HIV screening model of “ELISA single-reagent test + NAT” is strongly recommended for the voluntary blood donators in the region, expecting to save the local blood resources and effectively reduce the risk of blood-borne HIV transmission.

In summary, the overall prevalence of HIV infections maintained in a relatively stable level among the voluntary non-remunerated blood donation population in Hefei city between 2017 and 2021. The local blood dona-

tion service providers should assess the risk of HIV infections according to the regional HIV epidemiological characteristics, strengthen the healthcare consultation to blood donators, and protect the susceptible or vulnerable population. The authorized laboratories are expected to carefully evaluate blood screening strategies and rationally select testing reagents to effectively reduce the risk of blood-borne diseases and simultaneously conserve precious blood resources.

Ethical Approval Statement:
This study was granted a waiver of informed consent as pre-existing, anonymized data from the institutional database were utilized without direct interaction with blood donators, and the institutional staff managed to de-identify all the donating information, maintaining the privacy and confidentiality before any data accessibility. The study abides by the guidelines of the Life Ethics Committee of Anhui Medical University and the Declaration of Helsinki.

Declaration of Interest:
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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