Comparison of three immunoassay systems for screening of HIV infection in blood donation in China

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Background: Preventing transfusion-transmitted HIV infection requires a sensitive and specific donor screening strategy. Currently, there is no public data to compare the performance of the two-different-EIAs strategy, the single-EIA and single-CLIA strategies for HIV donor screening in China. The aim of this study is to evaluate the performance of the three strategies.

Methods: HIV Ag/Ab chemiluminescence microparticle immunoassay (ARCHITECT CMIA, Abbott Diagnostics, a version of CLIA) was performed on 2,138 samples. Western blot (WB) was used to confirm all samples with one or both EIA reactive or grey zone results and all samples with reactive Abbott CMIA results. The performance of the three strategies was evaluated by comparing testing results to HIV infection status as defined by WB positive results.

Results: The positive and the negative coincidence rates of CMIA HIV Ag/Ab tests with confirmed HIV infection status were 98.41% (95% CI: 91.47–99.59%) and 99.32% (95% CI: 98.87–99.63%) respectively, equal or higher than EIAs. CMIA results yield the strongest correlation with HIV infection status.

Conclusions: This is the first study to compare the performance of recently allowed single-EIA, single-CLIA donor screening strategy to the currently used two-different-EIAs strategy for HIV donor screening in China. Our results indicate that CMIA performed better than EIAs with a strong correlation with WB confirmed infection status. Implementation of one-CMIA strategy can further reduce the risk of transfusion transmitted HIV infection while decrease unnecessary waste of blood and permanent deferral of donor caused by false positive results.

Keywords: Blood screening; China; CMIA; strategies

Introduction

HIV continues to be one of the major threats to public health worldwide as well as one of the major transfusion-transmitted infections. WHO reported there were approximately 36.7 million people suffering from HIV at the end of 2016, among which, 1.8 million were newly infected (1). Chinese Center for Disease Control and Prevention reported approximately 64 thousand people living with HIV infection as of May 2016, and a rapid increase of sexual transmission of HIV which signals a potential risk of the virus spreading to the general population (2). In China, HIV prevalence in the first-time blood

donors was estimated to be 66/100,000 (95% CI: 59–74) and HIV incidence among repeat donors was estimated to be 9/100,000 (95% CI: 7–12) person-years (3).

Serological screening for HIV antibody/antigen was performed routinely using two EIAs in parallel in China for decades as required by government policy (4). If either of the two EIAs demonstrated reactive result, the blood donation will be discard and the blood donor will be deferred permanently. In 2015, the new Guidelines for Blood Banks Operation Procedures issued by the National Health and Family Planning Commission of the People's Republic of China permitted the use of one EIA (single-EIA strategy) or one CLIA (single-CLIA strategy) for HIV antibody/antigen testing in parallel with nucleic acid testing (NAT). Chemiluminescent immunoassay (CLIA) has been extensively used for clinical diagnosis in China for years (5). Compared to EIAs, CLIA offers several advantages

Figure 1 The geographical distribution of the four blood centers.

including (I) reduced testing time to less than one hour due to shorter incubation and reaction times by fully automated analyzers and (II) being suitable for use in random access mode, with ability to process specimens one at a time as per need, rather than in batches.

Given that there is no reported data comparing the recently allowed single-EIA, single-CLIA strategies to the two-different-EIAs strategy, we conducted testing using the Chemiluminescence microparticle immunoassay (CMIA). CMIA is a CLIA assay that is being used for door screening in the majority of blood centers in the developed world. We compared the performance of CMIA with the single-EIA and two-different-EIA testing results using as a gold standard the confirmatory results from the Western Blot test.

Methods

This study has obtained ethics approval from the ethics committee of the Institute of Blood Transfusion, Chinese Academy of Medical Sciences and the number of the approval is 201620.

Specimens

Blood donor samples were obtained from blood centers from four geographically diverse Chinese regions (*Figure 1*). Plasma samples from a total of 2,138 donors were collected. All samples had have gone through routinely screening for HBsAg, anti-HCV, anti-syphilis and HIV-1/2 Ab/Ag by two different licensed EIAs at the blood centers. Six different EIA kits used by the four blood centers are listed in *Table 1*.

As showed in *Table 2*, among the 2,138 samples, 63

Table 1 Kits for the detection of HIV Ab/Ag used by the four blood centers

Blood center	Kits for the detection of HIV Ab/Ag	Number of the samples
Chongqing	EIA kit for the detection of Anti-HIV (1+2), Shanghai Kehua Bio-engineering Co., Ltd. (Shanghai)	2,033
	Diagnostic Kit for Antibodies to Human Immunodeficiency Virus (ELISA), KINGHAWK PHARMACEUTICAL (Beijing)	
Mianyang	Diagnostic Kit for Antibodies to Human Immunodeficiency Virus (ELISA), ZHUHAI LIVZON DIAGNOSTIC INC. (Zhuhai)	9
	GENSCREEN ULTRA HIV Ag-Ab, BIO-RAD (France)	
Urumqi	Antibody to HIV 1+2 ELISA, Beijing Wantai Biological Pharmacy Enterprise CO., LTD. (Beijing)	20
	Diagnostic Kit for Antibodies to Human Immunodeficiency Virus (ELISA), Biomerieux (Shanghai)	
Guangxi	EIA kit for the detection of Anti-HIV (1+2), Shanghai Kehua Bio-engineering Co., Ltd. (Shanghai)	76
	GENSCREEN ULTRA HIV Ag-Ab, BIO-RAD (France)	

were tested reactive by both of the two EIAs, 102 samples were reactive by only one EIA and 1,973 samples were nonreactive by both of the two EIAs including 42 samples with gray zone results by one EIA and nonreactive by another.

HIV Ag/Ab screening by CMIA

The collected plasma samples were blinded to EIA results and assigned unique new sample identification numbers before tested testing with Abbott® HIV Ag/Ab Combo test (Abbott Diagnostics, Lake Forest, IL, USA) on the automated chemiluminescent microparticle immunoassay analyzer ARCHITECT i2000 system (CMIA, Abbott

Diagnostic, Lake Forest, IL, USA). HIV Ag/Ab Combo assay is based on a two-step sandwich chemiluminescent microparticle immunoassay for the testing of anti-HIV antibody and HIV p24 antigen in plasma samples as previously described (5). All procedures were conducted in accordance with manufacturer's instruction. Testing results were expressed as a signal to cut-off (S/CO) and S/CO >1.0 is considered reactive. The sensitivity and the specificity of this assay are 100% and 99.89% (95% CI: 99.68–99.98) respectively according to manufacture's instruction.

Confirmatory testing by Western blot

Samples tested reactive (or gray zone) by either of the EIAs or CMIA were tested further by Western blot (WB, HIV Blot 2.0, MP Diagnostics, Singapore), an assay widely used for anti-HIV antibody confirmatory testing. Confirmed HIV infection status is defined by a positive result in WB testing. No further testing was performed on the samples tested nonreactive by both EIAs and CMIA. The algorithm of the screening and confirmatory testing is presented in *Figure 2*.

Statistical analysis

Statistical analysis was conducted using statistical software SAS 9.13 (SAS Institute, Cary, USA). Agreement between different strategies was interpreted as Kappa value, and the

Table 2 Details of the samples included in this study#

Routine HIV Ab/Ag screening results by two EIAs	Number of the samples
Reactive by two EIAs	63
Reactive by one EIA	102
Nonreactive by two EIAs	1,931
Gray zone* by one EIA	42
Total	2,138

#, all samples were freshly frozen after collection before shipped to IBT and restored at −80 ℃; *Gray zone: samples tested with border line results (0.5< S/CO <1) by EIA.
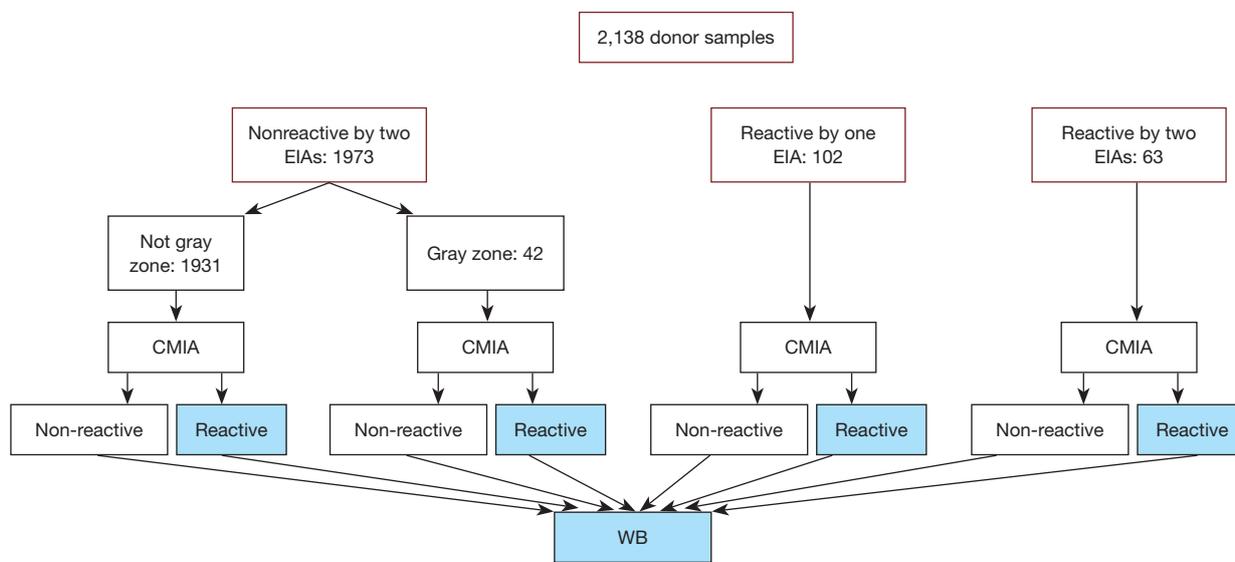
Figure 2 The algorithm of the screening and confirmatory testing. WB, Western blot.

Table 3 WB testing results of different samples

Routine HIV Ab/Ag screening results by two EIAs	Results of CMIA	Results of WB, n (%)			Total
		P	N	IND	
Two EIAs reactive	Reactive	55 (94.83)	1 (1.72)	2 (3.45)	58
Two EIAs reactive	Nonreactive	1 (20.00)	4 (80.00)	0 (0.00)	5
One single EIA reactive	Reactive	2 (22.22)	6 (66.67)	1 (11.11)	9
One single EIA reactive	Nonreactive	0 (0.00)	90 (96.77)	3 (3.23)	93
Two EIAs nonreactive	Reactive	4 (33.33)	5 (41.67)	3 (25.00)	12
Gray zone* by one EIA	Reactive	1 (33.33)	2 (66.67)	0 (0.00)	3
Gray zone* by one EIA	Nonreactive	0 (0.00)	39 (100.00)	0 (0.00)	39
Total		63 (28.77)	147 (67.12)	9 (4.11)	219

*Gray zone: samples with border line results (0.5< S/CO <1). WB, Western blot; P, positive; N, negative; IND, indeterminate.

Kappa value more than 0.75 was considered as substantial agreement.

Results

Of the total 2,138 samples tested for HIV Ag/Ab by CMIA on the ARCHITECT i2000 system, 82 samples were reactive and 2,056 were nonreactive. All EIA reactive or gray-zone samples and all the CMIA reactive samples (219 in total) were tested further by WB. As shown in *Table 3*, 55 (55/58, 94.83%) of the 58 samples reactive by both EIAs and CMIA were confirmed by WB. Five samples (5/63, 7.94%) reactive by both of the two EIAs were nonreactive by CMIA, of which 1 (1/5, 20%) was confirmed reactive by WB. For the 102 samples tested reactive by only one of the two EIAs, nine (9/102, 8.82%) were reactive by CMIA and two of these (2/9, 22.22%) were confirmed positive and one (1/9, 11.11%) was indeterminate by WB. Of the 93 samples reactive by only one EIA and nonreactive by CMIA, none was confirmed positive by WB. Of the 42 samples with gray zone result by one EIA, three (3/42, 7.14%) were tested reactive by CMIA and 1 (1/3, 33.33%) were confirmed positive by WB. Of the 39 samples reactive with gray zone result by one EIA and nonreactive by CMIA, none was confirmed positive by WB.

As showed in *Table 4*, 2,102 samples including 60 HIV-infected samples and 2,042 HIV-uninfected samples were tested by both Kehua ELISA and CMIA. Kehua ELISA correctly identified 54 samples as HIV-infected with a positive coincidence rate of 90.00% (95% CI: 79.49–96.24%) and 2,021 samples as uninfected with a negative

coincidence rate of 98.97% (95% CI: 98.43–99.36%). A total of 2,027 samples including 47 HIV-infected samples and 1,980 HIV-uninfected samples were tested by both Kinghawk ELISA and CMIA. Kinghawk ELISA and CMIA correctly identified 43 (43/47, 91.49%) and 46 (46/47, 97.87%) HIV infected cases, respectively. Of the 17 HIV uninfected cases tested by both Wantai ELISA and CMIA, 15 (15/17, 88.24%) were correctly identified by CMIA while only one (1/17, 5.88%) was correctly identified by Wantai ELISA. Of the 7 HIV uninfected samples tested by both LIZON ELISA and CMIA, the CMIA correctly identified all (7/7, 100%) the seven samples while LIZON ELISA identified only 1 (1/7, 14.29%) samples correctly. The overall positive coincidence rate and the negative coincidence rate of HIV Ag/Ab CMIA were 98.41% (95% CI: 91.47–99.59%) and 99.32% (95% CI: 98.87–99.63%), respectively. The agreement between HIV infection status and different screening assays including CMIA and six different EIAs were compared, and the best consistency (99.38%) was found between CMIA and HIV infection status with a kappa value of 0.90.

Discussion

Before 2016, all Chinese blood donors underwent HIV screening by two-different-EIAs. Recently, CLIA has been authorized by the Chinese government for the detection of HIV infection in blood donors. CLIA offers several advantages over traditional EIA screening, including less labor-intensity, higher throughput and faster turn-around time (6). For instance, the Abbott CMIA (one type of CLIA)

Table 4 Performance of different assays for HIV infected and HIV-uninfected cases

CMIA and different EIAs	HIV infected cases	Positive coincidence rate (%)	95% CI	HIV uninfected cases	Negative coincidence rate (%)	95% CI	Consistency (%)	Kappa
Group 1[a]	60			2,042				
CMIA correctly identified	59	98.33	91.06–99.96	2,030	99.41	98.98–99.70	99.38	0.90
Kehua correctly identified	54	90.00	79.49–96.24	2,021	98.97	98.43–99.36	98.62	0.79
Group 2[b]	47			1,980				
CMIA correctly identified	46	97.87	88.71–99.95	1,974	99.7	99.34–99.89	99.65	0.93
Kinghawk correctly identified	43	91.49	79.62–97.63	1,922	97.07	96.23–97.77	96.71	0.56
Group 3[c]	2			17				
CMIA correctly identified	2	100	15.81–100.00*	15	88.24	63.56–98.54	89.47	0.61
Wantai correctly identified	2	100	15.81–100.00*	1	5.88	1.49–28.69	15.79	0.01
Group 4[d]	14			69				
CMIA correctly identified	14	100	76.84–100.00*	63	91.3	82.03–96.74	92.77	0.78
Bio-Rad correctly identified	12	85.71	57.19–98.22	65	94.2	85.82–98.40	92.77	0.76
Group 5[e]	1			7				
CMIA correctly identified	1	100	2.50–100.00*	7	100	59.04–100.00*	100	1.00
LIZON correctly identified	1	100	2.50–100.00*	1	14.29	3.61–57.87	25.00	0.04
Group 6[f]	3			16				
CMIA correctly identified	2	66.67	9.43–99.16	15	93.75	69.77–99.84	89.47	0.60
Biomerieux correctly identified	2	66.67	9.43–99.16	16	100	79.41–100.00*	94.44	0.76
Group 7[g]	63			2,066				
CMIA total correctly identified	62	98.41	91.47–99.59	2,052	99.32	98.87–99.63	99.29	0.89

[a], samples with definite HIV infection status tested by both CMIA and Kehua ELISA; [b], samples with definite HIV infection status tested by both CMIA and Kinghawk ELISA; [c], samples with definite HIV infection status tested by both CMIA and Wantai ELISA; [d], samples with definite HIV infection status tested by both CMIA and Bio-Rad ELISA; [e], samples with definite HIV infection status tested by both CMIA and LIZON ELISA; [f], samples with definite HIV infection status tested by both CMIA and Biomerieux ELISA; [g], all the samples with definite HIV infection status tested by CMIA; *, one-sided, 97.5% confidence interval.

platform used in this study has a throughput of 200 tests per hour with a turn-around of 28 minutes (7). In contrast, EIA needs a long incubation time which results in delays in results reporting time and might increase the overall testing cost for some small blood centers. Numerous studies have reported that the HIV Combo CLIA has excellent sensitivity and specificity. A study of 10,995 specimens from different US studies funded by the Centers for Disease Control and Prevention reported that the sensitivity of CLIA for the detection of HIV Ag/Ab was 99.94% (95% CI: 99.79–99.99) and the specificity was 98.78% (95% CI: 98.51–99.01), and 48 (83%) of 58 HIV RNA positive but

EIA nonreactive specimens were detected by CLIA (8). Clinical performance of HIV Ag/Ab combination CMIA was investigated in China by testing of 88,000 clinical specimens and reported the sensitivity as 100% and specificity as 99.93% (95% CI: 99.73%–99.99%) (9). A study of 35,420 samples from a Chinese hospital compared the performance of EIA and CLIA. Eight (80%) of the 10 samples tested CLIA reactive but EIA nonreactive were confirmed positive by WB while no WB positive samples were missed by CLIA (10). Ouyang *et al.* (11) reported that among the 59 acute HIV infected specimens (37 were collected from a follow-up study of men who have sex with

men in China, and the others were from seroconservation panels of BBI and NABI and NIBSC companies), the sensitivity of CLIA (96.61%) was significantly higher than that of EIA (83.93%), and among the 703 specimens from clinical patients, the specificity of CLIA and EIA was 100% and 99.71%, respectively.

In the present study, we evaluated the performance of HIV 1/2 antibody/antigen CMIA in volunteer blood donors from four Chinese blood centers using WB as the confirmatory test for HIV infection status. We found that the HIV 1/2 antibody/antigen CMIA has a positive coincidence rate of 98.41% (95% CI: 91.47–99.59%) and a negative coincidence rate of 99.32% (95% CI: 98.87–99.63) with WB results, both higher than or equal to that of EIAs except for Bio-rad EIA and BioMerieux EIA. Bio-rad EIA showed a lower positive coincidence but a higher negative coincidence than CMIA while BioMerieux EIA showed the same positive coincidence with CMIA but a slightly higher negative coincidence rate. However, given that the relatively small sample size in these two groups (69 samples in group 4 and 19 samples in group 6), further study need to be conducted to gain more convincing results. In addition, good agreement (Kappa value higher than 0.75) with HIV infection status was found by CMIA (0.90), Kehua ELISA (0.79), BioMerieux ELISA (0.76) and Bio-Rad ELISA (0.76), among which CMIA has the best agreement with confirmed HIV infection status.

To compare the three strategies of two-different-EIAs, single-EIA and single-CMIA, the confirmed infection status of the samples with discrepant results by different strategies were further analyzed. The two-different-EIAs strategy demonstrated a very high false positive rate (all 93 samples disqualified by only one of the two EIAs were nonreactive by CMIA and negative by WB). Both single-CMIA strategy and single-EIA strategy demonstrated a lower false positive rate than the two-different-EIAs strategy. Of the 219 samples with discrepant results by EIAs and CMIA, 4 samples were reactive by single-CMIA but were missed by two-different-EIAs strategy and only one sample was missed by single-CMIA while reactive by the two-different-EIAs strategy. Therefore, the single-CMIA strategy demonstrated a lower false negative rate than two-different-EIAs and single-EIA strategy. To improve the sensitivity of two-different-EIAs, many blood centers in China set gray zone as S/CO between 0.5 and 1.0 and disqualify all donations with a gray zone or reactive result by either of the two EIAs. Only one of the 219 samples tested as gray zone was confirmed positive and this sample was detected as reactive by the single-CMIA strategy. Our results indicate that even though the use of a gray zone does increase the sensitivity of HIV screening with EIAs, it also significantly increased the rate of disqualifying donations based on false negative results.

There are several limitations relating to interpretation of results from this study. Firstly, only samples with screening reactive or gray zone EIA results were utilized in this study. Thus, our results cannot be used to calculate the true sensitivity and specificity of EIAs and CMIAs. Secondly, since different EIA kits were used by the four blood centers, the numbers of samples tested by a certain EIA kit are relatively small. This can affect the evaluation of the performance of different EIAs. The low agreement between HIV infection status with LIZON EIA and Wantai EIA in this study may be related to the small numbers of samples screened by these two assays included in this study. Thirdly, we didn't include the NAT results of these samples because the aim of this study is to compare the performance of three serological screening strategies. Moreover, the study was performed prior to implementation of NAT in China and hence NAT data is not available on all the samples included in this evaluation. The data on subset of samples where NAT data was available identified two samples that were negative by NAT, however, positive by CMIA and confirmed by the WB (data not shown). Whereas, the study also identified two samples that were NAT positive but negative by CMIA. These may be due to below the antigen detection limit of the CMIA with low viral load (data not shown). In addition, there were 48 samples that were NAT positive and all were detected by the CMIA (data not shown). We believe that improving the effectiveness of both serological and NAT screening is important to maximize blood safety.

This is the first study to compare the performance of the recently approved single-EIA, single-CLIA donor screening strategies with the currently used two-different-EIAs strategy for HIV blood screening in China. Our results suggest that single-CLIA performed better than both EIA strategies according to the agreements of results from these assays with the confirmed HIV infection status. The implementation of the single-CMIA strategy may serve to further reduce the risk of transfusion transmitted HIV infection and avoid the need for multiple assays, while decrease unnecessary waste of blood caused by false positive results.

Acknowledgments

Footnote

Conflicts of Interest: The authors Sushil Devare, Jian-Fang Liu, Xiao-Ting Lv, and Peng Yin are employees of Abbott Diagnostics. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Individual informed consent was waived. This study has obtained ethics approval from the ethics committee of the Institute of Blood Transfusion, Chinese Academy of Medical Sciences and the number of the approval is 201620.

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